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المحتويات

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MIP-1α Is More Sensitive than MCP-1, and CXCL10 Immune Markers in Diagnosis of Pediatric Sepsis

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Keywords: Pediatric sepsis, MIP-1α, MCP-1, CXCL10, cytokines, chemokines, CRP, neutrophil-to lymphocyte

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Abstract

Background: Pediatric sepsis is a leading cause of mortality among children both in Iraq and globally. The underlying causes of pediatric sepsis are diverse, encompassing a broad range of pathogenic mechanisms and varying responses to these triggers. However, a common characteristic of pediatric sepsis is the secretion of cytokines and chemokines.

Objectives: To evaluate the sensitivity and specificity of macrophage inflammatory protein-1 alpha (MIP-1α) in a comparison with monocyte chemotactic protein-1 (MCP-1) and C-X-C motif chemokine ligand 10 (CXCL10) immune markers in the pediatric sepsis and effort an evidence-based linking between the three biomarker and the laboratory value of neutrophil, lymphocyte and CRP.

Patients and Methods: This case control study includes 100 participants (50 patients and 50 control). Sandwich ELISA test used to evaluate the MIP-1α, MCP-1, and CXCL10 immune markers in pediatric sepsis and control groups.

Results: Present study shows high level of the neutrophil (62.45%), lymphocyte (47.90%) and CRP (50.52 mg / L) in the cases of pediatric sepsis compared with the normal range. Furthermore, this study shows that MIP-1α is more sensitive (82%) than MCP-1, and CXCL10 (56% and 54% respectively).

Conclusions: At the end, this study suggests that MIP-1α has the highest diagnostic sensitivity value, so it could be concerned as essential immunodiagnostic marker for pediatric sepsis.

MIP-1α اكثر حساسية من العلامات المناعية MCP-1 و CXCL10 في تشخيص الانتان عند الأطفال

سمر عطاالله سهيل، علي جليل علي الياسري , عبير ظاهر ناجي الحسناوي

الخلاصة

المقدمة: يعتبر الانتان أحد الأسباب الرئيسية للوفاة لدى الأطفال في العراق والعالم أجمع. الأسباب الكامنة وراء الانتان لدى الأطفال متنوعة وتشمل مجموعة واسعة من الآليات المرضية واستجابات مختلفة لهذه المحفزات. ومع ذلك، فإن السمة المشتركة للانتان لدى الأطفال هي إفراز السيتوكينات والكيموكينات

الهدف: تقييم حساسية وخصوصية البروتين الالتهابي البلاعم 1 ألفا **(MIP-1α)** مقارنة مع البروتين الكيميائي احادي الخلية 1 **(MCP-1)** ورابط الكيموكين من نوع **CXC 10 (CXCL10)** كعلامات مناعية في الانتان لدى الأطفال، والسعي لإيجاد علاقة بين هذه العلامات الحيوية الثلاثة والقيم المخبرية لعدد العدلات والخلايا اللمفاوية و بروتين سي التفاعلي.

الطرق والعينات: تتضمن دراسة الحالات والسيطرة 100 مشارك (50 مريض و 50 مجموعة سيطرة). تم استخدام اختبار الإليزا الساندويتش لتقييم علامات**MIP-1α** ، **MCP-1**، و **CXCL10** المناعية في حالات الانتان ومجموعة الاشخاص الاصحاء

النتائج: أظهرت الدراسة الحالية ارتفاعًا في مستوى العدلات (62.45%) والخلايا اللمفية (47.90%) وبروتين سي التفاعلي (50.52 ملغم/لتر) في حالات الانتان مقارنة بالمعدل الطبيعي. علاوة على ذلك، أظهرت هذه الدراسة أن **MIP-1α** أكثر حساسية (82%) من **MCP-1 (56%) , CXCL10** (54 %)على التوالي .

الاستنتاجات: في النهاية، تشير هذه الدراسة إلى أن **MIP-1α** لديه أعلى قيمة حساسية تشخيصية، لذلك يمكن اعتباره علامة مناعية تشخيصية أساسية للإنتان لدى الأطفال.

1. Introduction

Sever paediatric sepsis is defined as two or more systemic inflammatory response syndrome criteria, confirmed or suspected invasive infection, and cardiovascular dysfunction, acute respiratory distress syndrome, or two or more organ dysfunctions (Andrea T. Cruz et al., 2020) . The prevalence of pediatric sepsis in Iraq was reported to be (8.9%)

which may stand in a bit lower than other country ware prevalence stands at (39%) in New Delhi and (45.9%) in Egypt (Weiss et al., 2015)

Common etiologies in pediatrics sepsis were urinary tract infection (UTI) and meningitis especially in the ages below 6 month and above 10 years. Meanwhile, pneumonia were the main etiologies in the period from 6 month and until 5 years (Pawar et al., 2016) Innate immune response in pediatric sepsis starts with the recognition on the pathogens by the host immune system pattern recognition receptors (PRRs) on innate immune cells monocytes and neutrophils and somatic tissues (Mithal et al., 2022). Adaptive immune response also plays various crucial roles. It is essential in controlling inflammation and preventing tissue damage following an infection, as well as restoring the overall balance of the host's immune system through multiple mechanisms, and these processes and functions are disturbed or become unregulated in sepsis, resulting in inadequate protection against infections and/or immune suppression (Brady et al., 2020). One of the main macrophage inflammatory protein 1 alpha (MIP-1) is MIP-1α/CCL3 which are secreted by variant of cells including monocytes, T and B lymphocytes, neutrophils, dendritic cell (Dc) and natural killer (NK). It plays a critical role in the T Lymphocyte Recruitment whereas MIP-1β/CCL4 tends selectively toward the activation of CD4+ T cells, while, MIP-1α/CCL3 is directed towards the selectively activation of CD8+ T cell (Bhavsar et al., 2015). The recent researches demonstrate that the influx of granulocytes and macrophages into the peritoneal cavity during polymicrobial sepsis is temporally associated with increases in peritoneal CXCL10 concentrations. Blockade of CXCL10 significantly reduces the influx and phagocytic activity of peritoneal granulocytes during polymicrobial sepsis and worsens survival, in addition to providing evidence that CXCL10 is critical for the effective recruitment of innate immune effector cells during neonatal sepsis, it was demonstrated that exogenous CXCL10 can directly stimulate granulocyte and macrophage phagocytic function in vitro (Cuenca et al., 2011). Furthermore, numerous studies have shown that MCP-1 is linked to polarized Th2 responses which boost the secretion of IL-4 by T cells. Moreover, a growing body of evidence suggests that MCP-1 plays a crucial role in the pathogenic processes that lead to sepsis, also controls the advancement of inflammation and the generation of pro-inflammatory cytokines, and other Multiple studies have revealed that MCP-1 levels are significantly elevated in sepsis and contribute to systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). (Wang et al., 2018)

For all of the above, this study aims to identify the relationship between the most important and most related laboratory values (neutrophil and lymphocyte values in addition to C-reactive protein) with these three biomarkers (Monocyte chemotactic Protein 1, Macrophage inflammatory protein 1 alpha and CXC motif chemokine Ligand 10) in addition to identifying the quality and sensitivity of these biomarkers and showing the most important differences between them.

2. Materials and Methods

Blood sample were collected from 100 participants which includes 50 cases of sepsis and 50 as healthy control group at age ranged from > 1-14 years old attending to Children's Teaching Hospital in Karbala /Iraq during the period extended from October to April (2024). The blood specimen collected on EDTA tube and shaken up then was examined as soon as possible in automated hematology analysers (Swelab Alfa, Sweden) to count white blood cells. Immunological markers examined and estimated by Sandwich ELISA technique.

2.1. Statistical Analysis

Measures of central tendency for demographic data and laboratory values were defined by using frequencies expressing the sample size and arithmetic means. Furthermore, Statistical dispersion was estimated using Standard deviation (SD) for both the demographic parameters of the sample and the laboratory values. Person's correlation test was used in order to identify the association between Serum biomarkers and laboratory values of neutrophils, lymphocytes and CRP, (0.05 and 0.01 probability) using SPSS software (Version 26.0). ROC Curve analysis was used to estimate the sensitivity and selectivity of the biomarker using MedCalc software (Version 22).

3. Results:

3.1. Demographical and Clinical Characteristics of The Study Patients

This study recruited a total of 100 individuals comprising of 50 pediatric sepsis patients at the Children Teaching Hospital in kerbala . The patients were aged-matched with 50 healthy control group with matched demographic features as the pediatric sepsis patients. The mean age of the pediatric sepsis patients was 5.46 ±3.5 years, comprising of 32 females, aged 4.97±3.9 years with a weight of 17.45±9.1 kg and temperature at 38.66±0.8, as well as 18 males, aged 6.31±2.7 years with a weight of 19.64±8.6 kg and temperature at 38.60±0.5. The control group had the mean age of 6.96±3.4 years, comprising of 29 females, aged 6.65±3.9 years with a weight of 17.10±7.3kg, as well as 21 males, aged 7.38±2.5 years with a weight of 18.19±5.3 kg. The age, gender, weight and temperature of the study subjects were highly significant results, as present in Table1.

Table1: Age, Gender, Weight and Temperature of the Study Subjects

Groups	Gender	n	Age (mean±SD) (years)	p value	Weight (mean± SD)/Kg	p value	Mean Temp (± SD) (C^{o})	p value
Pediatric sepsis	**Female**	32	4.97±3.9	0.000*	17.45±9.1	0.028*	38.66±0.8	0.000*
	Male	18	6.31±2.7		19.64±8.6		38.60±0.5	
	Total	50	5.46±3.5		18.24±8.9		38.64±0.71	
Control	**Female**	29	6.65±3.9		17.10±7.3			
	Male	21	7.38±2.5		18.19±5.3			
	Total	50	6.96±3.4		17.56±6.5			

* Significant *p value* < 0.05, SD (standard deviation)

3.2.Laboratory Data of Patients Group

Regarding Laboratory data Table2, the Neutrophil mean of the pediatric sepsis patients was 62.45±24.6 cell (range, 10 – 97 cell), lymphocyte mean 47.90±33.4 cell (range, 6 – 103cell) and the mean of CRP was 50.52±44.1mg/L (range, 0.88 – 221mg/L).

Table2: Laboratory Data of Pediatric Sepsis Patient

Laboratory data	Mean	SD	Min	Max
Neutrophil	62.45	24.6	10	97
Lymphocyte	47.90	33.4	6	103
CRP mg/L	50.52	44.1	0.88	221
CRP: C-Reactive Protein				

3.3. Analysis of Serum Biomarker and Laboratory Data

Table3 shows Pearson's correlation matrix indication the association between the Serum biomarkers (MCP-1, MIP-1α and CXCL10) and the laboratory data (Neutrophils, Lymphocyte and CRP) of the pediatric sepsis studied subjects. Weak negative correlation was observed between Lymphocyte and the three biomarkers (r = -0.060, r = -0.099, r = -0.015 for MCP-1, MIP-1α and CXCL10 respectively). While a weak positive correlation was observed between MCP-1 and the Neutrophils, CRP (r = 0.093, and r = 0.020 respectively), and between CXCL10 and the Neutrophils, CRP (r = 0.092, and r = 0.202 respectively).

Table3: Pearson's Correlation Matrix for The Serum Biomarkers (MCP-1, MIP-1α And CXCL10) And the Lab Data (Neutrophils, Lymphocyte And CRP) For the Study Subjects

Parameters	Neutrophils	Lymphocyte	CRP
MCP-1	0.093	-0.060	0.020
MIP-1α	0.084	-0.099	0.068
CXCL10	0.092	-0.015	0.202
MCP-1: monocyte chemotactic protein-1, MIP-1α: macrophage inflammatory protein-1 alpha, CXCL10: C-X-C motif chemokine ligand 10			

3.4. ROC Analysis of MCP-1, MIP-1α And CXCL10 Serum Biomarker and Gender

The ROC curves Fig.1 data showed that all of the three-biomarker had significant results ($p < 0.001$) with efficiency very good AUC curve with specificity of 100% for MCP-1 and 52%, 96%for MIP-1α and CXCL10 respectively, and with the sensitivity of 82% for MIP-1α, and 56%, 54%for MCP-1 and CXCL10 respectively Table4.

Table4: ROC Curve Data for the Measured MCP-1, MIP-1α and CXCL10

Parameters	MCP-1	MIP-1α	CXCL10
AUC	0.770	0.696	0.758
p-value	<0.0001	<0.001	<0.0001
Best Cut-off	>981.17	>109	>142
Sensitivity (%)	56	82	54
Specificity (%)	100	52	96
AUC: Area Under Curve			

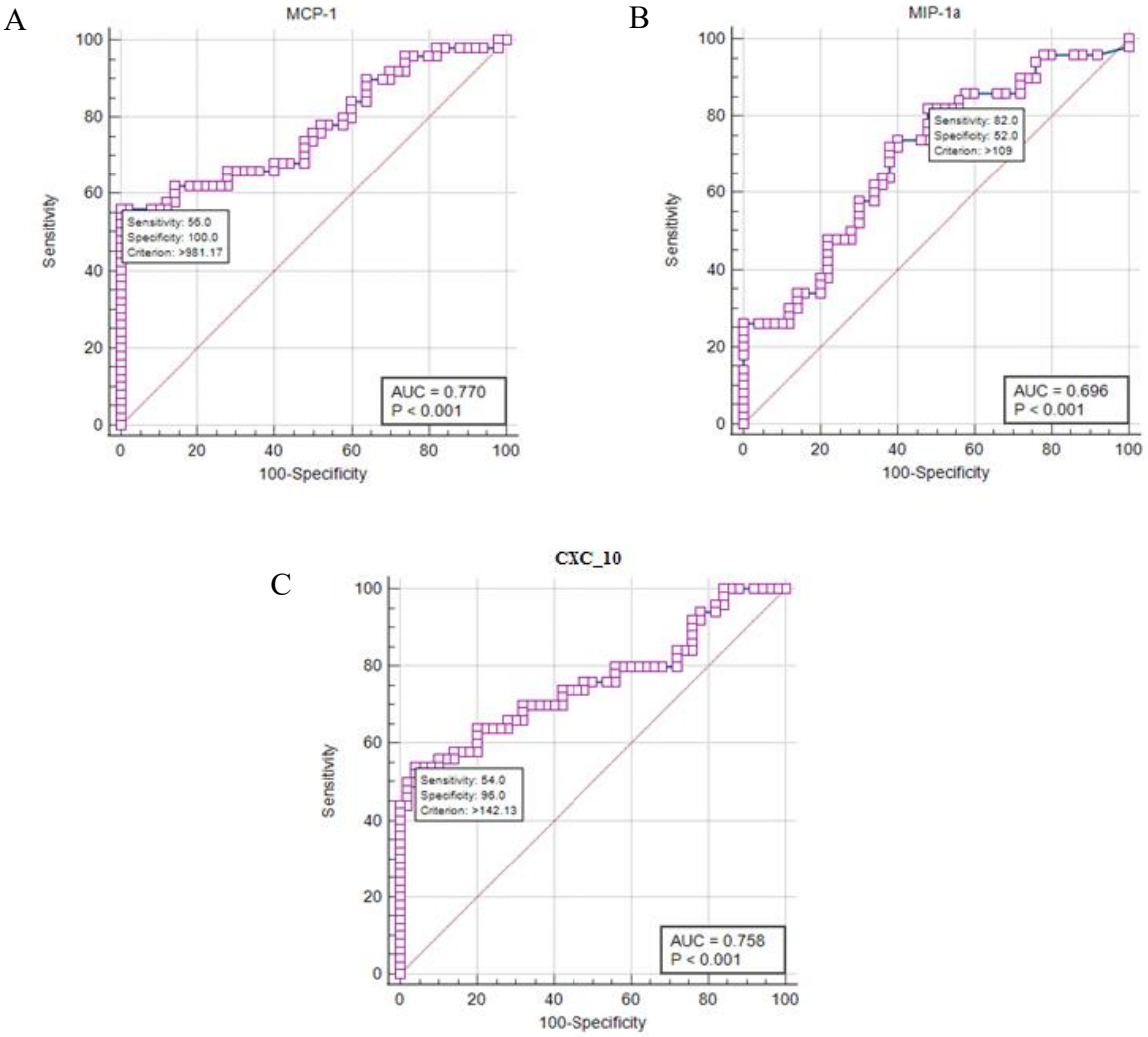

Figure1: Represents ROC Curve for The Measured of:
A) MCP-1, **B**) MIP-1α and **C**) CXCL10

4. Discussion

Differential diagnosis is considered very difficult in the case of pediatric sepsis due to the presence of many possible diagnoses that must be differentiated between them, especially congenital heart disease and metabolic disease in newborns, which are very common and can be confused with the features of pediatric sepsis shock (Hilarius et al., 2020). In addition, Age and underlying cardiovascular condition associated with the increasing in the odds ratio of the prevalence of pediatric sepsis with were estimated by 1.4 time for each variable in the United states (US) hospitalized pediatric patient between 2004 to 2012. Factors mentioned above were considered significant prognostic and risk factors side by side even with the organ dysfunction (Ruth et al., 2014). The current study showed a range of age results among male and female children with sepsis as shown in Table1, where the average age for females was (4.97 ± 3.9). (Charlson et al., 2018; Li et al., 2023) found that most cases of sepsis infection occur in children under the age of 10 years, Miura *et al* noted

in a study conducted in Japan using a national patient database where the study included children under the age of 20, and among 761 affected children, the age mean was (3), while, (McMullan et al., 2016) conducted a study on children under the age of about 18 years of age and they found that among the children with data, 1,873 children were infected, and the age mean was 57 months. Also, (Watson et al., 2003) found in a study conducted by the American Journal that the incidence of sepsis among males were (15) times higher than among females, this result was disagreeing with result of present study and this difference may be due to the sex-related differences in immunity and infection-related outcome that which have been found previously in animal and adult studies (40–47) suggesting that sex-related differences may be hormonal in origin. Najman *et al*., 2020 noted in a study of a group of children with fever between the ages of 1 month and 16 years, who had ≥ 1 warning sign for sepsis and their body temperature with a mean of 38.5(38.0-39.0). This result was consistent with current study result. Rapid diagnosis is considered one of the most important factors affecting the severity of pediatric sepsis. In order to achieve a rapid and effective diagnosis, there are several methodologies proposed (Andrea T Cruz et al., 2020), dysfunction of neutrophils which its leads to serious dangerous consequence that include TLR signaling deficits and altered apoptosis signaling pathways leading to immune dysfunction (Delano and Ward, 2016) . This immune system responds at a slower pace, but has the ability to identify specific antigens and use immunological memory to boost the immune response upon subsequent encounters with the same antigens, and it can depend on a smaller variety of cell types for its functions, which are lymphocytes, specifically T cells and B cells. In turn, B cells play a crucial role in producing antibodies and plasma cells essential for long-term immune defense, while T cells can be further categorized into various subclasses, each with distinct roles, such as CD4+, CD8+, gamma delta (γδ), and regulatory T cells (Tregs). (Brady et al., 2020). A study accomplished by (Ng et al., 2002)who found the neutrophil count in children with sepsis was 4.89(3.02-8.33). Also, another study who stated about children with mild sepsis, the neutrophil mean was 4.47(3.01-6.95) (da Silva et al., 2020; Sumitro et al., 2021). While in children with severe sepsis, the neutrophil mean was 5.52 (3.05-9.37). Also, there were increased in laboratory values, as the CRP mean was (50.5±44.1). A study conducted by the library International Medical Center on pediatric patients with a mean age of (6-169) months, where the CRP mean was (10.2±119.51), $p = 0.006$. In another study conducted on children, many children were found to have fever, as a high temperature is a warning sign of sepsis. This study included 291 children whose CRP value was 24 ml/l (Nijman et al., 2020). The present study showed that the Pearson correlation matrix indicated a set of associations between serum biomarkers (MCP-1, MIP-1α, and CXCL10) with laboratory data including neutrophils, lymphocytes, and CRP, for a pediatric sepsis subject Table3. (Inaba et al., 1997) reported the serum levels in children with meningitis showed that MIP-1α levels were strongly correlated with the number of neutrophils within the cerebrospinal fluid., $r = 0.750$ with $p < 0.001$, and there were significant correlations between MIP-1α levels and total lymphocyte count or neutrophil count ($r = 0.682$, $r = 0.478$) with ($p < 0.001$, $p < 0.001$) respectively. The present study showed that all three biomarkers had significant results ($p < 0.001$) with very good AUC curve efficiency with specificity of 100 for MCP-1 and 52, 96 for MIP-1α and CXCL10 respectively with sensitivity of 82 for MIP-1α, 56 and 54 for MCP. -1 and CXCL10, respectively Table4. A study

conducted by Li et *al* (2021b) showed that the levels of CXCL-10 in the serum of the sepsis children were high. It was also possible to measure serum levels using an area under the curve (AUC) of (0.885), a sensitivity of 81, and a specificity of 83.3. Also, (Song and Zheng, 2024) explained the value of the MCP-1. In the sepsis children, where the data of 82 children suffering from acute inflammatory conditions were analyzed according to the diagnostic criteria for sepsis, and their effectiveness, and the levels of use of this serum were analyzed using the area under the curve (AUC). The results were MCP-1 = 452.32 ± 2.97 pg/ Ml. AUC = 0.9406 with $p < 0.001$, as the results were significantly higher than those in the control group. In addition, (Gilholm et al., 2023) screened 3473 children for sepsis, and 523 children (15.1%) were found to have sepsis. The area under the receiver operating characteristic curve were (0.80, 95 CI 0.78 to 0.82) to reach a sensitivity of 90% the final model achieved a specificity of 51% confirmed by sensitivity analyzes using sepsis-related organ dysfunction scores and shock.

5. Conclusion

Our study suggests that MIP-1α have the highest sensitivity, so this marker seems to be exemplified an additional marker for immunodiagnostic of pediatric sepsis.

6. Ethical approval

The study protocol will be sent to the relevant ethical committee in the health directorate. Also, verbal approval will be taken from each participant before taking the sample. During samples collection, health measures and safety will be taken.

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Isolation and Identification of *Acinetobacter Baumannii* from Different Clinical Sources and Determine Antibiotic Resistance in Karbala City

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Abstract

Background: *Acinetobacter baumannii* one of the important multidrug-resistant opportunistic nosocomial pathogens, in part due to its high capability of acquiring resistance to different antibiotics groups.

Objective: The objective of this study was to determine the bacterial infection with *A.baumannii* and antibiotics sensitivity pattern in Karbala city.

Patients and Methods: Two hundred different Clinical specimens were collected from various sources from patient who admitted to Imam Hussein Medical City, the study beginning from the period January 2024 till the August 2024. The specimens which included sputum, wound, urine, blood and fluid and all specimens was collected as 40 cases.

Results: The collection specimens from patients included 40(20%) positive specimens distributed as 25 (62.5%) from females, and 15 (37.5%) from males and they were divided into: 17(42.5%) sputum, 10(25%) wound, 7(17.5%) urine,5(12.5%) blood ,1(2.5%) fluid specimen. After cultured on Blood and MacConkey agar, the isolates were identified via VITIK 2 compact system (Biomérieux, France). All isolates were tested for their resistance to 18 different antibiotics and the results exhibited that highest level of resistance in *A.baumannii* isolates to total antibiotics used in this study except Minocycline, Colistin and Tigecycline . Most isolates were resistant to Ticarcillin 100%, Ticarcillin/ Clavlanic Acid, Piperacillin and Meropenem (39)97.5%, Piperacillin/Tazobactam, Cefotaxime, and Ceftazidime (38) 95%, Imipenem, Ciprofloxacin and Ampicillin /Sulbactam showed resistance rate 37 (92.5%) and Cefepime 35(90%), Amikacin and Tobramycin34(85%), Gentamicin 82.5%, Trimethoprim/sulfamethoxazole 75%. Our study showed the Colistin, Minocycline and Tigecycline were sensitive in the rate36 (90%), 35 (87.5) and 30(75%) respectively.

Conclusion: The study concluded that the most common cases of infection with *A.baumannii* bacteria in sputum, and that this bacteria has a high resistance to most antibiotics.

عزل وتشخيص بكتريا *A. baumannii* من مصادر سريرية مختلفة وتحديد مقاومة المضادات الحيوية في مدينة كربلاء

دعاء حسين كاظم الوحيلي، هيام عبد الرضا العواد

الخلاصة

المقدمة: تعد بكتيريا *Acinetobacter baumannii* واحدة من أهم مسببات الأمراض الانتهازية المقاومة للأدوية المتعددة، ويرجع ذلك جزئيًا إلى قدرتها العالية على اكتساب المقاومة لمجموعات المضادات الحيوية المختلفة.

الهدف: الهدف من هذه الدراسة هو تحديد نمط العدوى البكتيرية وحساسية المضادات الحيوية في مدينة كربلاء.

العينات وطرائق البحث: تم جمع 200 عينة سريرية من مصادر مختلفة من المرضى الذين ادخلوا الى مدينة الامام الحسين الطبية، وبدأت الدراسة من كانون الثاني 2024 حتى آب 2024. العينات التي شملت البلغم والجرح والبول والدم والسوائل وجمعت جميع العينات 40 حالة.

النتائج: شملت عينات الجمع من المرضى 40 (20%) عينة إيجابية، موزعة بواقع 25 (62.5%) من الإناث و15 (37.5%) من الذكور وتم تقسيمها إلى: 17 (42.5%) من البلغم، 10 (25%).) الجرح، 7 (17.5%) بول، 5 (12.5%) دم، 1 (2.5%) عينة سوائل. بعد زراعتها على أجار الدم وأجار ماكونكي، تم التعرف على العزلات باستخدام نظام VITIK 2 compact (Biomérieux، فرنسا). تم اختبار مقاومة جميع العزلات لـ 18 مضاد حيوي مختلف، وأظهرت النتائج أن أعلى مستوى مقاومة لعزلات *A.baumannii* لجميع المضادات الحيوية المستخدمة في هذه الدراسة ماعدا Minocycline وColistin وTigecycline. أظهرت معظم العزلات مقاومة لمضادات تيكارسيلين 100%، تيكارسيلين/حمض الكلافلانيك، بيبيراسيلين وميروبينيم (39)97.5%، بيبيراسيلين/تازوباكتام، سيفوتاكسيم، سيفتازيديم(38) 95%، إيميبينيم، سيبروفلوكساسين وأمبيسلين/سولباكتام بنسبة مقاومة 37 (92.5). ٪) و سيفيبيم 35 (90%)، أميكاسين وتوبراميسين 34 (85%)، جنتاميسين 82.5%، تريميثوبريم/سلفاميثوكسازول 75%. أظهرت دراستنا أن الكوليستين والمينوسيكلين والتيجيسايكلين كانت حساسة بنسبة 36 (90%)، 35 (87.5) و30 (75%) على التوالي.

الاستنتاج: استنتجت الدراسة إلى أن أكثر حالات الإصابة ببكتيريا *A.baumannii* في البلغم هي الأكثر شيوعاً، وأن هذه البكتيريا لديها مقاومة عالية لمعظم المضادات الحياتية.

1. Introduction

A.baumannii are characterized by abilities to spread, and capacities to survive in most ecological surfaces and it was surprising ease with which it obtain antimicrobial multiple resistances. Because *A.baumannii* is resistant to antibiotics, rapidly spreads, and possesses virulence factors, it is considered a cause of nosocomial infection. For the previous 30 years, strains of *A*. *baumannii* have acquired resistance to anew developed antimicrobial drugs; these strains are recognized as multidrug-resistant organisms (MDR) *A*. *baumannii*. It became prevalent in several hospitals all over the world and has been lately documented there as a leading nosocomial pathogen (Agyepong et al., 2023; Zhang et al., 2022). Antibiotics resistance has become a community health problem, with high morbidity and mortality rates affecting largely countries with developing economies. In the United States of America (USA) the Centers for Disease Control and Prevention (CDC) approximation that infections associated to antibiotic resistant microorganisms are accountable for at least 23,000 deaths per year. In 2050, infections related to antimicrobial resistance will be accountable for 10 million deaths each year according to the World Health Organization (WHO). The WHO published in February 2017, a list of antibiotic resistant microorganisms for which the advance of novel antimicrobial treatments is considered urgent. This list contains microorganisms from the ESKAPE group: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter spp (Ahmed et al., 2024; Luo et al., 2023; Zhang et al., 2022)*.

The aimed of study was isolation and identification of bacterial isolates *A.buamannii* from diverse clinical sample by culturing and VITEK-2 system as well as to investigate of the occurrence of MDR and antibiotic susceptibility in *A.baumannii* isolates by VITEK-2 system.

2. Materials and Methods

2.1. Study Design

A cross-sectional study from 40 cases were collected from different types of specimens obtained from 200 clinical cases as total which Includes wounds, urine, sputum, fluid and blood of infections from both male and female in different ages, diverse local regions, the study beginning between January 2024 to August 2024.

2.2. Clinical Specimens

All Specimens collection was directly transferred to the laboratory and inoculation on culture media, macckonkey agar and blood agar by using streaking method, the samples were cultured aerobically at 37°C for duration of 24 hrs, after incubation period, the growth was examined daily.

2.3. Inclusion and Exclusion Criteria

The inclusion criteria filled out by the patients participating in our study and included knowledge of their age, gender, symptoms**,** while the exclusion criteria for persons with Cancer and smokers.

2.4. Identification of Bacterial Isolates

The isolates from pure colonies were phenotypically identified based on morphological, cultural, and biochemical properties by using gram negative card (GN) cards (ID) of the VITEK 2 system (Biomérieux, France), Antibacterial sensitivity testing on isolated bacteria was also accomplished using the compact automated system VITEK 2 (Pincus, 2010).

2.5. Antibiotics Susceptibility Test

Antibiotics susceptibility testing (AST) for isolates was determines the susceptibility to a group of antibiotics. The cards were laden into the VITEK 2 compact system automatic reader-incubator afterward being inoculated via card (AST). Used turbidity meter to make sure the number and density of microorganisms inoculated into the VITEK 2 cards were right (Ambaraghassi et al., 2019; Funke et al., 1998).

2.6. Statistical Analyses

The results were analyzed statistically in SPSS version 22 to observe Chi-square and the Probability levels were less than 0.05 was significant ($p < 0.05$).

3. Results and Discussion

3.1. Isolation of *A.baumannii*

The results in the present study revealed that a total number of 40 (20%) specimens of *A. baumannii* were found from (200) clinical specimens including wounds, urine, sputum, fluid and blood of infections from both males and females, diverse ages, varied local regions, while 160 specimens showed negative result for *A.baumannii* Fig.1. The collection was from hospitalized patients from Al-Hussien Medical City in Karbala. Our study greed with previous studies, in which the researchers collected 200 clinical samples from three main hospitals in the province of Babylon, out of 40 *A.baumannii* isolates and it is different with (Odih et al., 2023; Pandey et al., 2021). On the other hand, it different with another study in hospitals of Mosul and Erbil cities/Iraq that only 41(14.4%) isolates were identified as *A. baumannii*, and generality of these isolates were from burns (36.5%), surgical wounds (34.1%), and sputum (14.6%). However, it was recognized in CSF, blood, and urine specimens with lesser percentages (7.3%, 4.8%, and 2.4%), respectively, (Raut et al., 2020; Vrancianu et al., 2020)was established that the isolation rate of *A.baumannii* was 13% and (Lee et al., 2022; Liu et al., 2016)was recognized that the isolation rate of *A.baumannii* was (3.09%), while (Khaled et al., 2021)found the isolation rate of *A.baumannii* was (55.6%), finally Chaudhury *et al*., (2018) found that the isolation ratio was (9.51%) who established that isolation ratio of *A. baumannii* was (84%).

The disparity in the isolation ratio levels for whole studies may be due to several factors such as collection place and date and collection period the percentage of isolation could be diverse rendering to variance in nearby patients' levels of contamination and ecological factors (Khaled et al., 2021; Lee et al., 2022; Liu et al., 2016; Vrancianu et al., 2020).

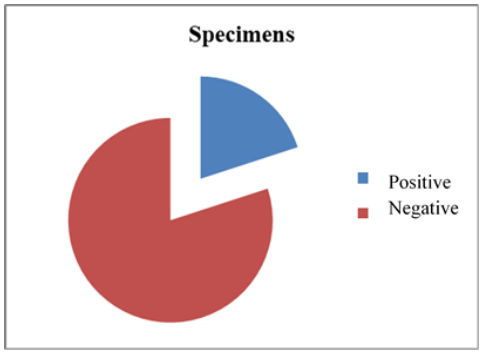

Figure1: Distribution of Tested Specimens Showing the Proportion of Positive and Negative Results. The blue segment represents **positive** specimens, while the red segment represents negative specimens. A larger proportion of the specimens tested negative.

3.2. Association Between The Occurance Of A.Baumannii With Types Of Specimen

The study found the isolation of *A.baumannii* from various clinical specimens, revealing important insights into its prevalence across different sources. Sputum specimens yielded the highest positivity rate at 42.5%, indicating a strong association with respiratory infections. This aligns with the known role of *A.baumannii* in respiratory tract infections, especially in patients who are critically ill or mechanically ventilated. On the other hand, Wound specimens followed, showing a positivity rate of 25%. This highlights the pathogen's significance in wound infections, particularly in hospitalized patients or those with surgical wounds, where *A.baumannii* can be a common contributor to complications. While, urine specimens had a positivity rate of 17.5%, suggesting that *A.baumannii* can also be involved in urinary tract infections, particularly in catheterized patients. While less common than in respiratory or wound infections, its presence in urine samples indicates a need for careful monitoring in these cases Table1. Blood specimens revealed a 12.5% positivity rate. Although lower than other specimen types, the isolation of *A.baumannii* from blood is concerning, as it can indicate serious conditions like bacteremia or sepsis, necessitating prompt clinical attention. Generally, Fluid specimens showed the lowest positivity rate at 2.5%. This suggests that *A.baumannii* is less frequently implicated in infections associated with body fluids, but its presence should still be regarded carefully in clinical assessments. Overall, the total positivity rate of 20% across all specimens underscores the significance of *A.baumannii* in this hospital setting. These findings emphasize the importance of choosing appropriate specimens for culture to ensure accurate diagnosis and effective treatment of infections caused by this pathogen Table1. The results indicated that sputum and wound specimens are the most common sources of *A.baumannii* infections. The Chi-square test result of 22.5 with a P-value of 0.00015 suggests a highly significant association between the specimen type and the presence of *A.baumannii.*

Table1: Types of Clinical Specimens with *A. Baumannii*

Types of specimens with *A. baumannii*						
Specimens	**Sputum**	**Wound**	**Urine**	**Blood**	**Fluid**	**Total**
Total	40	40	40	40	40	200
Positive	17	10	7	5	1	40
Percentage	42.5%	25%	17.5%	12.5%	2.5%	20%
Chi-square test: 22.5, P-value =0.00015, DF= 4						

In various studies, on *A.baumannii* isolation has shown the results are similar and different to our study, found the most common sources of *A.baumannii* was blood stream infections and lower percentage obtained from sputum ,urine and wound infections .The study in Hilla Teaching Hospital by Jabur, (Adewoyin et al., 2021; Blasco et al., 2019; Cartwright, 2010) found the highest percentage of isolation was obtained from urine samples ,the other source was wound , burn and sputum samples were low percentage of isolation, When analyzing the strains of *A.baumannii* antibiotics in patients, it was discovered that patients with specimens from the lung are more than those who have specimens from urine, blood, or even wound fluid. This raises the likelihood of these patients being harboring infections of the respiratory tract. It has been noted that the organism is associated with pneumonia due to introduction

of bacteria through mechanical ventilation towards seriously ill patients which could account for the larger number of pathogens isolated from the lungs, and moreover the prevalence of *A.baumannii* may indicate a capacity to develop and retain antibiotic resistance which makes the treatment of the infected individuals difficult and may improve the chances of their morbidity and mortality. In addition, other reasons for the higher rates of sputum collection include the possibility of high cut-off points for effective mould infection control interventions specifically among areas with high influx and out flux of patients or high levels of contamination devoid of appropriate measures (Aldali, 2023; Baral et al., 2019).

3.3. Association of occurrence A.bumanni with gender

The results showed that females account for the majority at 62%, and males make up 38% of the group, the gender breakdown of the patients depicted in the pie chart reveals a clear predominance of female individuals, who account the most common of the sample. In contrast, male participants constitute a smaller proportion, form the total Fig.2. The higher rates of *A.baumannii* infections in women compared to men might have multiple causes, the biology could explain this difference, as women's immune systems may react to infections. Hormones like estrogen could affect how the immune system works. Also, things like age other health problems, and existing medical conditions might lead to this gap. If more women in the study had these risk factors, it could explain why they got infected more often, how much time people spend in hospitals matters too. If women went to the hospital more or had more medical procedures, they'd be more likely to get *A.baumannii* and the people act can make a difference. Women might have different ways of staying clean or going to the doctor, which could change their chances of getting infected (Dias et al., 2022).

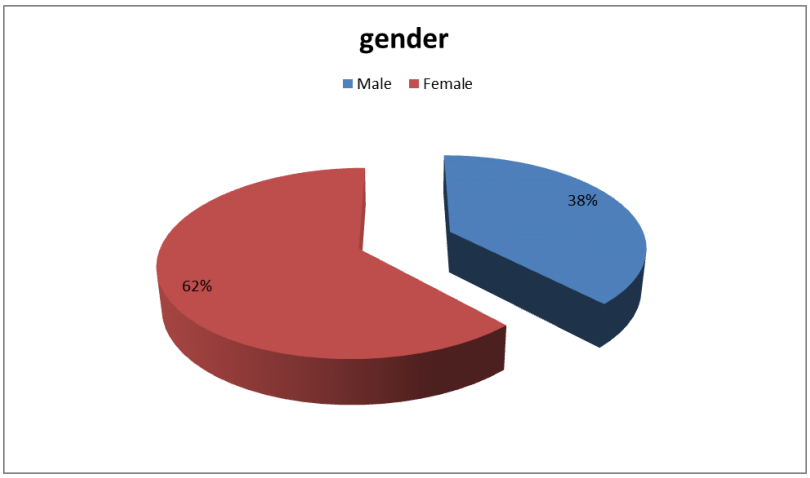

Figure2: Distribution of Participants by Gender. The chart illustrates the gender distribution of the study participants. Female participants represented 62% of the total sample, while male participants accounted for 38%.

3.4. Association of A.Bumanii Related With Seasonal Studies

This Table2 showed the variability in *A.baumannii* isolated from clinical samples during the study period has been reported. sputum consistently experienced the highest incidence of disease, ranging from 9.7% to 15.4% in different months. wound samples also showed a significant positive trend, at 13.6% in March. Urine and blood cultures showed low but consistent positivity, usually in the 1%. fluid sampling was the least overall, with only one case confirmed in

August. The overall quality across all sample types was 20%, with sewage being the most common source of *A.baumannii* isolates. These findings emphasize the importance of close monitoring of respiratory and wound infections in the management of this opportunistic pathogen.

Table2: Study Season Collecting Specimens According to The Study Season

Study Season	Total	Sputum	Positive (%)	Wound	Positive (%)	Urine	Positive (%)	Blood	Positive (%)	Fluid	Positive (%)
January	31	7	3 (9.7%)	5	2 (6.5%)	6	1 (3.2%)	7	1 (3.2%)	6	0
February	26	6	4 (15.4%)	6	2 (7.7%)	5	2 (7.7%)	6	1 (3.8%)	3	0
March	22	5	2 (9.1%)	5	3 (13.6%)	6	1 (4.5%)	3	0	3	0
April	29	6	3 (10.3%)	6	2 (6.9%)		2 (6.9%)	6	2 (6.9%)	7	0
May	18	4	2 (11.1%)	4	1 (5.6%)	4	0	4	1 (5.6%)	2	0
June	23	5	3 (13%)	5	0	5	1 (4.3%)	3	0	5	0
July	19	3	0	4	0	6	0	6	0	0	0
August	32	4	0	5	0	4	0	5	0	14	1 (3.1%)
Total	200	40	17 (42.5)	40	10 (25%)	40	7 (17.5%)	40	5 (12.5%)	40	1 (2.5%)
Chi-square test: 47.482 ; P value = 0.01217699; DF= 28											

Many *Acinetobacter* infections vary according to the season, which develops in damp conditions with more humid ambient air. Several outbreaks have been traced to liquid or wet environmental sources that have aided Acinetobacter species spread. There are numerous factors that are a significant cause of *A. baumannii* including environments, broad variety of PH, The link between humidity and infection rates points to *A.baumannii* thriving in wet environments. The experts have tracked outbreaks to wet or liquid sources in the environment. This shows that contaminated water and surfaces can harbor these bacteria. It underscores how crucial it is to watch and clean healthcare spaces to stop outbreaks as well as the, changes in seasons might affect how common infections are. This is because humidity levels go up at certain times of the year. This can make it easier for the bacteria to spread in hospitals and other medical facilities (Carvalheira et al., 2021). *A. baumannii* in places where cleaning isn't up to par. This is a big problem in intensive care units and surgical wards where patients are at higher risk and knowing these environmental factors

helps to create good infection control plans. To lower the chances of *A. baumannii* infections, hospitals can do a few key things. They can put strict cleaning rules in place, keep a closer eye on where germs might be hiding, and teach their staff why it's so important to keep things dry (Kyriakidis et al., 2021; Vázquez-López et al., 2020).

3.5. Determine The Sensitivity and Resistant Isolates In A.Baumanni

The specimen's data was entered on to a unique form, and they included: 17(42.5%) sputum, 10(25%) wound, 7(17.5%) urine,5(12.5%) blood ,1(2.5%) fluid specimen. After cultured on Blood and MacConkey agar, the isolates were recognized via Vitik 2compact system. All isolates were tested for their resistance to 18 different antibiotics and the results appeared that highest level of resistance in *A.baumannii* isolates to completely antibiotics used in this study except Minocycline, Colistin and Tigecycline . Most isolates were resistant to Ticarcillin 40(100%), Ticarcillin/ Clavlanic Acid, Piperacillin and Meropenem 39(97.5%), Piperacillin/Tazobactam, Cefotaxime, and Ceftazidime 38(95%), Imipenem, Ciprofloxacin and Ampicillin /Sulbactam showed resistance rate 37 (92.5%) and Cefepime 35(87.5%), Amikacin and Tobramycin 34(85%), Gentamicin 82.5%, Trimethoprim/sulfamethoxazole 75%. Our study showed the Colistin, Minocycline and Tigecycline were sensitive in the rate36 (90%), 35 (87.5) and 30(75%) respectively Table3.

Table 3: Analysis of Antimicrobial Susceptibility in Different Specimens

Specimens Antimicrobial	AST	Sputum	Wound	Urine	Blood	Fluid	Total
Ticarcillin	S	0	0	0	0	0	0
	R	17	10	7	5	1	40(100%)
Ticarcillin/ Clavlanic Acid	S	0	0	1	0	0	1
	R	17	10	6	5	1	39(97.5%)
Piperacillin	S	1	0	0	0	0	1
	R	16	10	7	5	1	39(97.5%)
Piperacillin/tazobactam	S	0	0	1	1	0	2
	R	17	10	6	4	1	38(95%)
Ceftazidime	S	0	0	1	1	0	2(5%)
	R	17	10	6	4	1	38(95%)
Cefepime	S	0	0	3	2	0	5(12.5%)
	R	17	10	4	3	1	35(87.5%)
Imipenem	S	0	0	2	1	0	3(7.5%)
	R	17	10	5	4	1	37(92.5%)
Meropenem	S	0	0	1	0	0	1(2.5%)
	R	17	10	6	5	1	39(97.5%)
Amikacin	S	0	4	0	2	0	6(15%)
	R	17	6	7	3	1	34(85%)
Gentamicin	S	3	0	2	2	0	7(17.5%)
	R	14	10	5	3	1	33(82.5%)
Tobramycin	S	2	2	2	0	0	6(15%)

	R	15	8	5	5	1	34(85%)
Ciprofloxacin	S	0	0	2	1	0	3(7.5%)
	R	17	10	5	4	1	37(92.5%)
Minocycline	S	16	9	7	3	0	35(87.5)
	R	1	1	0	2	1	5(12.5%)
Colistin	S	16	9	6	4	1	36(90%)
	R	1	1	1	1	0	4(10%)
Trimethoprim/sulfamethoxazole	S	6	0	2	2	0	10(25%)
	R	11	10	5	3	1	30(75%)
Ampicillin /Sulbactam	S	0	0	2	1	0	3
	R	17	10	5	4	1	37(92.5)
Cefotaxime	S	0	0	1	1	0	2(5%)
	R	17	10	6	4	1	38(95%)
Tigecycline	S	16	7	0	6	1	30(75%)
	R	1	3	5	1	0	10(25%)

Table3. showed that elevated level of resistance in *A.baumannii* isolates to all antibiotics used in this study except Minocycline, Colistin and Tigecycline .Most isolates were resistant to Piperacillin/Tazobactam 97.5% , 95% respectively , which was similar with local study in Babylon province. Results of another study showed the clinical isolates of *A.baumannii* were determined to be 95.6% resistant to piperacillin, 89.1% to ceftazidime, 97.8% to ceftriaxone, 95.6% to cefepime, 80.4% to ciprofloxacin, , 63% to meropenem and 54.3% to tetracycline , Imipenem showed resistance rate 37 (92.5%) and resistance rate to Meropenem was 39(97.5%) similar with study from diverse hospital in Thailand by (Aminul et al., 2021; Bhatta et al., 2021; Thirapanmethee et al., 2020). Another study found that 83 out of 91 (91.2%) isolates were resistant to imipenem and meropenem. Results establish that *A.baumannii* clinical isolates developing 100% resistance to ceftriaxone, cefotaxime, 95.45% to cefepime, chloramphenicol, aztronam and 40.90% to imipenem. Upon these local studies, we can observe interestingly the increase of resistance to imipenem antibiotic in our hospitals. Imipenem and Meropenem are from the Carbapenem antibiotics set. The cause for the emergence of resistance via bacteria to the antibiotic of this set is the ability of the bacteria to yield two types of β-lactamase enzymes, those are Carbapenem hydrolyzing class D of β-lactamase and Metallo β-lactamase enzymes, that hydrolysis and destroy carbapenems antibiotics (Gallego, 2015; Kitano et al., 2019; Thirapanmethee et al., 2020). Scientists showed was Cefepime highest resistance rate 20(100%) which was similar to our study, Further more in this study, high resistant ratio *A.baumannii* to Amikacin, ticarcillin 85%, 100% respectively. The resistance for Ciprofloxacin also displayed elevated resistance rate 92.5%, (Kareem, 2020). And other study found resistance rate (78.19%) for Ciprofloxacin. Our study showed the resistance rate to Colistin (10%) which was less than the result of found resistance rate to Colistin (66.96%). In our study the percentage of Ceftazidime (90%). These results were like and quite an agreement to the earlier studies in Iraq (Kareem, 2020; Stewart et al., 2018; van Duin & Bonomo, 2021). In conclusion: The study concluded that the most prevalent cases of infection with *A.baumannii* were found in sputum

samples. Additionally, it was noted that this bacterium exhibits high resistance to most antibiotics, posing significant challenges for treatment.

4. Conclusion

This study highlights the clinical and epidemiological significance of Acinetobacter baumannii infections in hospitalized patients at Al-Hussien Medical City, Karbala. The organism was isolated from 20% of clinical specimens, with sputum and wound samples being the most common sources. A significant association was found between the type of clinical specimen and the presence of A. baumannii, with the highest isolation rates observed in respiratory samples. Seasonal variation suggested increased prevalence during more humid months, emphasizing the potential role of environmental conditions in its transmission. The antimicrobial susceptibility profile revealed alarmingly high resistance rates to most tested antibiotics, particularly beta-lactams, aminoglycosides, and carbapenems, underscoring the multidrug-resistant nature of these isolates. Only Minocycline, Tigecycline, and Colistin retained considerable activity, indicating their potential utility as last-resort treatments. The emergence of resistance is likely driven by enzymatic mechanisms such as the production of carbapenemases and metallo-beta-lactamases. The findings underscore the need for continuous surveillance, strict infection control practices, and judicious use of antibiotics to combat the spread of multidrug-resistant A. baumannii. Additionally, targeted therapeutic strategies and further molecular studies are recommended to understand resistance mechanisms and guide effective clinical management.

5. Ethical Approval

Before the specimen was collected, written permission was obtained from each study patients, and all subjects involved in this experiment were informed. The university of Kerbala, College of Education for Pure Science Ethics Committee gave its approval to this study.

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Role of Bacteria as A Causative Agent of Cause Acute Appendicitis and Its Resistance to Antibiotic in Karbala

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Abstract

Background: Acute appendicitis is an inflammation of the appendix and is a common acute surgical emergency; however, the pathogenesis of appendicitis remains poorly understood. The bacteria is increasingly thought to play a key role in appendicitis.

Objective: The objective of this study was to determine the bacterial infection and antibiotics sensitivity pattern and study the distribution of appendicitis with gender and age groups in addition to clinical signs in patients with acute appendicitis in Karbala city.

Methods: Patients with acute appendicitis presenting between January 2024- June 2024 were studied. At surgery, 1cm rim of appendix was cut from the base and transferred into the Stuart's transport medium. The specimen was cultured on different type of culture media to identification bacteria. Antibiotic sensitivity test was performed.

Results: Samples collected from patients between (8-57) years old, the majority rate of appendicitis were between (16-30) ages with a percentage 44%, (1-15) age with a percentage 40%, (31-45) age with 14% and the minority rate was 2 % which belong to age (46-60). All specimens were positive to bacterial culture, gram positive bacteria were isolated at a lower rate (6%) than gram-negative bacteria (94%). The rate of infection was 54% in males and 46% in females. All patients have abdominal pain. Results found that *Escherichia coli* was the predominant aerobes, all species of gram-positive bacteria were resistance to the Benzylpenicillin and Oxacillin (100%), and sensitive to Rifampicin, Ticarcillin, Vancomycin and Penicillin (100%), all species of gram-negative bacteria isolated were sensitive to Amikacin and Imipenem (100%).

Conclusion: From this study, we can conclude that there was a relation between bacterial infections and *Escherichia coli which* was predominant and it was recorded that the infection in males were more than females in patients with acute appendicitis. gram negative bacteria showed to be more resistance to antibiotics than gram positive bacteria.

دور البكتيريا كعامل مسبب لالتهاب الزائدة الدودية الحاد ومقاومتها للمضادات الحيوية في كربلاء

ندى جاسم الكروي ,علاء عبد الحسين الدعمي , علي رحيم حنظل

الخلاصة

المقدمة

التهاب الزائدة الدودية الحاد هو التهاب حاد في الزائدة الدودية وهو حالة جراحية شائعة , ومع ذلك لا يزال المسبب في التهاب الزائدة الدودية غير مفهوم جيدا. يعتقد بشكل متزايد أن البكتيريا تلعب دورا رئيسيا في التهاب الزائدة الدودية الحاد .

الهدف

الهدف من هذه الدراسة هو تحديد العدوى البكتيرية ومدى حساسيتها للمضادات الحيوية ودراسة توزيع التهاب الزائدة الدودية مع الفئات الجنسية والعمرية بالإضافة إلى العلامات السريرية في مرضى التهاب الزائدة الدودية الحاد في مدينة كربلاء.

طرائق العمل:- تمت دراسة المرضى الذين يعانون من التهاب الزائدة الدودية الحاد واجريت الدراسة في الفترة من كانون الثاني 2024 الى حزيران 2024 في الجراحة ، تم قطع حافة 1 سم من الزائدة الدودية من القاعدة ونقلها إلى المختبر بواسطة وسط نقل **Stuart ,** تم زرع العينة على أنواع مختلفة من الوسائط لتحديد البكتيريا**,** ثم تم إجراء اختبار الحساسية للمضادات الحيوية.

النتائج

تم جمع العينات من المرضى الذين تتراوح أعمارهم بين (8 -57) سنة , وكانت نسبة الإصابة بالتهاب الزائدة الدودية في الغالب بين (16 - 30) سنة بنسبة 44% من إجمالي الحالات ، (1- 15) سنة بنسبة 40% ، (31-45) سنة بنسبة 14% والنسبة الأقل 2% والتي تنتمي إلى الفئة العمرية (46 - 60) ، كما أظهرت هذه الدراسة أن التهاب الزائدة الدودية الحاد يؤثر على كلا الجنسين مع عدم وجود فروق ذات دلالة إحصائية بين المجموعتين ولكنه يؤثر على الذكور (54%) أكثر من الإناث (46%) , حيث أظهرت جميع العينات نموا إيجابيا, تم عزل البكتيريا الموجبة لصبغة جرام بمعدل أقل (6٪) من البكتيريا السالبة لصبغة جرام (94٪) .وجدت النتائج ان اكثر الانواع البكتيرية شيوعا في مرضى التهاب الزائدة الدودية الحاد هي الإشريشيا القولونية ، وكانت جميع أنواع البكتيريا المعزولة الموجبة لصبغة جرام مقاومة **Benzyl penicillin** و **Oxacillin** بنسبة (100٪) ، وحساسة **Rifampicin , Ticarcillin , Vancomycin** و **Pencillin** بنسبة (100٪) ، وكانت جميع أنواع البكتريا المعزولة السالبة لصبغة جرام حساسة **Amikacin** و **Imipenem** بنسبة (100٪).

الاستنتاج

من هذه الدراسة يمكننا أن نستنتج أن هناك علاقة بين الاصابات البكتيرية والإشريشيا القولونية التي كانت سائدة وتم تسجيل أن الاصابة عند الذكور كانت أكثر من الإناث كما أظهرت العزلات السالبة لصبغة جرام أنها أكثر مقاومة للمضادات الحيوية من العزلات الموجبة لصبغة جرام لدى مرضى التهاب الزائدة الدودية الحاد.

1. Introduction

Acute Appendicitis (AA) is one of the common causes in the emergency unit due to abdominal pain and that appendectomy is one of the most surgical procedures performed in the world, diagnosis of Acute appendicitis is still challenging and some controversies on its management are still present among different settings and practice patterns worldwide (Moris, Paulson and Pappas, 2021).Diagnosis of appendicitis is clinical and combined with laboratory investigations, supplemented with selectively focused imaging, delayed diagnosis lead to problems such us punctured of appendix and sepsis. Obstruction and microorganisms are the important reasons in the most patients with acute appendicitis (Horattas, Haller and Ricchiutti, 2003; Alelyani *et al.*, 2021). Bacterial infection is believed to be crucial for inflammation of the appendix (Takahashi *et al.,*2021). Some bacteria can pass through appendix wall before perforation, whereas progressive infection and tissue damage with the necrosis allow the bacteria to enter the abdominal cavity. Studies on appendicitis are few, as studies related to identifying bacterial isolates associated with appendicitis and their role in increasing the complications of inflammation are limited, so the aim of this study was to investigate the bacterial infections in patients with acute appendicitis and study the distribution of appendicitis according to the sex, age groups, and clinical signs (Toumi *et al.*, 2010; Fabi *et al.*, 2022).

2. Patients & Methods

2.1. Study Design

Between January 2024 and June 2024, a total of 50 specimens (27 males and 23 females) and the age of patients ranged from (8 to 57) were collected from patients who attended in the operating room at Imam Hussein medical city in Karbala and diagnosed by physicians as acute appendicitis.

2.2. Clinical Samples

Usually, all patients were under the follow up after the operation. The specimens were placed in 1 mL of the normal saline 0.9% in a sterilized screw-capped container (Bio-Rad, 2014). Specimens directly has been transferred to the laboratory. In the hood, 1 gram of specimen was taken and crushed and placed in screw capped (glass tube), the tubes containing specimens of appendices were mixed by Vortex following inoculation on culture media, macckonkey agar, blood agar and mannitol salt agar by using streaking method, the samples were cultured aerobically at 37°C for duration of 24 hrs. for Storage of Bacteria the remain of specimen placed in screw capped (glass tube) contain brain heart infusion broth (BHI) and stored at -20°C (MacFaddin, 2000). and after incubation period, the growth was examined. If no growth were detected, then the plates were re-incubated for a further 24 hrs before discarding as negative result.

2.3. Questionnaire Sheets

The questionnaire sheet was filled out by the patients participating in our study and included knowledge of their age, gender, symptoms, medical and genetic history**.**

2.4. Identification of the Isolates

The isolated from pure colonies was phenotypically identified based on morphological, cultural, and biochemical properties. by using GN cards (ID) and GN cards (ID) of the VITEK 2 system (Biomérieux, France), As the protocol for institution, the ID results obtained using this traditional workflow were used as the standard for comparison. (Ha *et al*., 2018).

2.5. Antimicrobial Sensitivity Test

Antimicrobial susceptibility testing determines a bacterial isolates susceptibility to a set of antibiotics. The cards were loaded into the VITEK 2 system automatic reader-incubator after being inoculated. Colony counts were used to make sure the number and density of microorganisms inoculated into the VITEK 2 cards were right (Bazzi *et al*., 2017).

2.6. Exclusion Criteria

We excluded only persons with abdominal pain at any location and with no particular suspicion of appendicitis, other patients excluded were those that had peritonitis from ruptured appendix and those that had incidental appendectomy whereby appendix was removed during laparotomy for indication other than acute appendicitis.

2.7. Statistical Analyses

The results were analyzed statistically in SPSS version 22 to find out Chi-square, ANOVA (One away). Probability levels were less than 0.05 is significant ($p < 0.05$).

3. Results and Discussion

3.1. Sex-Based Distribution of Acute Appendicitis and Comparison with Previous Studies

The results of our study showed that the prevalence of acute appendicitis in males were 27 (54%) whereas in females it was 23 (46%) in Fig.1., there were no significant differences ($P \geq 0.05$) between both genders. The results indicate that the majority of cases were be in males then females, this result agree with study reported by (Abdulla *et al*.,2023). It has been demonstrated a statistically significant difference that males by 77.2% outnumbered females with 27.8% which is in accordance with these studies. (Daldal and Dagmura, 2020)showed different results, which found (59.7%) of females and (40.3%) of male patient. Distribution differences of appendicitis between both sexes might be due to the different in specimens' size that involved in each research or due to exclusion of certain patients' cases, or may be due to the misdiagnosis with other diseases in females such as gynecological diseases (Zhong *et al.*, 2014).

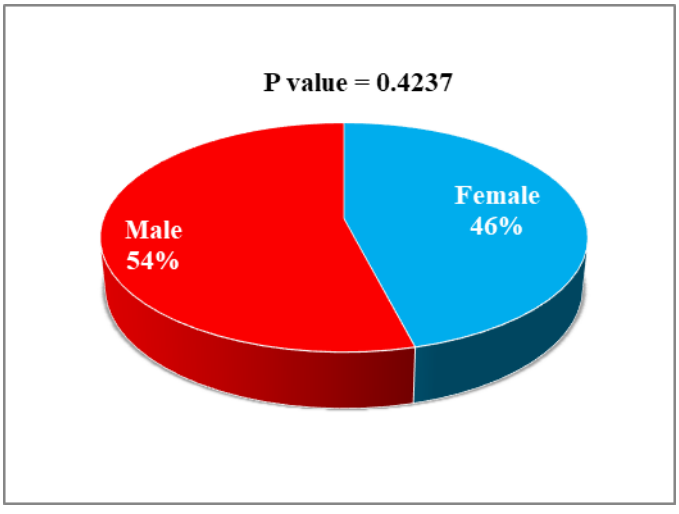

Figure1: Shows the Distribution of Appendectomy Specimens According to Gender

Ages of people who took part in this study varied from (7 to 52) years old in the healthy control and (8-57) in the patients. The age means of the patients (21.12±10.86 years), while the age means of the healthy control (21.76±11.79 years). Furthermore, there was no significant difference between the two groups according to age (p-value =0.7783). As shown in the Table1. Second research also discovered no statistically significant differences in age between the control and patients (Dinc *et al.*, 2015).In contrast, other research conducted in the same method comprised a total of 200 participants; however, the results of this investigation showed that there was strong significant difference in age between the two groups (Boshnak, Boshnaq and Elgohary, 2018; Haghi, Pourmohammad and Rabiee, 2019).

Table1: Distribution of Study Groups According Age

Groups	No.	mean ± SE	Median	Range
Healthy	50	21.76±11.79	19	7 – 52
Patients	50	21.12±10.86	19	8 – 57
P value	0.7783			

The majority rate of appendicitis was between (16-30) ages with a percentage 44%, (1-15) with a percentage 40%, (31-45) with a percentage 14 % and the minority rate was 2 %which belong to age (46-60), as in Fig.2. Our results agree with (Abdulla *et al.*, 2023). Also, study was done on 90 cases and the majority ratio were (46.09%) (35.65%) in age group with mean (15 & 25.5) respectively (Zhong *et al.*, 2014). Another study recorded that the age group ranging from 10 to 25 years was the most group affected by appendicitis (63%) (Karim, Shah and Durrani, 2019). (Almaramhy, 2017)concluded that increasing in the incidence of appendicitis in the age (15-25) might be because the occurrence of appendicitis due to the obstruction of appendix as a result of lymphoid hyperplasia because appendix contains extreme amount of lymphoid tissue in sub mucosa increasing in the number and size with increasing age, reaching extreme number and size through teenager with a higher probability of developing Appendicitis, or may be because an increased number of people in this group are exposed to the pathogens, which is transmitted through the digestive tract as a result of various foods**,** children are four times as likely than adults to get appendicitis Because of their physiological and metabolic traits and their comparably immature immune system, children are exposed to ambient climatic factors more than the adults .Appendicitis is most common between 10-20 years, yet, can occur at any age (Hancerliogulları *et al.*, 2017). Therefore, it is feasible to refer to the fact that appendicitis is more common in younger than in elder people, also elucidated that lower ratio was in age above 35 years that might be due to the regression in a mount of lymphatic tissue in the appendix. Differences in prevalence of appendicitis between age groups may be related to family history and genetics, as a family member is more likely to become infected (if previously infected) than in families that have not had infectious diseases before (Ross and Pawlina, 2011; Kleif, Vilandt and Gögenur, 2016; Goel, 2022).

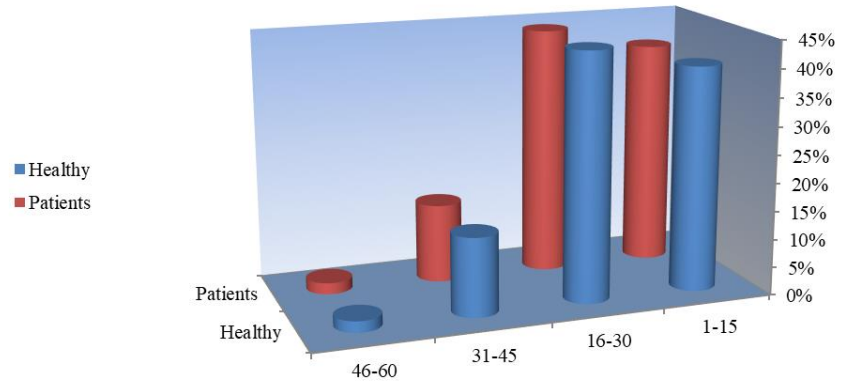

Figure2: Represents the Age Groups of the Study Sample

The clinical features correlated with acute appendicitis are showed in the Table2. 50 (100%) showed abdominal pain, vomiting was seen in 38(76%) patients, while 37(74%) patients were with nausea, Other features were fever 13(26%) and diarrhea 5(10%), the differences were significant between the symptoms and patients. From the results described above one can concluded that the abdominal pain is the commonest symptoms which may draw attention to the case as suspected appendicitis, these results were in accordance with related study who notice that abdominal pain was the most common feature of acute appendicitis (Goudie, 2023).

Table2: Distribution of the Acute Appendicitis According to Symptoms (N=50)

Clinical feature	No. (%)
Abdominal pain	50 (100%)
Vomiting	38 (76%)
Nausea	37 (74%)
Fever	13 (26%)
Diarrhea	5 (10%)

In this study all specimens yielded positive results for bacterial growth , a total of nine genera bacterial appendicitis, seven genera were gram- negative and two genera were gram- positive as shown in the Table3, gram negative bacteria was the common causes of acute appendicitis compared with that of gram positive, they were 47(94%) isolates and 3(6%) isolates respectively and there where highly significant difference between gram positive bacteria and gram negative bacteria (p-value= 0.00001**) show in Fig.3. The most common microorganism was *E. coli* which accounted for 29(58%) isolates, followed by *Klebsiella pneumoniae* isolates 8 (16 %), 5(10%) isolates of *Psedomonas aeruginosa*, 2(4%) isolates of *Enterococcus faecalis*, 1(2%) isolates of *Enterobacter aerogenes*, 1(2%) isolates of *Salmonella typhi* and 1(2%) of *Proteus mirabilis*. *Staphylococcus aureus* was the most frequent microorganism among gram-positive bacteria which accounted as 2(4%). While *Staphylococcus epidermmidis* was accounted as 1(2%). Our results being found were in agreement with other results recorded by (Rasmussen *et al.*, 2024). Those results were accepted and suspected since *E. coli* is the most common organism multiplying and quickly adheres on the surfaces of tissue. *E. coli* has other virulence factors such as host cell surface modifying factors, toxins, hemolysin and cytotoxic necrotizing factor type I (CNFI) (Garcia *et al.*, 2013).

Klebsiella pneumonia isolates were 8 (16 %) , this organism has a capsule that plays an essential role through the initial steps of the pathogenicity by interact with the mucus producing cells, mucus membranes colonization by bacteria is enjoyed to an adhesion process involved specific adhesions on the surface of bacterial. In addition to several pili involved in adhesion to the epithelial cells of intestine (Riwu, Effendi and Rantam, 2020; Abbas *et al.*, 2024). *Klebsiella pneumoniae* strains are an emerging threat in medical center and should be targeted for early identification and stringent control of infections brought on by *Klebsiella pneumoniae*. The explanation for the detection of *Pseudomonas aeruginosa* in appendix as causative agent of appendicitis due to the ability of this organism to attach and colonize epithelial tissue probably by pili and by a gene layer surrounding bacterial cells, also *Pseudomonas aeruginosa* possesses other virulence factors (enzymes and toxins), enable it to cause infection (Riwu *et al.*, 2022). Other gram- negative *Enterococcus faecalis*, *Salmonella typhi*, *Proteus mirabilis* and *Enterobacter aerogenes* were also detected in acute appendicitis but in low frequencies in compared with the other gram-negative bacteria. The implication of these bacteria in acute appendicitis are suspected, as they belong to the enteric group and all these bacteria have virulence factors permitting them to cause disease (Wang *et al.*, 2023). In our study several gram-positive bacteria characterized by *Staphylococcus aureus*, *Staphylococcus epidermmidis* also isolated and identified from patients with acute appendicitis but in low frequencies in comparative with gram negative bacteria. Gram positive bacteria are rarely reported due to adhesive and colonizer factor being less in gram positive in compared with gram negative furthermore most of gram-positive bacteria are fastidious require for special growth factors (vitamin, amino acids, etc) and growth condition (O_2, $CO_{2\ ...etc}$). However qualitatively, gram positive infections are more serious such as infection with bacteria *Clostridium spp*. The correlation between bacterial infection and appendicitis is characterized by an increase in bacterial presence leading to a higher incidence of appendicitis, studies have shown that specific bacteria, such as *Escherichia coli* and *Streptococcus spp.* are commonly found in patients with appendicitis, particularly in complicated cases (Zachos *et al.*, 2023).

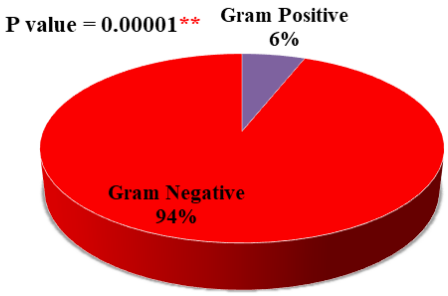

Figure3: Distribution of Bacteria Isolated from Appendicitis Patients

Table3: The Type of Bacteria Isolated from Appendicitis Patients

Bacteria	No.	Percentage (%)
Gram Positive		
Staphylococcus aureus	2	4 %
Staphylococcus epidermmidis	1	2 %
Total	**3**	**6 %**
Gram Negative		
Escherichia coli	29	58 %
Enterobacter aerogenes	1	2 %
Klebsiella pneumonia	8	16 %
Enterococcus faecalis	2	4 %
Proteus mirabilis	1	2 %
Psedomonas aeruginosa	5	10 %
Salamonella typhi	1	2 %
Total	**47**	**94 %**

3.2. Sensitivity Patterns to Antimicrobial Agents

This is an academic and practical study that determines the effect of different types of antimicrobial on microorganisms isolated from patients' specimens. Also taken into consideration is the extent to which bacteria respond to these antimicrobial, and to determine what alternatives are available for Iraqi surgeon to use in cases like these.

3.2.1. Antimicrobial Susceptibility for Gram Positive Bacteria

From observation the results of antimicrobial susceptibility profile for gram positive bacteria in patients with acute appendicitis were found all species isolated were resistance to the benzylpenicillin and oxacillin (100%), and all species isolated were sensitive to the rifampicin, ticarcillin, vancomycin and penicillin (100%). Isolates of gram-positive aerobic cocci were resistant to clindamycin, fusaric acid, erythromycin and tetracycline (66.6%) ,and resistant to gentamicin and ciprofloxacin 33.3% Fig.4.

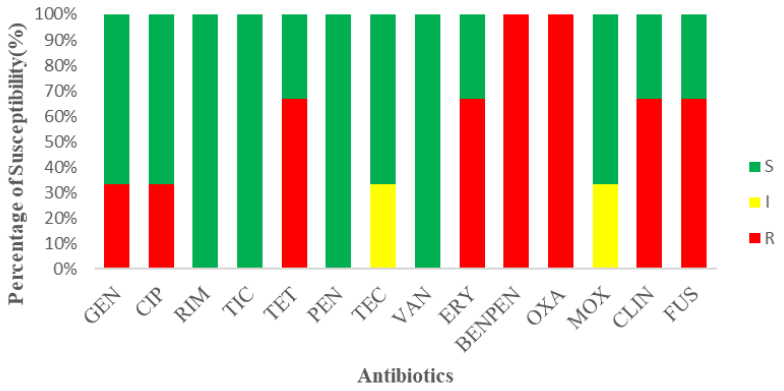

Figure4: Percentage of Bacterial Susceptibility to Various Antibiotics.
The bar chart illustrates the susceptibility profile of bacterial isolates against a range of antibiotics. Susceptibility (S) is shown in green, intermediate resistance (I) in yellow, and resistance (R) in red. Antibiotics such as ciprofloxacin (CIP), vancomycin (VAN), and teicoplanin (TEC) exhibited high effectiveness with 100% susceptibility, while penicillin (PEN), benzylpenicillin (BENPEN), erythromycin (ERY), oxacillin (OXA), and clindamycin (CLIN) showed high resistance rates. These results highlight the variability in antibiotic efficacy and emphasize the importance of antimicrobial susceptibility testing in guiding appropriate therapy.

3.2.2. Antimicrobial Susceptibility for Gram Negative Bacteria:

From the observed of the results of the antimicrobial susceptibility of gram-negative bacteria showed that all species had be isolated were sensitive to amikacin and imipenem (100%) while most isolates were resistance to ticarcillin and piperacillin 92.8%, aztreonam 78.5%, ciprofloxacin72.7%ticarcillin/clavulanicacid71.4%, cefepime60%, gentamicin50%, tobramycin57.1%, trimethoprim/sulfamethoxazole 55.5%, piperacillin/tazobactam55% as shown in Fig.5.

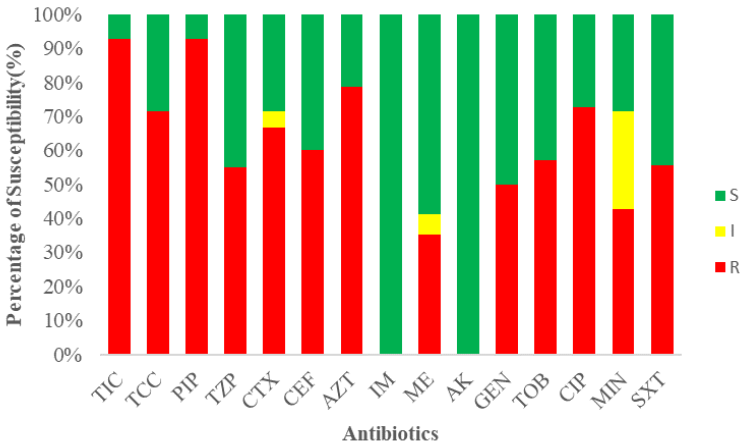

Figure5: Percentage of Bacterial Susceptibility to Various Antibiotics
This bar chart illustrates the susceptibility profiles of bacterial isolates tested against multiple antibiotics. Susceptibility (S) is shown in green, intermediate resistance (I) in yellow, and resistance (R) in red. High resistance rates were observed for several antibiotics, including ticarcillin (TIC), piperacillin (PIP), cefotaxime (CTX), ciprofloxacin (CIP), minocycline (MIN), and trimethoprim-sulfamethoxazole (SXT). In contrast, carbapenems such as imipenem (IM) and meropenem (ME) exhibited 100% susceptibility. These findings emphasize the prevalence of multidrug-resistant strains and the critical need for targeted antimicrobial therapy based on

our results showed that both Imipenem and Amikacin are more effective antimicrobial against all gram-negative bacteria. Our results agreement with a new study where both imipenem and Amikacin were well effective against gram negative bacteria. gram positive bacteria in our study showed sensitivity to penicillin, vancomycin, rifampicin and ticarcillin which agree with a recent study (Heo, 2021; Sahra *et al.*, 2021). gram positive bacteria which have been characterized to be sensitive to most common antibiotics in comparison with gram negative bacteria due to the difference in the outer membrane structure which looks to be permeable to most antibiotics in gram positive bacteria than gram negative bacteria. Our study found that all species of gram-positive bacteria had be isolated were resistance to benzylpenicillin and oxacillin, most gram-negative isolates were resistance to ticarcillin and piperacillin 92.8%. Gram negative bacteria have broad spectrum resistance for antimicrobial agent, the production of β lactamase is the main mechanism for this resistance (Bryskier, 1997; Jubeh, Breijyeh and Karaman, 2020). Resistance may be attributed to the continuous and excessive intake of the antimicrobial by the patient that results in the development of the bacterial resistance. Iraqi patients are well known for taking antimicrobial for everything without doctor consultation so this is a very strong reasons for this resistance. In some time use lower dose of antimicrobial gives the appearance that bacteria are resistant whereas in actual fact they are not affected by lower doses of antimicrobial given or use higher doses of antimicrobial to patient with low immunity. Generally, combination of antimicrobial can lead

to declined efficiency of drugs or sometimes increased effect on bacteria as it was by (Leus *et al.*, 2023; Salam *et al.*, 2023). Also, problems of resistance occur in patients susceptible to colonization as in hospital which associated to presence of drug resistant bacteria that may originate in hospital. Scientists observed that acquired drug resistant can also be the result of therapy failure. The importance of the use of prophylactic antimicrobial before operation has been demonstrated in a number of studies who found the aim of this treatment line is to prevent post-surgical infections following open appendectomy. The optimum type and dose of antimicrobial is unknown, so this carries the possibility of either under treatment with increased risk of post-operative infection or over treatment which could result in the microbial resistance. There are many reasons to which the differences in the antimicrobial sensitivity reported in our study can be attributed. The unnecessary prophylactic use of antimicrobial should be discouraged since this may result in increased selection of resistant variants or super infection with resistant flora, the poor quality of the antimicrobial source, absence quality control for imported antimicrobial and poor storage conditions all these participate in change of results (Andersen, Kallehave and Andersen, 2005). Antibiotic resistance significantly affects the management of bacterial infections in appendicitis, leading to increased complications and necessitating careful selection of empirical antibiotic therapy. Ongoing surveillance of resistance patterns and adaptation of treatment protocols are crucial to improving patient outcomes (Andersen, Kallehave and Andersen, 2003).

4. Recommendations

Conduct an extensive study on pathogens from other microorganisms and determine their proportion in causing appendicitis and study the possibility of the present of human leukocyte antigen (HLA) that may determine susceptibility of persons for acute appendicitis.

5. Acknowledgements

We express our genuine appreciation to the hospital staff and study participants.

6. Ethical Approval

Before the specimen was collected, written permission was obtained from each study participant, and all subjects involved in this experiment were informed. The university of Kerbala, College of Education for Pure Science Ethics Committee gave its approval to this work.

7. Conflict of Interest

The authors declare there is no conflict of interests

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HLA-DQ2 And HLA-DQ8 Haplotypes Influence Circulating Levels of Anti-Tissue Transglutaminase and Anti-Gliadin Antibodies in Celiac Disease: A Review

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Abstract

Celiac disease indicates symptoms like damage to small intestinal mucosa, and challenges with nutrient absorption when gluten is consumed by individuals who are genetically predisposed. Genetic, immunological, and environmental factors play important roles in the pathogenesis of the diseases. Here, we briefly reviewed how specific haplotypes linked with genetic risk of celiac disease influence the levels of serological markers associated with the disease. By understanding this link, personalised medicine methods based on genetic risk could be created, thereby potentially improving early disease diagnosis, accuracy of diagnosis, patient care and treatment methods.

تأثير الأنماط المتعددة من HLA-DQ2 و HLA-DQ8 على مستويات الأجسام المضادة للجلوتاميناز النسيجي والأجسام المضادة للجليادين لدى مرضى السيلياك: مراجعة

بان وحيد حسين بدير , ستار جبار راهي الگريطي

الخلاصة

يشير مرض السيلياك إلى أعراض تشمل تلف بطانة الأمعاء الدقيقة وصعوبات في امتصاص المغذيات عند تناول الغلوتين من قبل أشخاص لديهم استعداد وراثي. تلعب العوامل الوراثية والمناعية والبيئية أدوارًا مهمة في تطور هذا المرض. في هذه المراجعة الموجزة، قمنا بتسليط الضوء على كيفية تأثير أنماط معينة من الجينات المرتبطة بالمخاطر الوراثية لمرض السيلياك على مستويات المؤشرات المصلية المرتبطة بالمرض. ومن خلال فهم هذا الارتباط، يمكن تطوير أساليب الطب الشخصي المعتمدة على الخطر الوراثي، مما قد يُسهم في تحسين تشخيص المرض المبكر، ودقة التشخيص، ورعاية المرضى وطرق العلاج.

1. Introduction

Gluten sensitivity in individuals with a genetic predisposition, results in a kind of enteropathy commonly known as celiac disease. It is typified by an overreactive immune response brought on by gluten consumption, cystic hyperplasia and small intestinal lesions resulting from villi atrophy (Rubin and Crowe, 2020). Growing awareness of the clinical manifestations of celiac disease as well as its increasing occurrence have led to the understanding of the global epidemiology of the disease. Although exact statistics may vary depending on genetic and geographic variables, celiac diseases is estimated to affect 1% of people worldwide (Lebwohl and Rubio-Tapia, 2021). Epidemiological studies conducted in four regions: Oceania, the Middle East, East Asia and South Asia showed that South Asia has the highest incidence of celiac disease i.e. 0.8% and 7% among low- and high-risk populations respectively. However, Middle Eastern countries have the highest seroprevalence of celiac disease i.e. 1.4% (Ashtari et al., 2021). It is estimated that the prevalence rate in Africa is 1.1% while it is noteworthy that since North Africa consumes more wheat relative to sub-Saharan Africa, and its population has a greater frequency of the HLA-DQ2 haplotype, the sub-region have a high prevalence of the disease (Rajput et al., 2021). Sub-Saharan Africa, on the other hand, has lower incidence rates; nevertheless, this data may be impacted by underdiagnosis and lack of awareness (Lebwohl and Rubio-Tapia, 2021). While individuals can be affected by celiac disease at any age, adult diagnosis are increasingly occurring, although many individuals learn about their diagnosis at a relatively young age (Raiteri et al., 2022). This tendency is due to increased awareness of the disease and its symptoms, extra intestinal abnormalities such as dermatitis herpetiformis, malabsorption and gastrointestinal difficulties. Since its symptom might be mistaken for those of other diseases, celiac disease is still underdiagnosed worldwide. Improving screening procedures and raising public and professional aware ness are crucial for prompt diagnosis, disease management and treatment (Besser and Khosla, 2023).

Celiac disease presents a wide range of symptoms which vary significantly among different individuals and since the disease primarily affects the small intestine, leading to malabsorption of nutrients, it can also have systemic effects (Laurikka et al., 2022). The symptoms can be gastrointestinal (chronic diarrhea, abdominal pain, bloating and gas, loss of weight, constipation, and exhaustion), extra-intestinal (dermatitis herpetiformis, as skin condition marked by itchy, blistering rashes, often found on the elbows, knees and buttocks), neurological (headaches, migraines, peripheral neuropathy and cognitive impairment), bone and joint (osteoporosis, osteopenia and joint pain) as a result of calcium and vitamin D malabsorption, reproductive (irregular menstruations, infertility and pregnancy complications), and enamel hypoplasia . Interestingly however, some individuals with celiac disease may be asymptomatic, meaning they do not exhibit any noticeable symptoms despite intestinal damage (de Graaf et al., 2024, Durazzo et al., 2022, Lupianez-Merly et al., 2022). This can lead to delayed diagnosis and increased risk of complications, such as intestinal lymphoma or other autoimmune disorders (Mulder et al., 2023, Khorsheed et al., 2022). According to available data, gliadin peptides present in dietary gluten have the ability to interact with tissue transaminase (tTG) and cause damage to the mucosa of the intestine. This may stimulate CD4+ T cells to respond immunologically, which may lead to immunological hyperactivity. The major histocompatibility complex (MHC) gene present in all vertebrates, may play a role in immunity in some circumstances. The human equivalent of MHC is called Human Leukocyte Antigen (HLA) system. The human MHC antigens are encoded by the DNA surrounding the centromere on the short arm of chromosome 6 (Espino and Núñez, 2021, AL-Ghuraby et al., 2022). The HLA antigens are categorised into three groups as class I, class II, and class III according to their structures and functions. Regarding the celiac disease, the basic determinants of the genetic susceptibility for

celiac disease are the MHC class II HLA-DQA and DQB genes (these genes are encoded by the histocompatibility region on the short arm of chromosome 6) (Aboulaghras et al., 2023, Espino and Núñez, 2021). Individuals are genetically predisposed to celiac disease if they possess particular haplotypes of the HLA, most notably HLA-DQ2 and HLA-DQ8. These haplotypes are essential in the pathophysiology of the disease because they help T lymphocytes present gluten-derived peptides, which cause the intestinal mucosa to become inflamed. In addition to being essential for the diagnosis of celiac disease, the existence of the HLA-DQ2 and HLA-DQ8 haplotypes affects the levels of circulating antibodies, particularly anti-gliadin (AGA) and anti-tissue transglutaminase (tTG) antibodies (Losurdo et al., 2021). Elevated levels of these antibodies are frequently employed as serological indicators for celiac disease, facilitating the diagnosis and continual monitoring of the disease. These haplotypes have different impacts on how the immune system reacts to gluten, hence, as reported in previous studies, individuals with the HLA-DQ2 haplotype generally, have higher levels of these antibodies than those with the HLA-DQ8 haplotype (Mansouri et al., 2021). This review briefly examines the current understanding of the influence of HLA-DQ2 and HLA-DQ8 haplotypes on the levels of anti-tTG and AGA in circulation with the objective to highlight the importance of genetic predisposition in the clinical presentation of celiac disease. To better understand the pathogenesis of celiac disease and possibly improve individualized treatment options, it is essential to decipher the connection between HLA haplotypes that confer genetic susceptibility too celiac disease, and serological levels of antibodies associated with the disease.

1.1.Genetic Basis of Celiac Disease

One of the most extensively studied regions of the human genome is the MHC region, which has polymorphic HLA genes. The MHC proteins are linked to a variety of complicated diseases and are essential for antigen-specific immunity (Espino and Núñez, 2021). Its genetic and genomic variability is still challenging to characterize and interpret, despite decades of research and numerous advancements in the field. This region is extremely gene rich and contains many additional protein-coding genes, some of which are immune-related and others unrelated, in addition to the MHC genes found in the majority of the species that have been investigated to date. Immune effector cells, such as cytotoxic T cells, are presented with short peptides by MHC proteins, which are produced on the surface of cells. They contribute significantly to the antigen-specific immune response by presenting potentially antigenic peptides that can originate from both self-tissue and infectious sources. The coding sequence of an MHC gene corresponds to the repertoire of peptides that a particular MHC protein can bind. Within the MHC region, the HLA genes are typically present in several gene copies that are located adjacent to one another. Depending on whether they encode for MHC class I or class II proteins, they are categorized into MHC class I and class II genes (Rubin and Crowe, 2020, Medrano et al., 2012). The MHC class I proteins consist of a monomorphic β2-microglobulin and an alpha chain, which are encoded by a single MHC class I gene. All nucleated cells have MHC class I proteins, which primarily present peptides from the intracellular matrix. MHC class II alpha chain gene (HLA-DRA) and MHC class II beta chain gene (HLA-DRB1) encode heterodimers of an alpha and a beta chain, which make up MHC class II proteins (e.g., HLA-DR). They primarily present peptides generated from the extracellular matrix and are only expressed on antigen-presenting cells, like macrophages (Del Pozzo et al., 2021). There are two other categories of MHC genes: classical and non-classical MHC genes. These genes are located inside the MHC region of a particular genome. Non-classical MHC genes are often limited in their expression and have low genetic variability, whereas classical MHC genes, such as the HLA-DRB1 and HLA-B, encode a functional MHC protein sequence, show important allelic variations within a specific

species, and are likely to be ubiquitously expressed (Matern et al., 2020, Dehghani et al., 2021). Over the course of the year, a considerable number of enlightening reviews have been published on multiple features of the MHC and its function in immunity. The HLA-DQ2 and HLA-DQ8 haplotypes are significant in the development of celiac disease. These haplotypes are particular HLA gene variations that are linked to the disease condition's pathological immune response. About 90% of individuals with celiac disease have HLA-DQ2, which is made up of two subunits: DQB1*02:01 as well as DQA1*05:01. DQA1*03:01 and DQB1*03:02 make up HLA-DQ8 (Matern et al., 2020). The presence of specific HLA haplotypes significantly influence susceptibility to celiac disease (presented in Table1), as they are responsible for presenting gluten-derived peptides to the immune system.

Table1: HLA Haplotypes and Level of Celiac Disease Risk

Samples#	HLA Haplotypes	Level of Disease Risk
1	DQ2.5/DQ2.2	Extremely high
2	DQ2.5 and DQ8	Extremely high
3	DQ2.5 & DQB1*02 (double dose)	Extremely high
4	DQ2.2 & DQB1*02 (double dose)	Moderately high
5	DQ2.5 & DQB1*02 (single dose)	Moderately high
6	DQ8 homozygous	Moderately high
7	DQ8/DQ2.2	Moderately high
8	DQ2/DQ7	Moderately high
9	DQ8/DQ7	Moderately low
10	DQ2.2 & DQB1*02 (single dose)	Moderately low
11	DQ8 heterozygous	Moderately low
12	DQX.5	Extremely low
13	DOX.x	Extremely low
14	DQ2.x	Extremely low
15	DQ7/DQX	Extremely low
16	DQX homozygous	Extremely low
17	DQ7 homozygous	Extremely low

Since celiac disease does not always emerge in individuals who carry HLA-DQ2 or HLA-DQ8, environmental factors may also play a role in the pathogenesis of the disease. According to a systematic review and meta-analysis by Aboulaghras et al., the homozygous and heterozygous status of HLA-DQ2 is present with increased frequency in the majority of adult patients, confirming that the DQ2 allele is the primary one associated with celiac disease due to its high frequency in adult patients in all studies (Aboulaghras et al., 2023). There is a close association between HLA haplotypes and celiac disease, which can be attributed to the pathogenic processes of gluten peptide presentation via DQ2 and strong immunodominance. A small percentage of celiac disease cases develop without having any predisposing HLA haplotypes, despite the fact that the traditional DQ2/DQ8 connections with celiac disease were validated in many meta-analyses (Aboulaghras et al., 2022). In clinical settings, genetic testing for HLA-DQ2 and HLA-DQ8 is typically used to help diagnose celiac disease. It is extremely unlikely that celiac disease will be diagnosed if these haplotypes test negative. On the other hand, the existence of these haplotypes, particularly in a person exhibiting symptoms indicative of celiac disease, justifies additional diagnostic

assessment, such as serological testing for particular antibodies against gliadin and transglutaminase (Gualandris et al., 2021).

1.2.Pathophysiology of Celiac Disease

The immune system, especially T and B cells, environmental variables, and genetic predisposition all play intricate roles in the pathophysiology of celiac disease. Celiac disease is largely influenced by T lymphocytes, more especially CD4+ T helper cells. Genetic markers, specifically the HLA-DQ2 or HLA-DQ8 haplotypes, are frequently present in individuals with celiac disease (Aboulaghras et al., 2022). These haplotypes encode molecules that present T lymphocytes with peptides produced from gluten. After consumption, gluten undergoes partial digestion in the digestive system, which produces immunogenic peptides. These peptides are delivered to CD4+ T lymphocytes on the surface of antigen-presenting cells (APCs) by HLA-DQ2 or HLA-DQ8 molecules in genetically susceptible individuals (Aboulaghras et al., 2022, Núñez and Rubio, 2021). After becoming activated, these T cells proliferate and release cytokines that promote inflammation, like TNF-α and interferon-gamma (IFN-γ). These cytokines support the intestinal mucosa's inflammatory response, which damages enterocytes, or intestinal epithelial cells, and results in the distinct villous atrophy linked to celiac disease. The recruitment of additional immune cells is encouraged by the activation of T cells, which intensifies the inflammatory response and also degrades the intestinal lining (Ge and Chen, 2024, Silvester et al., 2021, Kagnoff, 2005). Fig.1 shows a flowchart that summarizes the pathogenesis of celiac disease.

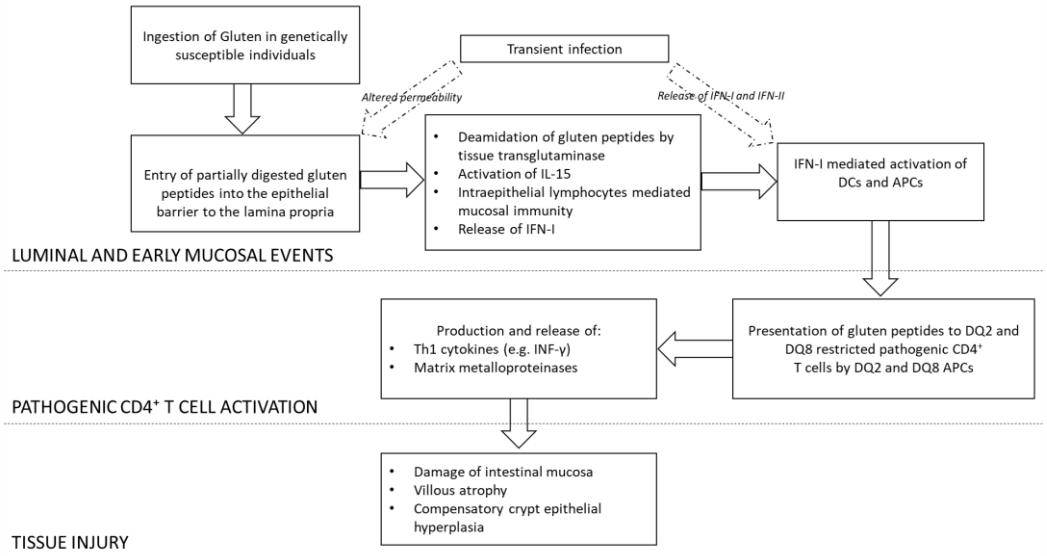

Figure1: Pathogenesis of Celiac Disease

Celiac disease pathogenesis involves three key events: luminal/mucosal processes, CD4+ T cell activation, and tissue injury. Initially, genetically predisposed individuals consume gluten, leading to the production of large, undigested gluten peptides. Tissue transglutaminase modifies these peptides, which are then presented by dendritic cells and antigen-presenting cells expressing DQ2 or DQ8. When these peptides cross the epithelial barrier into the lamina propria, they activate CD4+ T cells. Subsequently, these activated T cells release mediators that cause tissue damage, contributing to the symptoms of celiac disease.

Additionally, B cells are essential to the development of celiac disease, mainly through the generation of antibodies. B cells have the ability to develop into plasma cells in response to the gluten peptides that T cells present, and they are also able to generate particular antibodies against gluten, which include immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies (Kagnoff, 2005, du Pré and Sollid, 2015). One diagnostic criterion for celiac disease is the presence of these antibodies in the serum, which is a characteristic of the condition. Anti-endomysial and anti-tissue transglutaminase (tTG) antibodies are the most often tested types of antibodies (EMA). Antibodies generated by B cells have dual roles in celiac disease i.e. as a disease marker as well as an effector of its pathophysiology (Kagnoff, 2005, Lindeman et al., 2024). For instance, anti-tTG antibodies bind to tissue transglutaminase that modifies gluten peptides, further triggering inflammation and immunological activation. This interaction may perpetuate the cycle of intestinal tissue damage and immune activation (Majeed et al., 2022). Moreover, the dysregulation of B cell responses may worsen the autoimmune component of celiac disease by causing the production of antibodies that may attack the tissues of the body (du Pré and Sollid, 2015, Lindeman et al., 2024). This aberrant immune response is likely to be influenced by a variety of factors, including the gut microbiome, which can modify immune response and possibly affect the severity of the disease (Ge and Chen, 2024).

1.3.Serological Markers in Celiac Disease

Antibodies are central to the diagnosis of celiac diseases. The most important of these antibodies is the anti-tTG antibodies which target gluten peptides by altering them post-translationally through the action of an immunoglobulin enzyme (Ribes-Koninckx et al., 2022). Gluten proteins are altered by the tTG when ingested, into peptides that are more immunogenic and hence stimulates the body to make antibodies against them. Since anti-tTG antibodies exhibit symptoms of the autoimmune response to gluten, they are a useful biomarker for celiac disease (Khan et al., 2020). An intestinal biopsy, the presence of specific symptoms, and a positive serological test can all lead to a definitive diagnosis. Ant-tTG antibodies are typically assessed in conjunction with anti-endomysial antibodies (EMA) and deamidated gliadin peptides (DGP) to increase diagnostic accuracy (Anbardar et al., 2022). Measuring anti-tTG antibody levels is also helpful for monitoring disease activity because they drop dramatically when gluten is eliminated from the diet. Moreover, persistently elevated levels may indicate ongoing gluten use or possible health challenges. Anti-tTG antibodies, which represent the autoimmune mechanism behind gluten ingestion and provide therapeutic possibilities, are the basis for both diagnosis and treatment of celiac disease (Volta et al., 2024). Routine screening and monitoring of serological levels of these antibodies are crucial for individuals who are at risk or have been diagnosed with celiac disease. Antigliadin antibodies (AGA) are specific immunoglobulins that target gliadin. AGAs can be evaluated as IgA and IgG subclasses and were one of the first serological indicators used to diagnose celiac disease (Green et al., 2022). Elevated levels of these antibodies can indicate abnormal immune response to glute, even though they are less specific relative to other serological indicators such as anti-tTG and EMA (Volta et al., 2024). The emergence of more sensitive and specific tests, like as anti-tTG antibodies, has reduced the diagnostic value of AGA testing, while it can still yield additional proof for celiac disease. Moreover, AGAs are more frequently found in those with various gastrointestinal diseases including non-celiac gluten sensitivity. Furthermore, individuals with celiac disease may not always test positive for AGAs, especially if they currently follow a gluten-free diet (Green et al., 2022, Anbardar et al., 2022). The existence of AGAs can nevertheless be helpful in clinical practice, despite its limitations, especially when screening for celiac disease or in individuals whose test results are unclear. Overall,

more trustworthy serological tests have essentially replaced anti-gliadin antibodies in the final diagnosis of celiac disease, despite the fact that these antibodies offer insight into gluten sensitivity and immune response. Frequent AGA monitoring may still be helpful in determining how each patient reacts to gluten exposure (Green et al., 2022).

Influence of HLA-DQ2 and HLA-DQ8 on Antibody Levels

One significant field of research in the understanding of autoimmune diseases, like celiac disease in this case, is the comparative measurement of antibody levels in individuals with different haplotypes. According to many studies, individuals with the HLA-DQ2 haplotype often have higher levels of anti-tTG antibodies than those with the HLA-DQ8 haplotype (Khudher et al., 2020, Kadhum et al., 2022). This variation could be explained by the different ways that these haplotypes expose T cells to gluten-derived peptides, which produce different immunological reactions. In general, gluten peptides bind to HLA-DQ2 more strongly than they do HLA-DQ8, which enhances antibody formation and immune system activation (Aboulaghras et al., 2022). People with different haplotypes have varying amounts of AGA in addition to different anti-tTG antibodies. Moreover, previous studies have reported that individuals with HLA-DQ2may have higher levels of IgA and IgG AGA, while those who have HLA-DQ8 would react more inconsistently (Taşkın and Anlaş, 2023). AGA is often associated with early stages of gluten exposure and may serve as a sign of gluten sensitivity before more specialized antibodies such as tTG are produced. Haplotypes also influence the timing of antibody production. While HLA-DQ8 carriers may react more slowly, HLA-DQ2 carrier may develop antibodies earlier in life, often when gluten is added to the diet (Taşkın and Anlaş, 2023, Khudher et al., 2020). Genetic studies have also identified additional factors that may influence serological responses in individuals with different HLA haplotypes. Antibody levels may be affected by differences in immune regulatory genes, such as IL-15, which have been connected to an increased risk and severity of celiac disease (Kara et al., 2021). D'Avino et al clarified the intricate relationship between genes and serological responses by highlighting the part that these genetic differences play in regulating the immune response to gluten (D'Avino et al., 2021).

1.4.Clinical Implications of HLA Typing in Celiac Disease

HLA typing has extensive and multifaceted clinical significance as a vital diagnostic, therapeutic, and management tool for celiac disease (Kadhum et al., 2022). Conventional diagnostic techniques, such as serological testing for particular antibodies like AGA and anti-tTG, might result in false negative results, especially in patients who have already begun a gluten-free diet (Deja et al., 2020). Since the development of celiac disease requires the presence of HLA-DQ2 or HLA-DQ8, HLA typing offers a genetic basis for diagnosis. More so, studies indicate that at least one of these haplotypes is present in more than 95% of individuals with celiac disease (Aboulaghras et al., 2023, Espino and Núñez, 2021). Thus, negative HLA typing essentially rules out celiac disease, which in some situations eliminates the need for invasive tests like intestinal biopsies. HLA typing can also be used to identify individuals who are at risk, especially first-degree relatives of patients who have been diagnosed. Whether these people have the HLA-DQ2 or HLA-DQ8 haplotypes can be ascertained through genetic testing, enabling early surveillance and intervention (Del Pozzo et al., 2021). This proactive approach is essential because long-term consequences such as nutritional deficits, osteoporosis, and an increased risk of certain cancers can be avoided with early diagnosis and management of the disease. Fundamentally, strict adherence to a gluten-free diet is the main treatment strategy for celiac disease. However, patient education and dietary recommendations may be influenced by information derived from HLA typing. Healthcare practitioners can stress

the significance of avoiding gluten-containing foods and offer resources for managing dietary adjustments to individuals who test positive for HLA-DQ2 or HLA-DQ8 (Del Pozzo et al., 2021, Dieli-Crimi et al., 2015). Furthermore, by helping patients and their families realize that the disease is lifelong, knowledge of the genetic predisposition might promote adherence to dietary restrictions. Also, HLA typing can help with managing celiac disease in relation to other comorbidities, such as autoimmune diseases, which may also have genetic components, and are common among individuals with celiac disease (Martina et al., 2018).

1.5.Current Research and Future Directions

Recent studies have attempted to decipher the exact mechanisms by which HLA haplotypes influence the immune response. For example, it has been reported that the specific pattern of T cell activation exhibited by individuals with HLA-DQ2 in response to gluten peptides was connected with the extent of intestinal injury (Risnes et al., 2024, Voisine and Abadie, 2021). The new trajectory emphasizes the importance of HLA typing in predicting the severity of the disease and its repercussions. Additionally, the relationship between specific antibody production and HLA haplotypes has been the subject of recent research. According to recent studies, individuals with HLA-DQ2 are more likely to develop anti-tTG antibodies, but those with HLA-DQ8 may exhibit a different antibody profile (Poddighe and Capittini, 2021, Ramakrishna et al., 2021, Lee et al., 2020). The potential to tailor therapy and diagnostic methods based on the unique HLA haplotype of the patient is highlighted by this variance in immune response. For example, patients with HLA-DQ2 may benefit from more regular monitoring for repercussions due to their increased possibility of developing serious illness. Recent studies have examined the potential of adjunct therapy, such as immunomodulation or enzyme supplementation, which may be more advantageous in particular genetic backgrounds (Valvano et al., 2023, Discepolo et al., 2024). Using personalized medicine can boost effectiveness and lower side effects, thereby achieving enhance therapeutic outcomes. Additionally, studies are being conducted to find additional genetic factors that influence the immune response in celiac disease. For instance, susceptibility to and severity of celiac disease have been associated with variations in immune-related genes such as CTLA-4 and IL-15 (Gaba et al., 2024). Improved understanding of these genetic relationships can aid in the creation of personalized treatment programs and provide more profound understanding of the pathophysiology of celiac disease. Recent studies have shown that, in addition to genetic predisposition, environmental factors like timing of gluten introduction and the composition of the gut flora also affect the immune response. These factors can interact to significantly impact the onset and progression of the disease (Catassi et al., 2024, Galipeau et al., 2024). For this reason, personalized medical approaches that take into account the environmental and genetic factors may lead to more effective treatments and preventative measures.

2. Conclusion

In conclusion, the pathophysiology of celiac disease can be better understood by comparing the antibody levels among individuals with various haplotypes. The HLA-DQ2 and HLA-DQ8 haplotypes have a significant impact on the immune response to gluten, leading to variations in antibody production, Additionally, the application of HLA typing in clinical settings paves the way for tailored medical approaches based on genetic predisposition, which could enhance patient outcomes, boost diagnostic accuracy and direct treatment strategies. Future studies should concentrate on elucidating the molecular processes that underlie these immune reactions and investigating how other environmental and genetic variables influence antibody levels in celiac disease.

3. Conflict of Interest

The authors declare there is no conflict of interests

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Letrozole in Treating Polycystic Ovary Syndrome-Associated Infertility

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Abstract

Letrozole was originally reported for the treatment of breast malignancy, however, it exhibited potential in treating infertility disorders linked with polycystic ovary syndrome (PCOS). The purpose of this research was to investigate the usefulness of letrozole in treating infertile females with PCOS. A randomized, prospective, clinical trial involving 105 females aged between 19 and 33 was performed. Over a couple of months, evaluations were completed on days two and twelve of the females who received letrozole tablets. Substantial statistical ovulatory increment, more endometrial thickness, and altered hormone levels were identified. These changes signify letrozole's potential as a useful choice for PCOS-associated infertility. To fully comprehend its therapeutic potential and optimize treatment regimens, additional future study is required.

الليتروزول في علاج العقم المرتبط بمتلازمة تكيس المبايض

رحاب كاظم، امال عمران موسى، حميدة هادي عبد الواحد

الخلاصة

تم الإبلاغ في الأصل عن الليتروزول لعلاج الأورام الخبيثة في الثدي، ومع ذلك، فقد أظهر إمكانات في علاج اضطرابات العقم المرتبطة بمتلازمة تكيس المبايض. كان الغرض من هذا البحث هو التحقيق في فائدة الليتروزول في علاج الإناث العقيمات المصابات بمتلازمة تكيس المبايض. تم إجراء تجربة سريرية عشوائية مستقبلية شملت 105 أنثى تتراوح أعمارهن بين 19 و33 عامًا. على مدار شهرين، تم الانتهاء من التقييمات في اليوم الثاني والثاني عشر للإناث المتقدمات اللاتي تلقين أقراص الليتروزول ومواضيع التحكم الصحية. تم تحديد زيادة كبيرة في التبويض الإحصائي، وزيادة سمك بطانة الرحم، ومستويات هرمون متغيرة. تشير هذه التغييرات إلى إمكانات الليتروزول كخيار مفيد للعقم المرتبط بمتلازمة تكيس المبايض. لفهم إمكاناته العلاجية بشكل كامل وتحسين أنظمة العلاج، هناك حاجة إلى دراسة مستقبلية إضافية.

1. Introduction

Midway through 1997, letrozole was initially registered for the treatment of breast cancer in France (Bhatnagar, 2007). Given that letrozole medicine is used to overcome PCOS-related infertility, its multifaceted role becomes even more imperative (Chen et al., 2024a). The principal form of estrogen, estradiol, is produced by the conversion of androgenic hormone synthesized by the ovaries (Eskew et al., 2019). Letrozole is a non-steroidal aromatase inhibitor that is rapidly and completely absorbed from the gastrointestinal tract, with its absorption unaffected by food intake (Rashdan et al., 2024). The drug has a large volume of distribution, approximately 1.9 L/kg, and is about 60% bound to plasma proteins, primarily albumin (Rashdan et al., 2024). Letrozole's terminal elimination half-life is approximately 42 hours, allowing for steady-state concentrations to be reached within 2 to 6 weeks of daily administration (Desai et al., 2024). The metabolism of letrozole primarily occurs through cytochrome P450 enzymes, particularly CYP3A4 and CYP2A6, resulting in the formation of an inactive carbinol metabolite (Desai et al., 2024, Rashdan et al., 2024). Approximately 90% of the administered dose is recovered in urine, with around 75% as the glucuronide conjugate of the carbinol metabolite (Kivrak et al., 2024). There are often substantial hormonal alterations in PCOS. By inhibiting this conversion, letrozole increases androgen levels in the blood. Growing follicles may increase almost as a result, possibly enhancing the quality of the discharged follicles (Chen et al., 2024a). A 2022 study by Gowri Vi. et al. confirm this idea more strongly by showing that the administration of letrozole is favorably connected with increased mean follicle size in PCOS individuals undergoing in-vitro fertilization (IVF) (Gowri et al., 2022). Letrozole has effects that go beyond the follicle quality. The imbalance between estrogen and androgens in females with PCOS can result in a thinning endometrium, leaving the uterus less conducive to implantation. Letrozole may trigger an increase in endometrial thickness by boosting the synthesis of growth-promoting factors through elevated testosterone ratios (Pritts et al., 2011). All these kinds of hormones work together to create a denser endometrial lining, which can increase the implantation process. Consistent with a recent study, letrozole administration might boost endometrial growth among ladies with PCOS, indicating it can be an appropriate substitute for cases suffering from thin endometrium (Alhibshi et al., 2021). The most prominent action of letrozole in PCOS cases is its physiological activity to trigger ovulation. In females, ovulation remains on hold by the ordinary hormonal path-ways hindered by the excess estradiol production in PCOS. This antagonistic feedback loop is suppressed by letrozole usage, which prevents the peripheral estradiol conversion (Chen et al., 2024a). Owing to the continuous hypophysial secretion of FSH, further released eggs become accessible for spermatic fertilization by promoting the evolution of plentiful follicles and over-ovulation (Chen et al., 2024a). Accordingly, letrozole treatment has been confirmed to raise PCOS-afflicted female's prospects of becoming pregnant (Yang et al., 2021a). Compared to clomiphene citrate letrozole is cheap, easier to acquire, and has minor adverse reactions, therefore it is seen as a valuable substitute for other medications (Yang et al., 2021b). (Reed BG). Letrozole lowers the odds of multiple pregnancies by promoting mono-follicular development, which results in singleton pregnancies. Furthermore, letrozole has demonstrated efficacy in increasing sperm qualities in infertile with low blood levels of testosterone and estrogen, which may improve fertility in men with oligospermia (AlJuboory et al., 2020). The wide range of manifestations that women with PCOS experience accounts for a large portion of the uncertainty surrounding the diagnosis of PCOS. Considering the large percentage of females who are suffering from PCOS and the substantial impact it has on patients, it is important to gain a greater awareness of the current burden in the vicinity of the Middle East (Liu et al., 2021). The majority of research investigations on PCOS have been carried out in developed countries, with limited data

available on the magnitude of the problem in developing countries, such as Iraq (Motlagh Asghari et al., 2022). A previous study estimated that a third of reproductive-aged females in Iraq have PCOS (Reed BG). Infertility is defined generally as the inability to conceive after one year or longer of unprotected sex. This condition can affect both men and women and may result from various factors affecting the process of conception, such as problems with ovulation, sperm quality, or the reproductive organs (Adnan A. H. Al-Bdairi, 2021, Adnan A. H. Al-Bdairi 2023). Infertility is a common issue, with about 1 in 5 women aged 15 to 49 in the United States being unable to have conception after one year of trying (Adnan A. H. Al-Bdairi 2022). Hence the current study aimed to fill the gap in the available data and to investigate the precise role of letrozole in Iraqi infertile females with PCOS.

2. Patients and Methods

2.1. Study Design

This was a prospective, single-center, randomized, controlled pragmatic clinical trial conducted at the Teba Center for Infertility and In-Vitro Fertilization, Babylon-Iraq, from September 2023 to February 2024.

2.2. Selection of Participants

A total of 105 female patients participated in the study, with an average age of 27.1 ± 4.9 years.

Inclusion Criteria: Females with a history of infertility (primary or secondary) and PCOS, were diagnosed at the Teba Center based on the modified Rotterdam criteria with an age range of 19-33 years old. The modified Rotterdam criteria for diagnosing Polycystic Ovary Syndrome (PCOS) were established to provide a standardized approach for clinicians. These criteria include the following components:

- Oligo- or Anovulation: This refers to irregular menstrual cycles or the absence of ovulation, which can lead to infertility.
- Clinical and/or Biochemical Signs of Hyperandrogenism: This includes symptoms such as hirsutism (excess hair growth), acne, and alopecia (hair loss), as well as elevated levels of androgens (male hormones) in the blood.
- Polycystic Ovaries: This is determined through ultrasound imaging, which reveals the presence of multiple small follicles (typically 12 or more) in one or both ovaries, often accompanied by an increased ovarian volume (Christ and Cedars, 2023).

To meet the diagnosis of PCOS using the modified Rotterdam criteria, a woman must present with at least two of the three components listed above. These criteria help in identifying PCOS effectively while considering its diverse manifestations.

Exclusion Criteria: any cases of tubal blockage or refusal to participate.

2.3. Data Collection

Baseline Assessment: On the second day of the menstrual cycle, the following data were collected for each participant: demographic information, clinical history, medical history, and ultrasound examinations by two separate specialist sonographers to assess egg size and endometrial thickness. Hormonal assessments for LH, AMH, and prolactin using Electro-Chemiluminescence Immunoassay (ECLIA) kits from Mindray® Medical International Limited, China. AMH assays (performed only on the second day of the cycle).

2.4. Intervention:

All the participants were randomly selected to receive letrozole treatment. Letrozole was administered as 2.5 mg tablets (Femara®, NOVARTIS®, Basel, Switzerland) once daily after a meal for five consecutive days, starting from the second day of the menstrual cycle, for two consecutive cycles (Reed BG).

2.5. Follow-up Assessment:

After two months of letrozole administration, on the 12th day of the cycle, the following assessments were repeated: ultrasound examination, and hormonal tests for LH and prolactin.

2.6. Sample Size Calculation Method

The sample size for this study was calculated using a power analysis method to ensure adequate statistical power for detecting differences in outcomes related to CYP3A4*18 genetic variations. The following steps were employed:

1. Effect Size: A medium effect size (Cohen's d = 0.5) was assumed based on previous studies.
2. Significance Level: A significance level (alpha) of 0.05 was established.
3. Desired Power: The power was set at 80% (0.8), indicating a 20% chance of Type II error.
4. Statistical Test: The Kruskal-Wallis test was selected for comparing multiple groups.
5. Sample Size Formula: The formula used for sample size estimation was: $n = ((Z\alpha/2 + Z\beta)^2 \times k) / d^2$, (Here, n is the sample size per group, $Z\alpha/2$ is the Z-value for the significance level, $Z\beta$ is the Z-value for power, k is the number of groups, and d is the effect size).
6. Calculation: For three genotype groups (TT, TC, CC), the calculation yielded a minimum requirement of approximately 95 participants.

2.7. Statistical Analysis

Statistical studies were completed using SPSS (V-27) and JASP (V- 0.18.3.0). Continuous variables were resented as mean and standard deviation (SD). *The* categorical variables were examined using Chi-square tests to assess associations. The normality of the data was tested and the data were normally distributed. Comparison of means was completed using independent samples t-tests to compare means between two study groups, and ANOVA was utilized to compare means between more than two groups. Pearson correlation coefficients were used to assess relationships between continuous data. A significance level below 0.05 was considered statistically significant.

3. Results

Important details on the clinical and demographic traits of the individuals under study are provided in Table1. An improved understanding of the age distribution within the population under study is made possible by the table's additional division of the age distribution into three categories: < 20 years (16%), 20 – 29 years (55%), and > 30 years (29%). The average age was 27.11 (4.9) years, and in the group over 30, the mean BMI rises with age. The patients' mean ± SD BMI and marriage length are 29.7±4.9 and 6.0±3.5 years, respectively. The patients in the study had 0.8 children and 0.6 miscarriages. The ultrasound measures on U/S Day 2 and the endometrial thickness on Days 2 and 12 are displayed in the table. The table also includes the amounts of other hormones, such as LH, Prolactin, and AMH.

Table 1: Basal Demographic Features of the Studied Patients

Variables	Mean	SD
Age	27.1	4.9
< 20 y	18.8	0.7
20 – 29 y	24.9	2.1
> 30 y	33.1	0.9
Marriage span/years	7.1	0.9
BMI kg/m^2	28.9	2.7
No. of miscarriage	0.7	0.9
No. of children	0.8	0.8
Endometrial thickness Day 2	3.9	5.1
Follicular size on U/S Day 2	3.9	1.2
Endometrial thickness Day 12	7.7	0.8
Follicular size on U/S Day 12	18.1	3.9
LH (mlU/mL)	8.8	3.3
Prolactin (ng/mL)	16.1	9.7
AMH (ng/mL)	4.4	1.9

Table2 displays the variations in important study variables among the age groups of the patients. Remarkably, the p-values for the majority of the variables are in proximity to the traditional significance threshold ($p < 0.05$), suggesting possible correlations between age groups and these factors. Nonetheless, p-values for a few variables are somewhat higher than this cutoff. Age-related increases in BMI seem to be modest, however they are not statistically significant ($p = 0.071$). The duration of marriage varies significantly ($p = 0.001$) among age groups, with older age groups showing longer marriages. There are substantial age-group variations in both the number of children and miscarriages ($p = 0.002$ and $p = 0.062$, respectively), with older age groups showing higher mean values. Follicular size and endometrial thickness vary slightly between age groups, but not to a statistically significant degree ($p > 0.05$).

Table2: Differences in the Study Variables According to the Studied Age Groups

Variables	Age classes/y	Mean± SD	P-value
BMI	< 20	29.4 ±5.1	0.073
	20 – 29	29.1 ±4.9	
	> 30	30.8 ±5.1	
Marriage span	< 20	2.9 ±3.2	0.001
	20 – 29	6.1 ±3.3	
	> 30	7.6 ±4.1	
No. of children	< 20	0.3 ±0.5	0.002
	20 – 29	0.8 ±0.6	
	> 30	1.2 ±0.9	
No. of miscarriage	< 20	0.4 ±0.8	0.068
	20 – 29	0.5 ±1.1	
	> 30	0.9 ±1.2	
Follicular size on U/S Day 2	< 20 y	4.0 ±1.3	0.92
	20 – 29 y	4.0 ±1.1	
	> 30 y	3.9 ±1.2	
Follicular size on U/S Day 12	< 20 y	18.8 ±4.1	0.91
	20 – 29 y	17.7 ±4.4	
	> 30 y	18.2 ±3.8	
Endometrial thickness Day 2	< 20 y	3.9 ± 0.7	0.079
	20 – 29 y	7.9 ± 0.8	
	> 30 y	3.7 ± 0.7	
Endometrial thickness Day 12	< 20 y	7.8 ±0.6	0.079
	20 – 29 y	7.8 ±0.3	
	> 30 y	7.7 ±0.8	

The ultrasonography results for endometrial thickness and egg size on days 2 and 12 are shown in Fig.1, which showed a highly significant increase on day 12 of the menstrual cycle

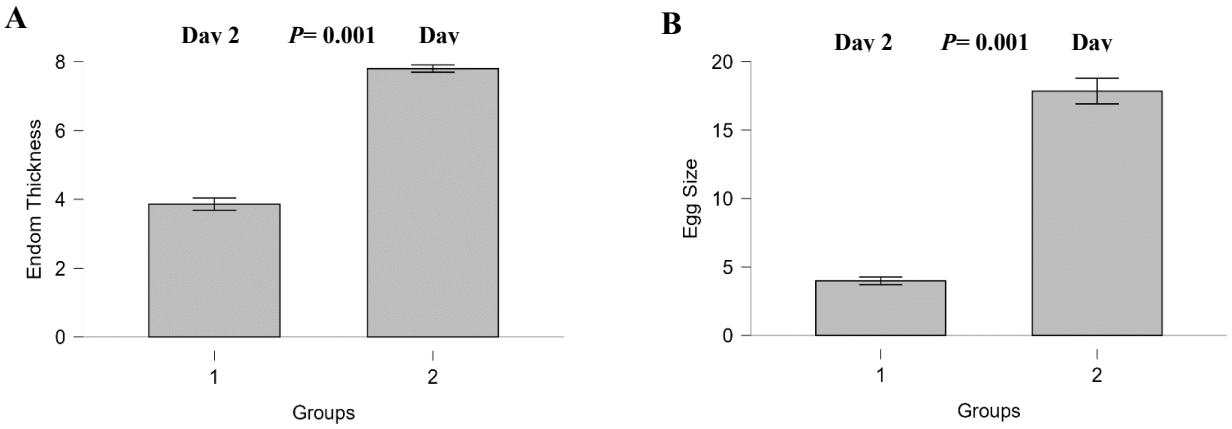


Figure1: Ultrasonography Results for Endometrial Thickness and Egg Size on Day 2 And Day 12 of the Menstrual Cycle

The left panel shows endometrial thickness, and the right panel shows follicular (egg) size in Groups 1 and 2. Both parameters were significantly higher in Group 2 compared to Group 1 on Day 12 (P = 0.001). Error bars represent standard error of the mean (SEM).

Hormonal results for LH and prolactin on days 2 and 12 of the menstrual cycle are shown in Fig.2A & Fig.2B. Between Day 2 and Day 12 of the menstrual cycle, there are significant differences in LH levels (p=0.001), but there are no significant alterations in prolactin levels ($p > 0.05$).

The variation in study parameters based on menstrual cycle regularity [regular (N=64) and irregular (N=41)] is seen in Fig.3A-R. The data suggests that although women with irregular menstrual cycles and those with regular cycles do not vary significantly in age, BMI, or most hormonal parameters, they do vary significantly in the duration of their marriages, with the latter group having a shorter average duration.

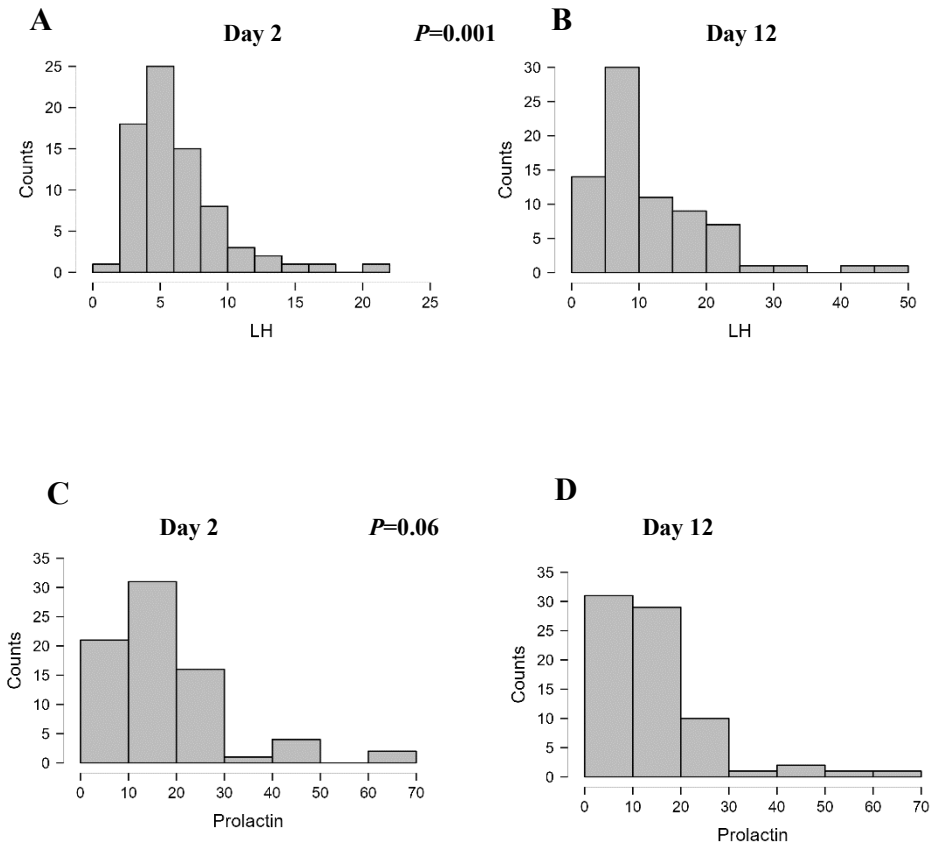
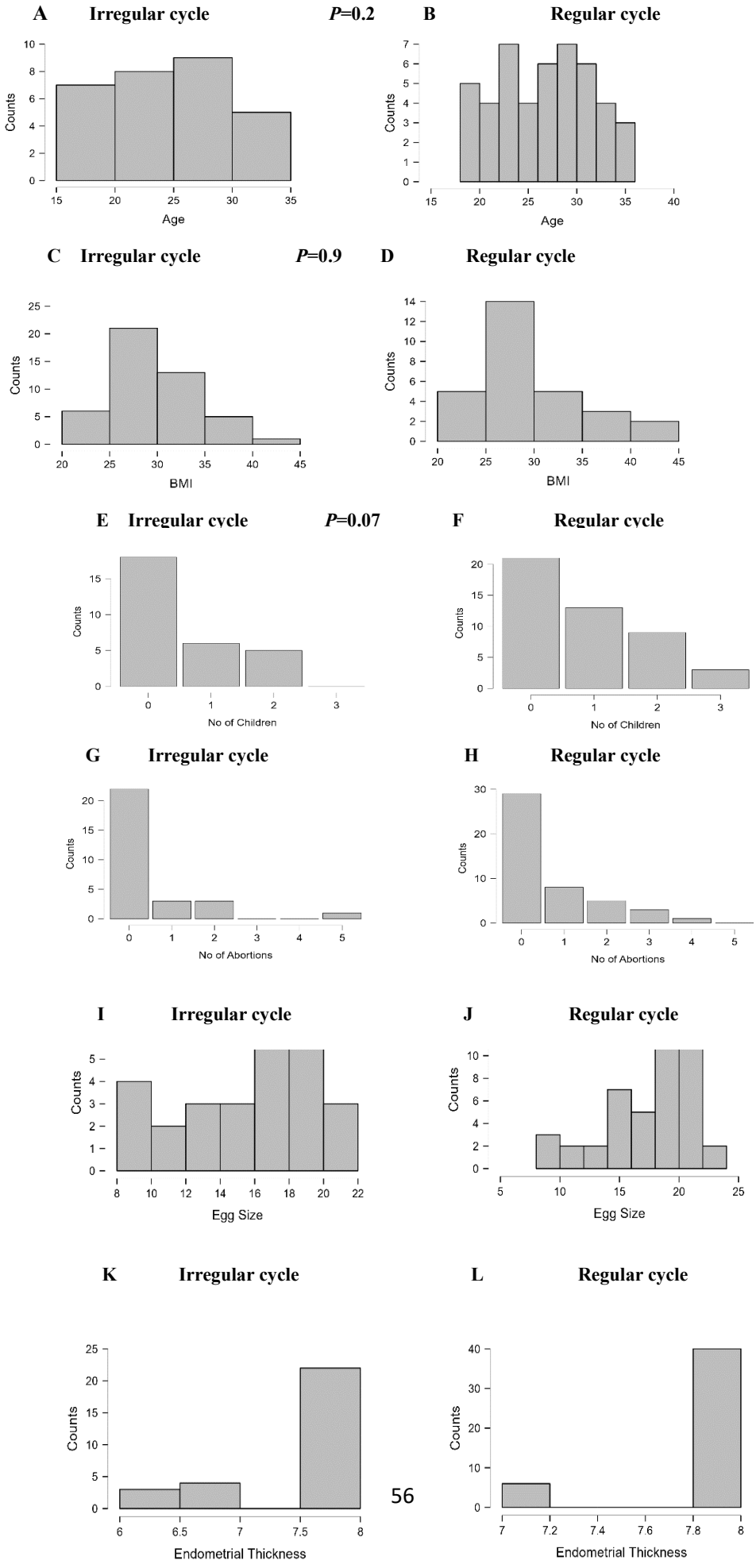


Figure 2. Hormonal Measurements on Day 2 And Day 12 of The Menstrual Cycle
A and **B** show the distribution of luteinizing hormone (LH) levels on day 2 and day 12, respectively, with a significant increase observed on day 12 (**P = 0.001**), consistent with the expected pre-ovulatory LH surge. **C** and **D** illustrate the distribution of prolactin levels on day 2 and day 12, showing a slight but non-significant decrease in prolactin levels on day 12 (**P = 0.06**).


A Irregular cycle
P=0.2
B Regular cycle
Counts
Age
C Irregular cycle
P=0.9
D Regular cycle
BMI
E Irregular cycle
P=0.07
F Regular cycle
No of Children
G Irregular cycle
H Regular cycle
No of Abortions
I Irregular cycle
J Regular cycle
Egg Size
K Irregular cycle
L Regular cycle
Endometrial Thickness

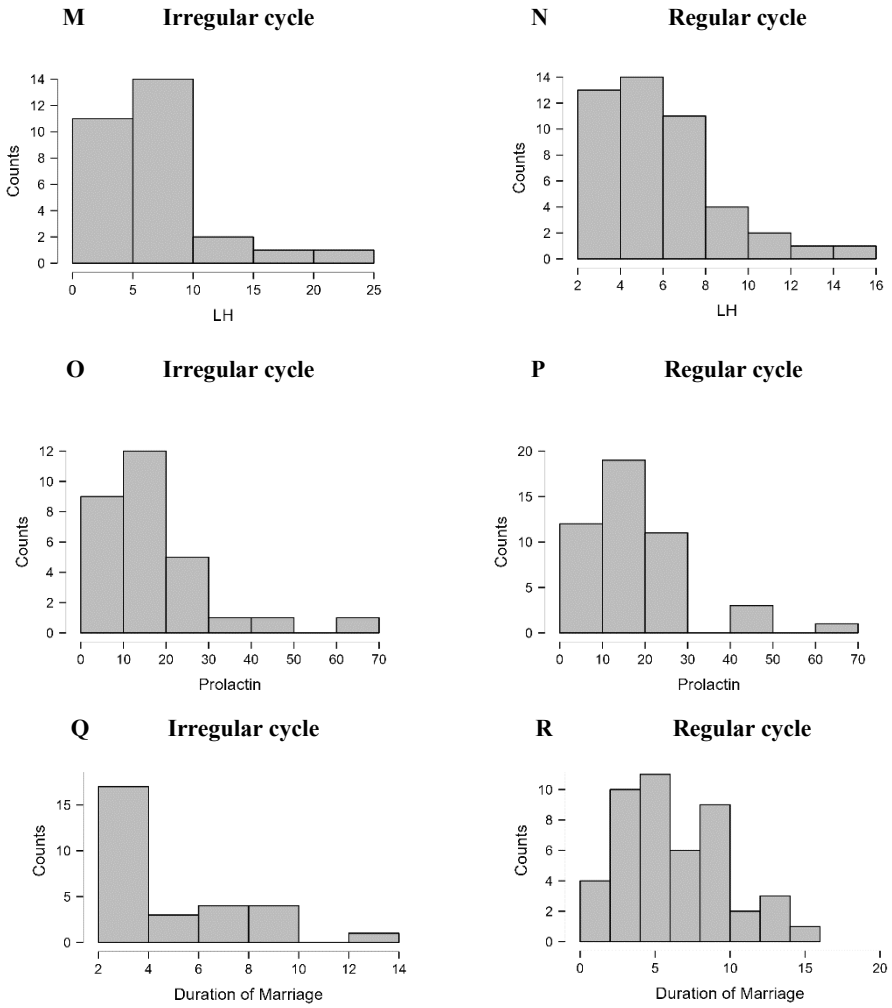


Figure3: Distribution of Demographic, Reproductive, Hormonal, and Clinical Variables Among Women with Irregular and Regular Menstrual Cycles

(A–B) Age distribution in women with irregular and regular cycles, respectively (**P = 0.2**). **(C–D)** Body Mass Index (BMI) distribution across both groups (**P = 0.9**). **(E–F)** Number of children, showing a near-significant difference (**P = 0.07**). **(G–H)** Number of abortions, with no significant variation. **(I–J)** Distribution of follicular (egg) size. **(K–L)** Distribution of endometrial thickness measurements. **(M–N)** Luteinizing hormone (LH) levels. **(O–P)** Prolactin levels. **(Q–R)** Duration of marriage in both groups. Each pair of histograms compares the respective variable between women with irregular cycles (left) and regular cycles (right), highlighting patterns and differences in reproductive and endocrine profiles.

The capacity of many factors, such as follicular size, prolactin, LH, and endometrial thickness, to differentiate between hormonal and ultrasonic changes on days 2 and 12 of the cycle, is shown in Table3 and Table4 and Fig.4. For each variable, the table gives the sensitivity, specificity, 95% confidence intervals, p-values, and area under the receiver operating characteristic (AUC) curve. Endometrial thickness and follicle size are highly effective markers for distinguishing changes, making them valuable for monitoring menstrual cycle-related changes after letrozole administration. With an AUC value of 0.515 and a non-significant p-value of 0.752, LH demonstrates low discriminative capacity. Additionally, the moderate sensitivity and specificity imply little value in differentiating between acoustic and hormonal changes. With the highest AUC value of 0.777, prolactin stands out as having good discriminative capacity. The statistical significance of the low p-value of 0.001 indicates that prolactin levels are useful in differentiating between changes that are hormonal and ultrasonic. Prolactin's usefulness in this situation is further supported by the comparatively high sensitivity and specificity values.

Table3: Ability of Endometrial Thickness, Follicular Size, LH, and Prolactin to Distinguish Between Hormonal and Ultrasonic Changes on Days 2 And 12 of The Cycle

Variables	AUC	P-value	Sensitivity	Specificity	95% Confidence Interval	
Endometrial thickness	1.0	0.001	1.00	1.00	1.00	1.00
Folicular size	1.0	0.001	1.00	1.00	1.00	1.00
LH	0.515	0.752	0.578	0.508	0.422	0.608
Prolactin	0.777	0.001	0.613	0.514	0.699	0.854

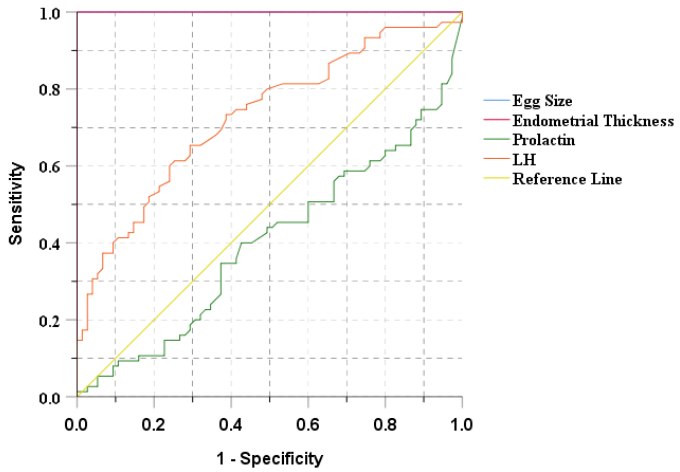


Figure4: Receiver Operating Characteristic (ROC) Curve Analysis of Egg Size, Endometrial Thickness, Prolactin, and Luteinizing Hormone (LH) As Predictors of Menstrual Cycle Regularity

The ROC curves illustrate the diagnostic performance of four parameters: egg size (blue), endometrial thickness (pink), prolactin (orange), and LH (green). The yellow diagonal line represents the reference line (no discrimination). Among the tested parameters, endometrial thickness shows the highest sensitivity and specificity, followed by prolactin. Egg size and LH display comparatively lower diagnostic accuracy. The analysis supports the potential clinical utility of endometrial thickness and prolactin as strong indicators for distinguishing between regular and irregular

Spearman's correlation analysis Table4 reveals numerous important relationships between the study variables, providing insights into the interplay between various factors associated with fertility and reproductive health.

Table4: Spearman's Correlations Evaluations of the Study Variables Among Each Other's

Parameters (means)	Analyses	Age	BMI	Follicular Size	Endometrial Thickness	LH	Prolactin	AMH
Age	Correlation	—						
	Significance	—						
BMI	Correlation	0.251**	—					
	Significance	0.033	—					
Follicular Size	Correlation	-0.046	-0.051	—				
	Significance	0.881	0.811	—				
Endometrial Thickness	Correlation	-0.110	-0.251	.898**	—			
	Significance	0.299	0.169	.000	—			
LH	Correlation	-0.181	-0.323	.375**	0.323**	—		
	Significance	0.452	0.445	.000	.000	—		
Prolactin	Correlation	-0.211	-0.201	-.152	-.149	-.030	—	
	Significance	0.318	0.105	.063	.069	.711	—	
AMH	Correlation	-0.309	-0.100	.027	-.108	.173	-.077	—
	Significance	0.071	0.560	.817	.355	.138	.509	—

** p < .01

4. Discussion

The study aimed to evaluate the effectiveness of letrozole in ovulation induction outcomes in infertile Iraqi females. The study found that letrozole was an effective ovarian stimulant, and achieved a significantly higher endometrial thickness. Letrozole, which is largely applied to treat breast tumors, has drawn attention due to its likely benefits in treating PCOS-related infertility. The existing investigation contributes to the growing body of trials supporting letrozole as a first-line choice for infertility in PCOS females. Letrozole's capacity to stimulate ovulation and increase gestation rates sheds light on its potential in the management of PCOS-related infertility (Yang et al., 2021b, Chen et al., 2024a, Franik et al., 2022a). To inhibit the negative feedback on the hypothalamic-pituitary-gonadal axis and encourage the development of new follicles, letrozole works by blocking the synthesis of estrogen (Eskew et al., 2019, Franik et al., 2022a, Pritts et al., 2011, Yang et al., 2021b).

Simultaneous modulation of other hormonal biomarkers, like LH and prolactin, was also detected. These hormonal shifts are crucial for regulating the complex processes of follicular growth, ovulation, and synthesis of corpus luteum—all of which must occur for a fruitful pregnancy (Duncan, 2021, Przygrodzka et al., 2021, Reed BG). Additionally, after letrozole was orally administered, favorable increases in endometrial thickness and morphology were exposed by sonography assessment. The observed rise in endometrial thickness, which reflects appropriate endometrial proliferation and responsiveness, was particularly significant. From this, it can be inferred that letrozole may improve endometrial dynamics and increase the chances of successful embryo implantation and subsequent pregnancy. While the current study indicates that letrozole is an effective ovarian stimulant, it is essential to consider that the evidence base for its use in ovulation induction is still developing. A recent systematic review included 41 randomized controlled trials involving 6,522 women, demonstrating that letrozole improves live birth rates compared to clomiphene citrate (CC) for ovulation induction (Franik et al., 2022b). However, the variability in study designs and populations underscores the need for caution in generalizing these findings. For instance, while some studies report significant improvements in endometrial thickness and pregnancy rates with letrozole, others indicate no substantial differences compared to CC (Chen et al., 2024b, Kar, 2013). Furthermore, the lack of consensus on optimal dosing regimens complicates the interpretation of results across different studies (Yang et al., 2021b). Thus, while letrozole shows promise as a first-line treatment for infertility in women with PCOS, further research with larger sample sizes and standardized protocols is necessary to confirm these findings and address existing inconsistencies (Kivrak et al., 2024). There is a significant difference ($p = 0.001$) in the duration of marriage across age groups. Similarly, while the search results fail to demonstrate a significant difference in the duration of marriage between age groups in couples experiencing infertility due to PCOS, they do indicate that PCOS can significantly affect a couple's satisfaction with their marriage and sexual life, as well as their connections with friends and family (Navid et al., 2018, De Frène et al., 2014). There were non-significant differences in age, BMI, and most hormonal parameters between women with irregular and regular menstrual cycles. However, there is a significant variation in the duration of marriage, with women experiencing irregular cycles having a shorter mean duration of marriage. The duration of marriage has no direct effect on PCOS women's irregular menstrual periods. Nevertheless, irregular periods after marriage can be caused by a variety of circumstances, including stress, changes in practice, weight variations, hormonal birth control, and pregnancy (Khalaf et al., 2015). The condition itself of PCOS can cause irregular menstrual cycles in women, which can impact menstrual regularity and reproductive health (Dason et al., 2024). To address any underlying concerns and receive the proper therapy or management, it is imperative that women who experience irregular periods after

marriage visit healthcare specialists (Navid et al., 2018). The follicular size and endometrial width on Day 12 had significantly enlarged compared to Day 2 of the cycle after administration of oral letrozole. This can be clarified by the fact that letrozole acts as an aromatase inhibitor, which upsurges intra-ovarian androgenic concentrations, particularly in the early antral follicles (6-8mm) at Day 3 or 5 of the menstruation (McGrail et al., 2020). These increased androgen measures in the early ovarian follicles stimulate granulosa cell mitosis, increase expression of FSH receptors, and make the ovarian follicles more resistant to atresia. The latter effect boosts follicular development and eventual ovulation (Rose and Brown, 2020). The increased level of estrogen caused by increased follicle development with early letrozole initiation leads to enhanced endometrial width and maturation (Franik et al., 2022a). Exactly, the published data indicate the endometrial thickness was meaningfully more if letrozole was initiated on Day 5 (9.0mm) compared to Day 3 (8.0mm). The enhanced endometrial response induced by letrozole initiation may contribute to the increased implantation and clinical pregnancy rates detected (Sakar and Oglak, 2020). Hormone replacement therapy led to an increase in proliferative thickening, which is evidence that endometrial growth and responsiveness to hormone stimulation are accepted. These findings might support a new function for letrozole in enhancing endometrial cohesiveness and establishing the perfect environment for the attachment-implantation signal of the embryo and, eventually, conception (15,16).

The search results revealed no studies have compared the impact of oral letrozole initiated on the second day of the cycle for two successive cycles with day 12^{th} of the second cycle regarding follicle size or endometrial thickness. However, data about the effects of letrozole on the endometrial thickness are still controversial. If letrozole was started on the fifth day of the cycle, compared to the third day of the cycle, there were greater rates of ovulation, endometrial thickness (on the day of the HCG intramuscular injection), gestation, and clinical pregnancy in PCOS females documented earlier (Roy et al., 2012, Shi et al., 2022). In contrast, a study on the effect of letrozole on endometrial width in IVF cycles indicated that using letrozole during stimulation reduced the endometrial width by 0.81mm (Ruiter-Ligeti et al., 2021). In comparison to patients who did not get letrozole during their initial IVF cycles, those who received it had a thinner epithelium on the trigger day. The study showed that letrozole thins the endometrium, but it is still unclear how letrozole affects endometrial function. This underscores the need for more research before letrozole is prescribed for new transfers (Ruiter-Ligeti et al., 2021).

The correlational analysis in the current work reveals many substantial correlations among the study parameters, providing insights into the relations between several factors related to reproductive health and fertility. Nevertheless, it's vital to exercise attention when understanding these results and to consider any further confounding issues that could influence the exposed links. The current findings have considerable therapeutic inferences for managing infertility. By clarifying the mechanisms through which letrozole medicine supports ovulation and endometrial receptiveness, healthcare providers can modify treatment schedules to optimize reproductive outcomes for affected individuals. Since letrozole is oral and has a proper safety profile, it is also an appropriate and well-tolerated choice for individuals receiving fertility therapies.

5. Conclusion

According to the research, taking letrozole orally on the second day of the menstrual cycle greatly promotes endometrial and follicular growth. The study considerably enhances our understanding of in what way letrozole aids females with PCOS-related infertility. The conclusions propose that letrozole intervention might be valuable for numerous fertility-related features, including ovulation stimulation, thick endometrium, and lower gestational rates. Prolactin hormone exhibits possible biomarkers to distinguish between hormonal and ultrasonic variations in the context of PCOS medicine. The findings highlight the worth of letrozole as a therapeutic choice that helps females with PCOS-related infertility, and they also stress the necessity for further research to expand treatment tactics and reproductive outcomes.

6. Ethical Consideration

The University of Karbala's College of Pharmacy accepted the study protocol, and the Karbala Health Directorate was also consulted for approval. Furthermore, consent was obtained from every patient following an explanation of the study's nature and goals.

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Letrozole Impact on Polycystic Ovarian Syndrome Subfertility Using Sonography and Hormonol Assay

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Abstract

Background: One of the main causes of infertility and anovulation is PCOS. the oral aromatase inhibitor letrozole has become a new and promising first-choice treatment for ovulation induction, especially in cases with PCOS and infertility due to effectiveness and acceptable adverse effect profile.

Objective: This clinical trial was intended to evaluate the impact of letrozole administration on the hormones, follicle size, and endometrial thickness among infertile women with PCOS

Patients & Methods

A study at Teba Centre for Infertility involved 100 female patients with PCOS and infertility. Data was collected before and after two months of letrozole administration, using modified Rotterdam criteria and hormone tests.

Results

Remarkable enhancements in the induction of ovulation and the modulation of hormones were noted after letrozole administration. Both follicle size and endometrial thickness increased dramatically. Relationships between the study variables were found by correlation analysis highlighting estrogen significance in differentiating between hormonal alterations.

Conclusion

Letrozole appears to be a promising treatment for infertility, by enhancing the hormonal, besides follicular, and endometrial sonographic features necessary for ovulation and successful pregnancy. To further understand causes and treatment plans, additional research is required.

تأثير ليتروزول على العقم الناتج عن متلازمة تكيس المبايض باستخدام التصوير بالموجات فوق الصوتية والفحص الهرموني

نوار رياض كريم , أمال عمران موسى ,حسن محمود أبو المعالي

الخلاصة

المقدمة

تعتبر متلازمة تكيس المبايض واحدة من الأسباب الرئيسية للعقم وعدم الإباضة. أصبح مثبط الأروماتاز الفموي "ليتروزول" خيارًا جديدًا واعدًا كعلاج أولي لتحفيز الإباضة، خاصة في حالات متلازمة تكيس المبايض والعقم، وذلك بفضل فعاليته وملفه الجانبي المقبول للآثار الجانبية.

الهدف

تهدف هذه التجربة السريرية إلى تقييم تأثير تناول "ليتروزول" على الهرمونات، وحجم الجريبات، وسمك بطانة الرحم لدى النساء المصابات بالعقم ومتلازمة تكيس المبايض.

العينات وطرق العمل

أجريت دراسة في مركز طيبة للعقم على 100 مريضة تعاني من متلازمة تكيس المبايض والعقم تم جمع البيانات قبل وبعد شهرين من إعطاء الليتروزول، باستخدام معايير روتردام المعدلة واختبارات الهرمونات.

النتائج

لوحظت تحسينات ملحوظة في تحفيز الإباضة وتعديل الهرمونات بعد تناول "ليتروزول". كما زاد حجم الجريبات وسمك بطانة الرحم بشكل كبير.

الاستنتاج

يبدو أن "ليتروزول" علاج واعد للعقم، من خلال تعزيز الخصائص الهرمونية، بالإضافة إلى تحسين الخصائص الجريبية والبطانية الرحمية اللازمة للإباضة والحمل الناجح. هناك حاجة لمزيد من الأبحاث لفهم الأسباب وخطط العلاج بشكل أفضل.

1. Introduction

Polycystic ovary syndrome (PCOS) affects an estimated 8–13% of reproductive-aged women and is considered the most common endocrinologic illness. Approximately, 70% of affected PCOS females remain undiagnosed universally. (Motlagh Asghari et al., 2022). The prevalence of PCOS in Iraqi women in Al-Hilla city was estimated at 33% of reproductive-aged women in a previous study. (Ban Aamer Mousa, 2020). PCOS is the most prevalent cause of anovulatory cycles and a principal cause of infertility among women ((WHO)). Polycystic ovarian syndrome is metabolic, endocrine and genetic disorders. Infertility is generally defined as the inability to conceive after one year or longer of unprotected sex. This condition can affect both men and women and may result from various factors affecting the process of conception, such as problems with ovulation, sperm quality, or the reproductive organs. (Adnan A. H. Al-Bdairi, 2021, Adnan A. H. Al-Bdairi 2023). Infertility is a common issue, with about 1 in 5 women aged 15 to 49 in the United States being unable to get pregnant after one year of trying. (Adnan A. H. Al-Bdairi 2022). Letrozole is a selective oral aromatase inhibitor for post-menopausal women with hormone-responsive breast cancer. It is a newer first-line treatment for the induction of ovulation mainly in infertile an-ovulatory women, often in conditions such as unexplained infertility and PCOS. (Chen et al., 2024). Being less harmful, more easily available, and a cheaper alternative to medications like clomiphene citrate, it has long been considered a treatment option when other traditional therapies have failed. (Yang et al., 2021). Letrozole induces mono-follicular development, and subsequently singleton pregnancies without related risk of multiple gestations. Letrozole, in addition, is beneficial in sperm motion characteristics in infertile men with pathologically reduced E2 serum profiles, resulting in improved fertility in oligospermic men. (AlJuboory et al., 2020). It also inhibits estrogen synthesis, which stimulates FSH secretion and the development of ovarian follicles. (Chen et al., 2024). Great confusion concerning the diagnosis of PCOS is caused by the broad heterogeneity of the warning symptoms experienced by patients with PCOS. Given the large number of females who suffer from PCOS and its substantial impact on the general well-being in the Middle East region, a better understanding of the contemporary burden is important. (Liu et al., 2021). Most clinic-epidemiologic surveys on PCOS have been carried out in developed states, with only restricted information existing on the burden in developing countries including Iraq. (Motlagh Asghari et al., 2022). This clinical was intended to evaluate the impact of letrozole administration on the hormones, follicle size, and endometrial thickness among infertile women with PCOS

2. Patients & Methods

2.1. Study Design

This study was designed as a prospective, randomized, single-center, controlled clinical trial, conducted at the Teba Center for Infertility and In-Vitro Fertilization, Babylon City-Iraq, from September 2023 to February 2024. It included 100 female patients with age ranges of 26.3±5.1 years who attended this center for infertility and PCOS or the diagnosis of PCOs was then made in the center. The gynecologists at the center diagnose, examine, assess, categorize, treat, and follow-up these females.

Any female with a history of infertility (primary or secondary) and PCOS, irrespective of the number of abortions they had or the regularity of their menses, age range of 18 – 35 y, was included. Any females with tubal blockage or who refused to participate were excluded from the current study. All the data about the demographic, clinical, and medical history of the included females were recorded. On the 2nd menstrual cycle day, ultrasound examinations performed by two separate specialist sonographers were reported about the follicle size and

endometrial thickness. In the meantime, hormonal assessments for FSH and estrogen were performed also. AMH assays were done only on the second day of the mens. On the 12th of the cycle and after two months of letrozole administration, ultrasound examination, and hormonal tests were repeated.

2.2. Diagnosis of PCOS

The final diagnosis of PCOS was confirmed by the gynecologists mainly in Teba Center based on history, clinical examination, hormonal studies, and ultrasound examinations, based on the "modified Rotterdam criteria" (Christ and Cedars, 2023).

2.3. Letrozole administration

Letrozole tablets (Femara®, NOVARTIS®, Basel, Switzerland) 2.5 mg, once daily after meal for 5 consequent days starting from the 2nd day of the mens for two consequent cycles. The medicine might be taken at any time throughout the day, nonetheless, it should be administered at the same time each day. The patients should swallow the tablet whole with a drink of water, and must not crush or chew it. It can be challenging to ensure that letrozole is taken precisely on "day two" of the MC in females whose MCs are irregular. (Chen et al., 2024). However, some policies can be adopted to estimate the time, such as progesterone challenge testing, serial ultrasound or hormonal monitoring, clinical decision-making, tracking the patient's own MC patterns, and baseline ultrasound and hormonal assessment. Eventually, clinical decision and flexibility are critical when dealing with patients with irregular MCs. Moreover, the team cooperates with patients, a reproductive endocrinologist, a fertility specialist, sonographers, and of course the gynecologists for optimal guidance and assistance in the management of irregular MCs.

2.4. Hormonal assays

Hormonal assessments of FSH, estrogen, and AMH were performed, which were evaluated by two-site immunoenzymometric assay using specific kits from TOSOH® Corporation, Tokyo, Japan.

2.5. Data Analyses

Statistical scrutiny was done by SPSS (V-27) and JASP (V- 0.18.3.0). The continuous parameters were written as (Means/SD). The chi-square test ($\boldsymbol{X^2}$) was utilized to expose the association between the categorical parameters. Independent samples t-test was applied to match any two groups' means. ANOVA was applied to compare the variation of the means among more than two groups. Pearson matrix correlation was applied to assess relationships between the continuous data. A p-value below 5% was significant in these analyses.

3. Results

Tables1 and Table2 show the clinical and demographic characteristics of the studied participants. The tables further break down the age distribution into three categories: < 20 years (16%), 20 – 29 years (55%), and ≥ 30 years (29%). The mean BMI increases with age, from 29.2 in the < 20 years group to 30.7 in the > 30 years group. The mean ± SD of BMI and marriage duration of the patients are 29.7±4.9, and 6.0±3.5years, respectively. The studied patients have undergone 0.6 abortions and have 0.8 children. Table 1 presents ultrasound measurements, including follicle size and number on U/S Day 2 and endometrial thickness on Day 2 and Day 12. Hormone levels, including FSH, Estrogen, and AMH, are also included in the tables. Table 2 provides insights into how various study variables vary across different age groups of the patients. However, the differences are not significant statistically ($p > 0.05$). There is a considerable variation ($p = 0.001$) in the duration of marriage across the age of the study groups. The frequency of abortions across age groups is relatively similar across all age classes ($p > 0.05$). The mean number of children increases significantly with age, from 0.2 in the < 20 years group to 1.1 in

the > 30 years group (p = 0.002). The mean follicle size and number on U/S at Day 2 across age groups are similar (p > 0.05). The mean endometrial thickness on the 2nd and 12th day of the MC across age groups remains relatively consistent (p > 0.05), for both measurements.

Table 1: Basal Demographic Features of The Studied Patients

Variables	Mean	Std. Deviation
Age	26.3	5.1
< 20 y	18.7	0.8
20 – 29 y	25.2	2.5
> 30 y	32.6	1.9
BMI	29.7	4.9
Duration of Marriage	6.0	3.5
No. of abortion	0.6	1.1
No. of children	0.8	0.9
Follicle size and no. on U/S Day 2	3.9	1.2
Endometrial thickness Day 2	3.9	5.1
Follicle size and no. on U/S Day 12	17.9	4.1
Endometrial thickness Day 12	7.8	0.5
FSH (mlU/mL)	7.2	3.1
Estrogen (pg/mL)	106.5	186.0
AMH (ng/mL)	4.5	2.9

Table2: Variations in study variables according to patient age groups: < 20 years (n = 16), 20–29 years (n = 55), and > 30 years (n = 29)

Variables	Age/year	Mean	SD	P value
BMI	< 20	29.2	4.3	> 0.05
	20 – 29	29.4	5.0	
	> 30	30.7	5.0	
Duration of Marriage	< 20	3.3	1.9	0.001
	20 – 29	6.0	3.1	
	> 30	7.4	4.0	
No. of abortion	< 20	0.4	0.8	> 0.05
	20 – 29	0.5	1.1	
	> 30	0.9	1.2	
No. of children	< 20	0.2	0.4	0.002
	20 – 29	0.7	0.8	
	> 30	1.1	1.1	
Follicle size and no. on U/S Day 2	< 20 y	4.0	1.1	> 0.05
	20 – 29 y	4.0	1.2	
	> 30 y	3.9	1.3	
Endometrial thickness Day 2	< 20 y	3.9	0.8	> 0.05
	20 – 29 y	7.9	0.7	
	> 30 y	3.7	0.7	
Follicle size and no. on U/S Day 12	< 20 y	18.9	4.0	> 0.05
	20 – 29 y	17.5	4.3	
	> 30 y	18.0	3.7	
Endometrial thickness Day 12	< 20 y	7.7	0.7	> 0.05
	20 – 29 y	7.9	0.3	
	> 30 y	7.7	0.6	

Table3 provides ultrasound findings regarding follicle size and mean endometrial thickness on the 2nd and 12th day of the MC. The data shows a substantial and statistically significant increase in follicle size from Day 2 to Day 12. The combined low p-values (0.001) for both the increase in follicle size and endometrial thickness reinforce the reliability of these changes. This rules out the likelihood that these results occurred by chance.

Table3: Ultrasound Findings of the Follicle Size and Endometrial Thickness on the 2nd and 12th Day of the Menstrual Cycle

Variable	Days of the menstruation	Mean	SD	P value
Follicle Size	Day 2	3.9	1.2	0.001
	Day12	17.8	5.1	
Endometrial Thickness	Day 2	3.9	0.8	0.001
	Day12	7.8	0.5	

Table4 presents hormonal findings of FSH and Estrogen at days 2 and 12 of the menstrual cycle. It indicates significant variations in estrogen levels between Day 2 and Day 12 of the menstrual cycle (p= 0.001), while FSH levels show no significant differences (p > 0.05).

Table 4: Hormonal Findings of FSH, and E2 at a Thickness on the 2nd And 12th Day of the Menstrual Cycle

Variable	Days of the menstruation	Mean	SD	P value
FSH	Day 2	7.1	2.6	> 0.05
	Day12	7.4	3.5	
E2	Day 2	41.4	31.4	0.001
	Day12	170.0	246.1	

Table5 presents the variation in study parameters according to the regularity of the menstrual cycle. It indicates that while there are non-significant alterations in age, BMI, and most hormonal parameters between women with irregular and regular menstrual cycles, there is a significant statistical alteration in the duration of marriage, with females experiencing irregular cycles having a shorter mean duration of marriage.

Table5: Variation in the study parameters according to the regularity of the menstrual cycle, [Regular cycle (N=61) and irregular cycle (N=39)]

Variables	Group	Mean	SD	P value
Age	Irregular	25.3	5.2	> 0.05
	Regular	27	5.0	
BMI	Irregular	29.5	4.8	> 0.05
	Regular	29.9	4.9	
Duration of Marriage	Irregular	4.9	3.2	0.03
	Regular	6.7	3.6	
No of Abortions	Irregular	0.5	1.1	> 0.05
	Regular	0.1	1.1	
No of Children	Irregular	0.6	0.8	> 0.05
	Regular	0.9	0.9	
FSH	Irregular	6.9	2.5	> 0.05
	Regular	7.2	2.7	
E2	Irregular	40.9	37.9	> 0.05
	Regular	41.6	26.9	

Numerous substantial correlations between the study variables are revealed by Spearman's correlation analysis Table6, offering insights into the interactions between various factors related to fertility and reproductive health.

Table 6: Spearman's Correlations Evaluations of the Study Variables Among Each Other

Variable		Age	Endometrial Thickness	BMI	Follicle Size	FSH	E2	AMH
Age	*R*	—						
	P	—						
Endometrial Thickness	*R*	-0.11	—					
	P	0.37	—					
BMI	*R*	0.26*	-0.16	—				
	P	0.02	0.17	—				
Follice Size	*R*	-0.03	0.898***	-0.04	—			
	P	0.83	< .001	0.72	—			
FSH	*R*	-0.08	-0.08	-0.05	-0.16	—		
	P	0.51	0.31	0.67	0.06	—		
E2	*R*	0.19	0.350**	0.09	0.36***	0.32***	—	
	P	0.10	0.001	0.46	< .001	< .001	—	
AMH	*R*	-0.21	0.06	-0.08	-0.10	-0.25*	0.17	—
	P	0.07	0.59	0.51	0.39	0.03	0.15	—
* p < .05, ** p < .01, *** p < .001								

The ability of endometrial thickness, follicle size, FSH, and estrogen to discriminate between hormonal and ultrasonic changes on days 2 and 12 of the MC following letrozole is displayed in Table7 and Fig.1 and Fig.2; along with the corresponding 95% confidence intervals (CI), specificity, sensitivity, and area under the curve (AUC). Endometrial thickness and follicle size are highly effective markers for distinguishing changes, making them valuable for monitoring menstrual cycle-related changes after letrozole administration. Estrogen has some utility but is less effective, while FSH is not a reliable marker for monitoring these changes, than endometrial thickness and follicle size.

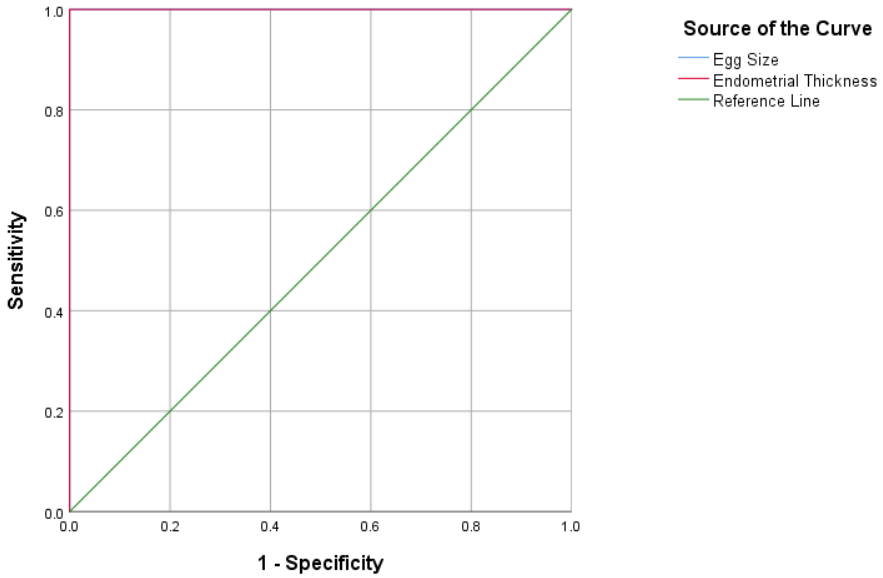

Figure1: ROC Curve Analysis of Egg Size And Endometrial Thickness as Predictors of Clinical Outcome.

The ROC curves compare the diagnostic performance of egg size (blue line) and endometrial thickness (red line), with the green diagonal representing the reference line (no discrimination). Endometrial thickness shows a perfect sensitivity and specificity (AUC = 1.0), indicating it may be an excellent predictor in this context, while egg size overlaps the same curve line, suggesting similar performance in this analysis.

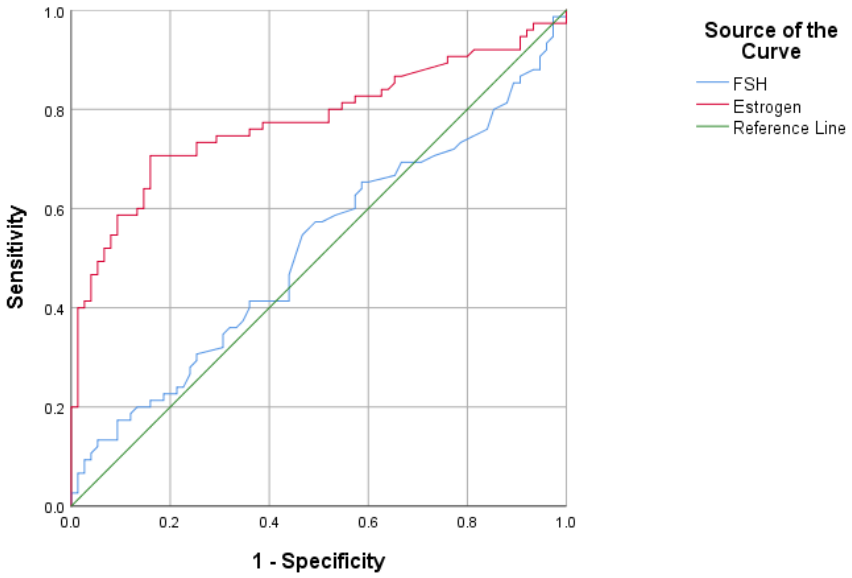

Figure2: Roc Curve Analysis of Follicle-Stimulating Hormone (**Fsh**) and Estrogen as Predictive Markers The ROC curves illustrate the diagnostic performance of FSH (blue line) and estrogen (red line) in relation to the studied outcome. The green diagonal represents the reference line indicating no discriminative ability (AUC = 0.5). Estrogen demonstrates superior diagnostic performance with higher sensitivity and specificity compared to FSH, suggesting a stronger predictive value in this clinical context.

Table 7: Ability of Endometrial thickness, follicle size, **FSH**, and Estrogen to distinguish between hormonal and ultrasonic changes on the 2nd and 12th day of the menstrual cycle

Variables	AUC	P-value	Sensitivity	Specificity	95% Confidence Interval	
Endometrial thickness	1.0	0.001	1.00	1.00	1.00	1.00
Follice size	1.0	0.001	1.00	1.00	1.00	1.00
FSH	0.515	0.752	0.578	0.508	0.422	0.608
Estrogen	0.777	0.001	0.613	0.514	0.699	0.854

4. Discussion

Letrozole therapy resulted in a marked rise in ovulation rates and endometrial receptivity, indicating a considerable gain in ovulatory and uterine activity. The current study was intended to find out the extent to which the aromatase inhibitor letrozole operates as a medication to treat infertility. Our results add to the growing volume of data that supports letrozole should be administered as a first-line treatment for infertility. (Yang et al., 2021, Chen et al., 2024, Franik et al., 2022). The study evaluated the effectiveness of letrozole to stimulate ovulation and improve fertility in women who have ovulation-related infertility. How letrozole works is it inhibits the enzyme aromatase which is responsible for synthesizing estrogen. This inhibition destroys the HPG axis' negative feedback loop. Therefore, estrogen mainly played the role of suppressing FSH in the regulation of the anterior

pituitary. Elevated FSH concentrations stimulate follicle growth and maturation, which eventually results in ovulation. (Franik et al., 2022, Eskew et al., 2019).

The authors reported that this effect was regulated simultaneously with the control of hormonal indicators such as estradiol and FSH. The hormonal oscillations regulate the complex mechanisms of growth and development of the follicles, ovulation, and the steroidogenic process finally yielding the corpus luteum, which are crucial actions for the setting up of a successful pregnancy. (Duncan, 2021, Przygrodzka et al., 2021, Reed BG).

The mean ±SD of the follicle size and endometrial thickness on Day 12 had significantly increased compared to Day 2 of the menstrual cycle after administration of letrozole. This can be explained by the fact that letrozole inhibits aromatase, which increases intra-ovarian androgen levels, especially in the early antral follicles (6-8mm) that are present on Day 3 or Day 5 of the cycle (McGrail et al., 2020). These higher androgen levels in the early follicles promote granulosa cell mitosis, increase FSH receptors, and make the follicles more resistant to atresia. This enhances follicular development and ovulation (Rose and Brown, 2020). The higher estrogen levels resulting from the increased follicular development with early letrozole initiation lead to better endometrial thickening and maturation (Franik et al., 2022). Specifically, the search results indicate the median endometrial thickness was significantly greater when letrozole was started on Day 5 compared to Day 3 (9.0mm vs 8.0mm). The improved endometrial response with early letrozole initiation may contribute to the higher conception and clinical pregnancy rates observed (Sakar and Oglak, 2020).

Proliferative thickening increased following the HRT - a rubber stamp on the fact that epithelial proliferation and reaction to hormone stimulation are taken for granted. These results may suggest a novel role for letrozole concerning improving endometrial cohesion and creating an environment that is ideal for the embryo attachment-implantation signal, and ultimately, conception (15,16).

The search results revealed no studies have compared the effect of oral letrozole starting on the second day of the menstrual cycle for two successive cycles with day 12^{th} of the second cycle regarding follicle size or endometrial thickness. However, data about the effects of letrozole on the endometrial thickness are still controversial. If letrozole was started on the fifth day of the menstrual cycle, compared to the third day of the menstrual cycle, there were greater rates of ovulation, endometrial thickness, pregnancy, and clinical pregnancy in PCOS women documented earlier. (Roy et al., 2012, Shi et al., 2022). In contrast, a study on the effect of letrozole on endometrial thickness in IVF cycles indicated that using letrozole during stimulation reduced the endometrial thickness by 0.81mm. In comparison to patients who did not get letrozole during their initial IVF cycles, those who received it had a thinner endometrium on the trigger day. The study showed that letrozole thins the endometrium, but it is still unclear how letrozole affects endometrial function. This underscores the need for more research before letrozole is prescribed for new transfers. (Ruiter-Ligeti et al., 2021).

There is a significant statistical alteration ($p = 0.001$) in the duration of marriage between age classes. Similarly, while the search results fail to demonstrate a significant difference in the duration of marriage between age groups in couples experiencing infertility due to PCOS, they do indicate that PCOS can significantly affect a couple's satisfaction with their marriage and sexual life, as well as their connections with friends and family (Navid et al., 2018, De Frène et al., 2014).

There were no significant differences in age, BMI, and most hormonal parameters between women with irregular and regular menstrual cycles. However, there is a significant difference in the duration of marriage, with women

experiencing irregular cycles having a shorter mean duration of marriage. The duration of marriage has no direct effect on PCOS women's irregular menstrual periods. Nevertheless, irregular periods after marriage can be caused by a variety of circumstances, including stress, changes in practice, weight variations, hormonal birth control, and pregnancy. The condition itself of PCOS can cause irregular menstrual cycles in women, which can impact menstrual regularity and reproductive health. (Dason et al., 2024). To address any primary worries and to obtain the proper management, females with irregular periods after marriage must visit health care specialists. (Navid et al., 2018).

Spearman's correlational investigation exposed substantial associations between different study variables, presenting clear insights into the relationship between factors affecting reproductive women's health. Nonetheless, the cautious clarification is rational to account for possible confusing confounders that may affect the associations.

5. Conclusion

The data shows that letrozole oral administration on the 2nd day of the menstruation, significantly enhances the growth of follicle and endometrium. This proposes that letrozole efficiently excites follicular development, promoting the growth of the dominant follicle(s). Additionally, letrozole boosts endometrial receptivity by stimulating endometrial maturation. These outcomes validate letrozole's worth in enhancing the main reproductive factors needed for fruitful ovulation and potential implantation in infertile females with PCOS. Moreover, the high specificity and sensitivity measures for follicular size and endometrial width highlight their ability as markers of the ultrasonic and hormonal variations induced by letrozole. Hence, these results support the latent role of letrozole among women suffering from PCOS in improving reproductive outcomes.

6. Ethical Approval

This study was approved by the Ethics Committee of the College of Pharmacy/University of Karbala on August 2, 2023. (Ref: 2023HU2). All patients provided informed consent before being enrolled in the study. The study was performed in accordance with the Declaration of Helsinki.

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Prevalence and Antimicrobial Resistance Patterns of *Escherichia Coli* in Pediatric Urinary Tract Infections in Karbala, Iraq: A Four-Year Retrospective Study

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Abstract

One of the primary microorganisms responsible for urinary tract infections (UTIs) is Uropathogenic *Escherichia coli* (UPEC). Recent years have shown an increase in the prevalence of multidrug-resistant bacteria and UPEC with high antibiotic resistance, which could make treatment more challenging. The study intended to determine the prevalence and antibiotic resistance pattern of E. coli from suspected pediatric urinary tract infections in Karbela, Iraq. A retrospective cross-sectional study was conducted at Department of Microbiology Laboratory at Karbala Teaching Hospital for Children, Iraq, from August 2023 to February 2024.Data for antimicrobial susceptibility test results collect from 409 pediatric patients among 2020 to 2023. Data were analyzed using excel software. The result show females had a higher prevalence of UTIs compared to males across all study years. The prevalence of UPEC isolates varied seasonally, with peaks occurring in different months for different years. Overall, resistance rates to many antibiotics were high, particularly Nalidixic acid, Ciprofloxacin, Levofloxacin, and Ceftriaxone. While some antibiotics showed slight variations in resistance between males and females. Amikacin and Nitrofurantoin demonstrated higher effectiveness against UPEC. The proportion of UPEC isolates varied by age group, with the 1–5-year age group showing the highest prevalence. The present study concluded high prevalence of UPEC with Multidrug-resistant (MDR) isolated from urinary tract infection in Karbala city, Iraq.

أنماط انتشار ومقاومة مضادات الميكروبات لبكتيريا الإشريكية القولونية في التهابات المسالك البولية عند الأطفال في كربلاء، العراق: دراسة استرجاعية لمدة أربع سنوات.

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الخلاصة

أحد الكائنات الحية الدقيقة الأساسية المسؤولة عن التهابات المسالك البولية هي الإشريشية القولونية المسببة للأخماج البولية .أظهرت السنوات الأخيرة زيادة في انتشار سلالات البكتيريا المقاومة للمضادات المتعددة و UPEC مع مقاومة عالية للمضادات الحيوية، مما قد يجعل العلاج أكثر صعوبة. تهدف الدراسة إلى تحديد انتشار ونمط مقاومة المضادات الحيوية للإشريشية القولونية من التهابات المسالك البولية المشتبه بها عند الأطفال في كربلاء بالعراق. أجريت دراسة مقطعية بأثر رجعي في مختبر الأحياء المجهرية / مستشفى كربلاء التعليمي للأطفال بالعراق، من أغسطس 2023 إلى فبراير 2024. تم جمع بيانات نتائج اختبار حساسية مضادات الميكروبات من 409 مريض أطفال بين عامي 2020 و2023. تم تحليل البيانات باستخدام برنامجExcel . تظهر النتيجة أن الإناث لديهن انتشار أعلى لالتهابات المسالك البولية مقارنة بالذكور في جميع سنوات الدراسة. تباين انتشار عزلات UPEC موسميًا، مع حدوث الذروة في أشهر مختلفة لسنوات مختلفة. بشكل عام، كانت معدلات المقاومة للعديد من المضادات الحيوية مرتفعة، وخاصة حمض الناليديكسيك، والسيبروفلوكساسين، والليفوفلوكساسين، والسيفترياكسون. في حين أظهرت بعض المضادات الحيوية اختلافات طفيفة في المقاومة بين الذكور والإناث. أظهر أميكاسين ونتروفورانتوين فعالية أعلى ضد UPEC تباينت نسبة عزلات UPEC حسب الفئة العمرية، حيث أظهرت الفئة العمرية من 1 إلى 5 سنوات أعلى معدل انتشار. وخلصت الدراسة الحالية إلى ارتفاع معدل انتشار UPEC مع البكتيريا المقاومة للأدوية المتعددة (MDR) المعزولة من عدوى المسالك البولية في مدينة كربلاء بالعراق.

1. Introduction

Children frequently experience urinary tract infections (UTIs). Up to 7% of children will have had a UTI by when they are 19 years old (Delbet *et al*., 2017). UTI prevalence may be influenced by a variety of variables, including age, sexes, immunosuppression, and urological equipment (Iqbal *et al*., 2010). Pediatric UTI in many instances, remain under-diagnosed because of the absence of specific symptoms and signs, particularly in infants and young children (Desai *et al*., 2016). The various regions and populations studied showed significant differences in the epidemiology, species distribution, and susceptibility patterns of uropathogen) Behzadi *et al*., 2021 (. *Escherichia coli* is the primary cause behind UTIs, and the patient's own feces serve as a reservoir for the pathogen (Nielsen *et al*., 2014).Numerous virulence factors, particularly those related to long-term survival in the urinary tract, are carried by Uropathogenic Escherichia coli (UPEC). However, little research has been done on their frequency or function among UPEC that causes pediatric UTIs (Ramos *et al*., 2011). Antimicrobial resistance and widespread antibiotic usage appear to be significantly correlated, according to the available data. Thus, prescribing and using antibiotics appropriately can lessen the disease burden of UTIs, which in turn will reduce the difficulties and expenses associated with them(Foxman, 2010). This study aimed to determine the prevalence of UPEC isolated from pediatric patients and analyze the antimicrobial susceptibility patterns (AST) results of these isolates to evaluate their multidrug resistance (MDR) in Karbala City, Iraq

2. Patients and Methodology

A retrospective cross-sectional study was conducted over a six-month period from August 2023 to February 2024. Data were collected from the medical records of patients referred to the Microbiology Laboratory of Karbala Teaching Hospital for Children, Iraq. A total of 409 urine samples data results confirmed to contain UPEC were obtained from patients of all ages and both sexes diagnosed with suspected urinary tract infections (UTIs) among 2020 and 2023. Patients were categorized into four age groups: under 1 year, 1 to 5 years, 6 to 10 years, and 11 to 16 years. Collected Antimicrobial susceptibility testing result was performed on locally available antibiotics using the disk diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines as routine work. The following antibiotics were included in the susceptibility testing of the isolate samples; ampicillin (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), nitrofurantoin (300 µg), nalidixic acid (30 µg), cefixime (5 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), trimethoprim-sulfamethoxazole (TMP-SMX) (5/250 µg), amikacin (30 µg), levofloxacin (5 µg), Chloramphenicol (30µg) and amoxicillin-clavulanic acid. Data Analysis by Microsoft Excel 2016 was used for the statistical analysis of our results.

3. Results

A total of 409 urine samples result from AST were collected from suspected UTI patients. The sex and age group distribution of patients from whom the urine samples were collected is shown in Fig.1., which the data appears to be a count of the number of patients suspected with UTI by sex and year. The ratio of female: male ,1.76 :1, 2.10:1 ,2.12:1 and 2.56:1 for 2020 to 2023 respectively with rang 2.17:1 for four years. Females had a higher number of UTIs than males across all four years. 2023 had the highest number of UPEC diagnoses (139) and 2020 had the lowest (83).

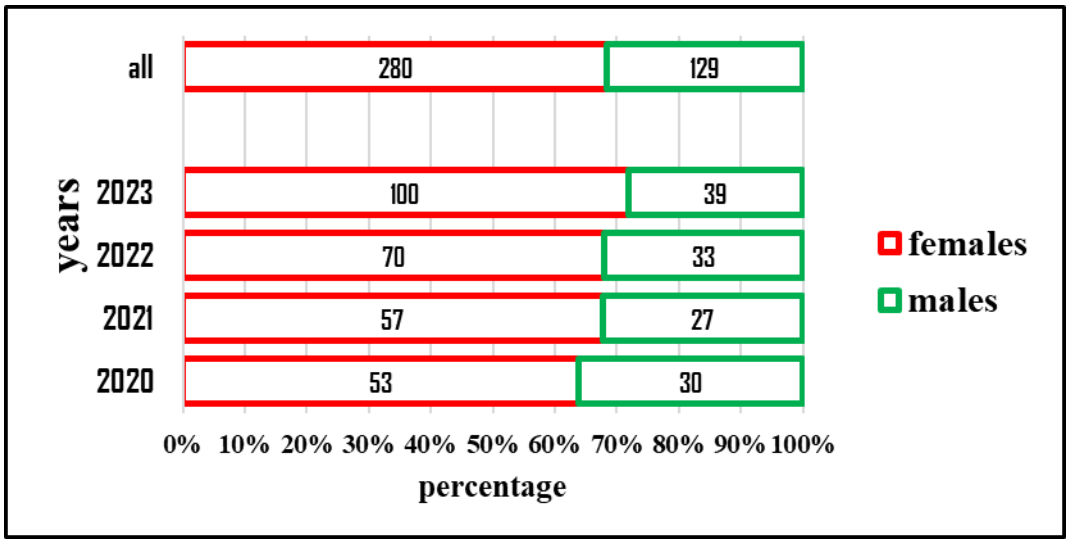

Figure1: Gender Distribution of Study Participants Across Years 2020 To 2023. The horizontal bar graph displays the number and percentage of female (red bars) and male (green bars) participants per year and in total (top row). Females consistently outnumbered males in each year, with the highest participation recorded in 2023. The total number of participants across all years was 409, comprising 280 females and 129 males.

The Table1 show age group <1 year proportion of isolates UPEC decreased over the years, from 34% in 2020 to 12% in 2023 ,1-5 years group had the highest overall percentage, with a peak in 2021 (42%), 6 –10 years percentage increased steadily, reaching 37% in 2023 and 11-16-year group maintained a relatively stable proportion, with a slight increase in 2023.

Table1: Prevalence of UPEC Isolates Among Age Group Pediatric Patients Through the Four Years.

AGE GROUP	2020 % (N)	2021 % (N)	2022 % (N)	2023 % (N)	TOTAL % (N)
< 1	34 (28)	19 (16)	19 (19)	12 (16)	19 (79)
1--5	23 (19)	42 (35)	35 (36)	29 (40)	32 (130)
6--10	25 (21)	26 (22)	29 (30)	37 (52)	31 (125)
11--16	18 (15)	13 (11)	17 (18)	22 (31)	18 (75)
TOTAL	100 (83)	100 (84)	100 (103)	100(139)	100 (409)

The Table2 show group under 1-year higher proportion of UPEC isolates in males (59%). Group 1-5 years and 6-10 years show significantly higher proportion in females (78% and 82% respectively)11-16 years females continue to have a higher proportion, but the gap narrows, (60% female vs. 40% male). Overall, the females account for the majority of UPEC isolates (68%). These trends indicate a higher prevalence of UTIs in females, particularly in the 1–10-year age groups, aligning with known anatomical and physiological susceptibilities.

Table2: Prevalence of UPEC Isolates According Sex and Age Group

Sex	Age group				
	< 1	1 - 5	6 - 10	11 - 16	Total
Female	41 (32)	78 (101)	82 (102)	60 (45)	68 (280)
Male	59 (47)	98 (29)	18 (23)	40 (30)	32 (129)
Total	100 (79)	100 (130)	100 (125)	100 (75)	100 (409)

Table3 presents the monthly prevalence of UPEC isolates. In 2020, peak distributions were observed in February 44% and December 31%. Conversely, 2022 exhibited high distributions in January39% and September 37%, with no cases recorded in July and August. The year 2022 also saw peak distributions in July 42% and December 44%. In contrast, 2023 demonstrated the highest prevalence in August 59%, maintaining a relatively consistent distribution throughout the year, except for December. Overall, 2023 exhibited a more consistent monthly spread of UPEC isolates compared to the previous years.

Table3: Monthly Prevalence of UPEC Isolates with Percentages and Frequency Over Four Years

Year	Jan % (N)	Feb % (N)	Mar % (N)	Apr % (N)	May % (N)	Jun % (N)	Jul % (N)	Aug % (N)	Sep % (N)	Oct % (N)	Nov % (N)	Dec % (N)	TOTAL % (N)
2020	15 (6)	44 (20)	20 (7)	25 (8)	10 (4)	19 (7)	8 (2)	13 (4)	17 (5)	15 (4)	15 (4)	31 (12)	20 (83)
2021	39 (16)	11 (5)	23 (8)	16 (5)	27 (11)	22 (8)	0 (0)	0 (0)	37 (11)	19 (5)	18 (5)	26 (10)	21 (84)
2022	12 (5)	18 (8)	26 (9)	25 (8)	20 (8)	33 (12)	42 (10)	28 (9)	17 (5)	19 (5)	26 (7)	44 (17)	25 (103)
2023	34 (14)	27 (12)	31 (11)	34 (11)	44 (18)	25 (9)	50 (12)	59 (19)	30 (9)	47 (13)	41 (11)	0 (0)	34 (139)
TOTAL	10 (41)	100 (45)	100 (35)	100 (32)	100 (41)	100 (36)	100 (24)	100 (32)	100 (30)	100 (27)	100 (27)	100 (39)	100 (409)

Fig.2 presents antibiotic effectiveness against UPEC for all four years. Amikacin, Nitrofurantoin, and Chloramphenicol demonstrated high efficacy. Ciprofloxacin, Gentamicin, and Levofloxacin exhibited moderate effectiveness. Nalidixic acid, Trimethoprim-Sulfamethoxazole, Cefixime, Ceftriaxone, Ceftazidime, Cefotaxime, Ampicillin, and Amoxicillin-Clavulanate showed low effectiveness. The data indicates that Amikacin, Nitrofurantoin, and Chloramphenicol are the most effective antibiotics against UPEC in this study population.

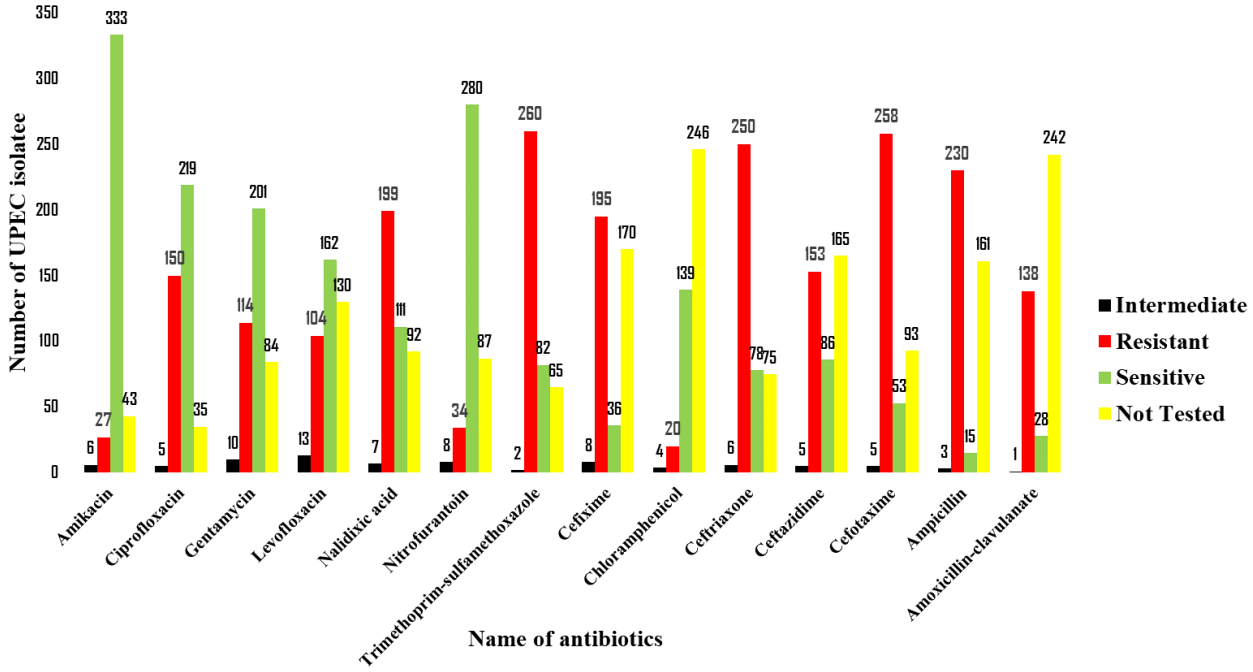

Figure2: Antibiotic Susceptibility Profiles of Uropathogenic *Escherichia Coli* (UPEC) Isolates Against 14 Antibiotics

The bar graph illustrates the number of UPEC isolates classified as sensitive (green), resistant (red), intermediate (black), or not tested (yellow) for each antibiotic. Amikacin showed the highest sensitivity (n = 333), whereas resistance was notably high for cefotaxime (n = 258) and ampicillin (n = 230). Variability in resistance and sensitivity patterns highlights the importance of local antibiograms in guiding effective treatment strategies.

Table4 provided data outlines the antibiotic resistance patterns of UPEC isolated from urine samples for four years (2020-2023). The data is categorized by antibiotic, year, and resistance pattern (R: resistant, S: sensitive, I: intermediate, NT: not tested). The analysis data show highly effective antibiotics: Amikacin, and Nitrofurantoin, moderately effective: Ciprofloxacin, Gentamycin, Levofloxacin and low effectiveness: Nalidixic Acid, Trimethoprim-Sulfamethoxazole, Cefixime, Chloramphenicol, Ceftriaxone, Ceftazidime, Cefotaxime, Ampicillin, and Amoxicillin-Clavulanate.

Table4: Antimicrobial Sensitivity Test Results for Isolated UPEC From Urine Within Four Years

Antibiotics	Pattern	2020 % (n)	2021 % (n)	2022 % (n)	2023 % (n)	TOTAL % (n)
Amikacin	I	1 (1)	1 (1)	1 (1)	2 (3)	1 (6)
	R	10 (8)	1 (1)	11 (11)	5 (7)	7 (27)
	S	85 (71)	62 (52)	84 (87)	89 (123)	81 (333)
	NT	4 (3)	36 (30)	4 (4)	4 (6)	11 (43)
Ciprofloxacin	I	2 (2)	0 (0)	1 (1)	1 (2)	1 (5)
	R	31 (26)	40 (34)	42 (43)	34 (47)	37 (150)
	S	57 (47)	60 (50)	55 (57)	47 (65)	54 (219)
	NT	10 (8)	0 (0)	2 (2)	18 (25)	9 (35)
Gentamycin	I	0 (0)	1 (1)	1 (1)	6 (8)	2 (10)
	R	40 (33)	11 (9)	11 (9)	18 (25)	28 (114)
	S	57 (47)	32 (27)	32 (27)	53 (74)	49 (201)
	NT	3 (3)	56 (47)	56 (47)	23 (32)	21 (84)
Levofloxacin	I	2 (2)	0 (0)	1 (1)	7 (10)	3 (13)
	R	15 (12)	17 (14)	35 (36)	30 (42)	25 (104)
	S	35 (29)	27 (23)	48 (50)	43 (60)	40 (162)
	NT	48 (40)	56 (47)	16 (16)	20 (27)	32 (130)
Nalidixic acid	I	1 (1)	1 (1)	0 (0)	4 (5)	2 (7)
	R	39 (32)	24(20)	65 (67)	57 (80)	49 (199)
	S	30 (25)	15(13)	28 (29)	32 (44)	27 (111)
	NT	30 (25)	50(50)	6 (7)	7 (10)	22 (92)
Nitrofurantoin	I	2 (2)	2 (2)	1 (1)	2 (3)	2 (8)
	R	17 (14)	7 (6)	2 (2)	9 (12)	8 (34)
	S	59 (49)	58 (49)	89 (92)	65 (90)	69 (280)
	NT	22 (18)	33 (27)	8 (8)	24 (34)	21 (87)
Trimethoprim-sulfamethoxazole	I	0 (0)	1 (1)	0 (0)	1 (1)	0 (2)
	R	40 (33)	64 (54)	77 (79)	68 (94)	64 (260)
	S	22 (18)	22 (18)	20 (21)	18 (25)	20 (82)
	NT	39 (32)	13 (11)	3 (3)	14 (19)	16 (65)
Cefixime	I	0 (0)	0 (0)	1 (1)	5 (7)	2 (8)
	R	0 (0)	12 (10)	83 (86)	71 (99)	48 (195)
	S	0 (0)	4 (3)	10 (10)	17 (23)	9 (36)
	NT	100 (83)	85 (71)	6 (6)	7 (10)	41 (170)
Chloramphenicol	I	0 (0)	0 (0)	0 (0)	3 (4)	1 (4)
	R	4 (3)	1 (1)	8 (8)	6 (8)	5 (20)
	S	2 (2)	7 (6)	64 (66)	46 (65)	34 (139)
	NT	94 (78)	92 (77)	28 (29)	45 (62)	60 (246)
Ceftriaxone	I	1 (1)	2 (2)	0 (0)	2 (3)	2 (6)
	R	53 (44)	50 (42)	81 (84)	58 (80)	61 (250)
	S	17 (14)	19 (16)	15 (16)	23 (32)	19 (78)

	NT	**29 (24)**	**29 (24)**	**2 (3)**	**17 (24)**	**18 (75)**
Ceftazidime	**I**	**0 (0)**	**5 (4)**	**1 (1)**	**0 (0)**	**1 (5)**
	R	**13 (11)**	**25 (21)**	**60 (62)**	**42 (59)**	**37 (153)**
	S	**0 (0)**	**11 (9)**	**30 (31)**	**33 (46)**	**21 (86)**
	NT	**86 (72)**	**59 (50)**	**9 (9)**	**25 (34)**	**41 (165)**
Cefotaxime	**I**	**0 (0)**	**2 (2)**	**0 (0)**	**2 (3)**	**1 (5)**
	R	**58 (48)**	**62 (52)**	**82 (85)**	**53 (73)**	**63 (258)**
	S	**11 (9)**	**10 (8)**	**15 (15)**	**15 (21)**	**13 (53)**
	NT	**31 (26)**	**26 (22)**	**3 (3)**	**30 (42)**	**23 (93)**
Ampicillin	**I**	**0 (0)**	**0 (0)**	**0 (0)**	**2 (3)**	**1 (3)**
	R	**0 (0)**	**31 (26)**	**84 (87)**	**84 (117)**	**56 (230)**
	S	**0 (0)**	**5 (4)**	**4 (4)**	**5 (7)**	**4 (15)**
	NT	**100 (83)**	**64 (54)**	**12 (12)**	**9 (12)**	**39 (161)**
Amoxicillin-clavulanate	**I**	**0 (0)**	**0 (0)**	**0 (0)**	**1 (1)**	**0 (1)**
	R	**15 (12)**	**87 (73)**	**27 (28)**	**18 (25)**	**34 (138)**
	S	**0 (0)**	**3 (3)**	**11 (11)**	**10 (14)**	**7 (28)**
	NT	**85 (71)**	**10 (8)**	**62 (64)**	**71 (99)**	**59 (242)**
R: Resistant, S: Sensitive, I: Intermediate, NT: Not Tested, n: number of UPEC						

The Table5 provides information on antibiotic susceptibility tested with UPEC isolated. Data presented in the table, detailing the number and percentage of UPEC isolated from urine samples, be analyzed to compare resistance patterns between males and females for various antibiotics. Most antibiotics show higher sensitivity in females, indicating better efficacy. Males generally exhibit higher resistance across several antibiotics

Table5: Antimicrobial Sensitivity Test Results for Isolated UPEC From Urine Based on Sexes

Antibiotics	**Pattern**	**Female % (n)**	**Male % (n)**	**Total**
Amikacin	**I**	**2 (6)**	**0 (0)**	**1 (6)**
	R	**5 (13)**	**11 (14)**	**7 (27)**
	S	**81 (227)**	**82 (106)**	**81 (333)**
	NT	**12 (34)**	**7 (9)**	**11 (43)**
Ciprofloxacin	**I**	**1 (3)**	**2 (2)**	**1 (5)**
	R	**34 (94)**	**43 (56)**	**37 (150)**
	S	**56 (158)**	**47 (61)**	**54 (219)**
	NT	**9 (25)**	**8 (10)**	**9 (35)**
Gentamycin	**I**	**2 (6)**	**3 (4)**	**2 (10)**
	R	**26 (72)**	**33 (42)**	**28 (114)**
	S	**51 (143)**	**45 (58)**	**49 (201)**
	NT	**21 (59)**	**19 (25)**	**21 (84)**
Levofloxacin	**I**	**3 (8)**	**4 (5)**	**3 (13)**
	R	**25 (71)**	**25 (33)**	**25 (104)**
	S	**45 (125)**	**29 (37)**	**40 (162)**
	NT	**27 (76)**	**42 (54)**	**32 (130)**
Nalidixic acid	**I**	**2 (6)**	**1 (1)**	**2 (7)**
	R	**46 (128)**	**55 (71)**	**49 (199)**
	S	**30 (85)**	**20 (26)**	**27 (111)**
	NT	**22 (61)**	**24 (31)**	**22 (92)**

Nitrofurantoin	**I**	**2 (5)**	**2 (3)**	**2 (8)**
	R	**6 (18)**	**12 (16)**	**8 (34)**
	S	**74 (207)**	**57 (73)**	**68 (280)**
	NT	**18 (50)**	**29 (37)**	**21 (87)**
Trimethoprim-sulfamethoxazole	**I**	**0 (1)**	**1 (1)**	**0 (2)**
	R	**65 (181)**	**61 (79)**	**64 (260)**
	S	**21 (58)**	**19 (24)**	**20 (82)**
	NT	**14 (40)**	**19 (25)**	**16 (65)**
Cefixime	**I**	**2 (5)**	**2 (3)**	**2 (8)**
	R	**48 (135)**	**47 (60)**	**48 (195)**
	S	**10 (27)**	**7 (9)**	**9 (36)**
	NT	**40 (113)**	**44 (57)**	**42 (170)**
Chloramphenicol	**I**	**1 (2)**	**2 (2)**	**1 (4)**
	R	**4 (12)**	**6 (8)**	**5 (20)**
	S	**35 (98)**	**32 (41)**	**34 (139)**
	NT	**60 (168)**	**60 (78)**	**60 (246)**
Ceftriaxone	**I**	**1 (3)**	**2 (3)**	**2 (6)**
	R	**61 (170)**	**62 (80)**	**61 (250)**
	S	**21 (60)**	**14 (18)**	**19 (78)**
	NT	**17 (47)**	**22 (28)**	**18 (75)**
Ceftazidime	**I**	**1 (3)**	**2 (2)**	**1 (5)**
	R	**35 (97)**	**43 (56)**	**38 (153)**
	S	**24 (66)**	**15 (20)**	**21 (86)**
	NT	**41 (114)**	**40 (51)**	**40 (165)**
Cefotaxime	**I**	**1 (3)**	**2 (2)**	**1 (5)**
	R	**62 (174)**	**65 (84)**	**63 (258)**
	S	**15 (41)**	**9 (12)**	**13 (53)**
	NT	**22 (62)**	**24 (31)**	**23 (93)**
Ampicillin	**I**	**0 (1)**	**2 (2)**	**1 (3)**
	R	**57 (159)**	**55 (71)**	**56 (230)**
	S	**4 (11)**	**3 (4)**	**4 (15)**
	NT	**39 (109)**	**40 (52)**	**39 (161)**
Amoxicillin-clavulanate	**I**	**0 (0)**	**1 (1)**	**0 (1)**
	R	**31 (88)**	**38 (50)**	**34 (138)**
	S	**8 (22)**	**5 (6)**	**7 (28)**
	NT	**61 (170)**	**56 (72)**	**59 (242)**
R: Resistant, S: Sensitive, I: Intermediate, NT: Not Tested, n: number of UPEC				

Table6 present data compare antibiotic resistance patterns across different age groups. Older age groups generally show higher sensitivity. Infants (<1 year) often exhibit higher resistance levels. Nitrofurantoin and Amikacin show higher effectiveness across age groups. Trimethoprim-Sulfamethoxazole, Cefixime, Ceftriaxone, and Ampicillin show high resistance across all ages.

Table6: Antimicrobial Sensitivity Test Results for Isolated UPEC From Urine Based on Age Group

Antibiotics	Pattern	Age group				
		< 1 % (n)	1 – 5 % (n)	6 – 10 % (n)	11 – 16 % (n)	Total % (n)
Amikacin	I	0 (0)	0(0)	0 (0)	0 (0)	0(0)
	R	13 (10)	5 (7)	4 (5)	7 (5)	7 (27)
	S	78 (62)	78 (101)	84 (105)	87 (65)	81 (333)
	NT	9 (7)	16 (21)	9 (11)	5 (4)	11 (43)
Ciprofloxacin	I	1 (1)	1 (1)	2 (3)	0 (0)	1 (5)
	R	47 (37)	32 (41)	39 (49)	31 (23)	37 (150)
	S	44 (25)	55 (73)	52 (65)	61 (46)	53(219)
	NT	8(6)	12 (15)	6 (8)	8 (6)	9 (35)
Gentamycin	I	4 (3)	2 (2)	4 (5)	0 (0)	2 (10)
	R	37 (29)	22 (29)	26 (33)	31 (23)	28 (114)
	S	44 (35)	47 (61)	53 (66)	52 (39)	49 (201)
	NT	15 (12)	29 (38)	17(21)	17(13)	21 (84)
Levofloxacin	I	6 (5)	2 (3)	2 (3)	3 (2)	3 (13)
	R	22 (17)	24 (31)	31 (39)	22 (17)	25 (104)
	S	19 (15)	40 (52)	45 (56)	52 (39)	40 (162)
	NT	53 (42)	34 (44)	22 (27)	23 (17)	32 (130)
Nalidixic acid	I	3 (2)	1 (1)	2 (3)	1 (1)	2 (7)
	R	51 (41)	45 (59)	51 (64)	47 (35)	49 (199)
	S	23 (18)	24 (31)	30 (37)	33 (25)	27 (111)
	NT	23 (18)	30 (39)	17 (21)	19 (14)	22 (92)
Nitrofurantoin	I	0 (0)	2 (2)	4 (5)	1 (1)	2 (8)
	R	13 (10)	5 (6)	7 (9)	12 (9)	8 (34)
	S	62 (49)	66 (86)	76 (95)	67 (50)	68 (280)
	NT	25 (20)	27 (36)	12 (16)	20 (15)	21 (87)
Trimethoprim-sulfamethoxazole	I	1 (1)	0 (0)	1 (1)	0 (0)	0.5 (2)
	R	62 (49)	65 (85)	70 (87)	52 (39)	63.5(260)
	S	14 (11)	19 (24)	18 (23)	32 (24)	20 (82)
	NT	23 (18)	16 (21)	11 (14)	16 (12)	16 (65)
Cefixime	I	1 (1)	3 (4)	2 (3)	0 (0)	2 (8)
	R	41 (32)	48 (63)	48 (60)	54(40)	47 (195)
	S	0 (0)	9 (11)	12 (15)	13 (10)	9 (36)
	NT	58 (46)	40 (52)	38 (47)	33 (25)	42 (170)
Chloramphenicol	I	1 (1)	0 (0)	2 (2)	1 (1)	1 (4)
	R	6 (5)	2 (3)	7 (9)	4 (3)	5 (20)
	S	25 (20)	35 (46)	38 (48)	33 (25)	34 (139)
	NT	67 (53)	63 (81)	53 (66)	62 (46)	60 (246)
Ceftriaxone	I	1 (1)	1 (1)	2 (3)	1 (1)	2 (6)
	R	72 (57)	60 (78)	59 (74)	55 (41)	61 (250)
	S	9 (7)	16 (21)	25 (31)	25 (19)	19 (78)
	NT	18 (14)	23 (30)	14 (17)	19 (14)	18 (75)
Ceftazidime	I	0 (0)	1 (1)	1 (1)	4 (3)	1 (5)
	R	46 (36)	32 (42)	41 (51)	33 (24)	37 (153)
	S	5 (4)	19 (25)	28 (35)	29 (22)	21 (86)
	NT	49 (39)	48 (62)	30 (38)	34 (26)	41 (165)
Cefotaxime	I	1 (1)	1 (1)	2 (3)	0 (0)	1 (5)

	R	**72 (57)**	**62 (81)**	**64 (79)**	**55 (41)**	**63 (258)**
	S	**3 (2)**	**9 (12)**	**19 (24)**	**20 (15)**	**13 (53)**
	NT	**24 (19)**	**28 (36)**	**15 (19)**	**25 (19)**	**23 (93)**
Ampicillin	**I**	**1 (1)**	**1 (1)**	**1 (1)**	**0 (0)**	**1 (3)**
	R	**43 (34)**	**55 (71)**	**65 (81)**	**59 (44)**	**56 (230)**
	S	**1 (1)**	**3 (4)**	**3 (4)**	**8 (6)**	**4 (15)**
	NT	**55 (43)**	**41 (54)**	**31 (39)**	**33 (25)**	**39 (161)**
Amoxicillin-clavulanate	**I**	**0 (0)**	**0 (0)**	**0 (0)**	**1 (1)**	**0 (1)**
	R	**38 (30)**	**38 (30)**	**26 (33)**	**33 (25)**	**34 (138)**
	S	**4 (3)**	**4 (3)**	**10 (12)**	**9 (7)**	**7 (28)**
	NT	**58 (46)**	**58 (46)**	**64 (80)**	**56 (42)**	**59 (242)**

Table6 shows the resistance pattern of MDR of UPEC. Among 409 isolates, 96% of isolates were found resistant to at least one antibiotic, and % 80 was found resistant at least to 3 or more classes of antibiotics, thus classified as MDR

Table 6: Isolated UPEC From Urine with Determine MDR According Antimicrobial Sensitivity Test

Number of antibiotics resistant	**2020 UPEC isolate % (N)**	**2021 UPEC isolate % (N)**	**2022 UPEC isolate % (N)**	**2023 UPEC isolate % (N)**	**Total % (N)**
0	**7 (6)**	**2 (2)**	**1 (1)**	**5 (7)**	**4 (16)**
1	**16.5 (14)**	**11.5 (9)**	**1 (1)**	**4 (6)**	**7.25 (30)**
2	**13.5 (12)**	**11.5(9)**	**4 (4)**	**7 (10)**	**9 (35)**
3	**16 (13)**	**15.5 (13)**	**1 (1)**	**11.5 (17)**	**11 (44)**
4	**16 (13)**	**11.5 (9)**	**11 (11)**	**13.5 (19)**	**13 (52)**
5	**16 (13)**	**14 (12)**	**11.5 (12)**	**9 (12)**	**12 (49)**
6	**6 (5)**	**24 (20)**	**9.5 (10)**	**12 (16)**	**12 (51)**
7	**5 (4)**	**4 (4)**	**16.5 (17)**	**6 (8)**	**8 (33)**
8	**4 (3)**	**2 (2)**	**13 (14)**	**10 (14)**	**8 (33)**
9	**0 (0)**	**3 (3)**	**13 (14)**	**10 (14)**	**8 (31)**
10	**0 (0)**	**1 (1)**	**9 (9)**	**9 (12)**	**5 (22)**
11	**0 (0)**	**0 (0)**	**6.5 (7)**	**2 (3)**	**2 (10)**
12	**0 (0)**	**0 (0)**	**1 (1)**	**1 (1)**	**0.5 (2)**
13	**0 (0)**	**0 (0)**	**1 (1)**	**0 (0)**	**0.25 (1)**
TOTAL	**100 (83)**	**100 (84)**	**100 (103)**	**100 (139)**	**100 (409)**

4. Discussion

One important bacterium that can cause potentially catastrophic urinary tract infections is *Escherichia coli*. Consideration of a number of variables, including host determinant, epidemiology, and antibiotic susceptibility, is necessary for accurate characterization of these diseases (Patil *et al*., 2023). In order to treat a UTI, it is essential to identify the organism and determine its susceptibility to antibiotics. It emphasizes how crucial close communication and coordination are between the microbiologist and the clinician (Moue *et al*., 2015). In the current study, the high rate of UPEC [n=280, 68.5%] was identified in female pediatric patients during the four years of the study period with a higher frequency in 2023 [n=100,72%].and this is conduct with Mexican study [n=86,78.2%] (Ramírez-Castillo *et al*., 2018) and Nepal study [n=132,88.3%] (Raya *et al*., 2020) Hormonal differences between males and females may influence susceptibility to infection and subsequent antibiotic resistance (Vasudevan, 2014) .The urinary tract anatomy varies between sexes, potentially affecting the likelihood of infection and the types of bacteria

involved (Minardi *et al*., 2011). In the current study the 1–5-year age group had the highest total percentage of UPEC isolates, indicating a significant burden in this age range. Infants and toddlers with their developing immune systems, are more susceptible to infections and may consequently receive antibiotics more frequently. This increased exposure to antibiotics can elevate the risk of antibiotic resistance (Lee, 2016). One study revealed that over the past ten years, mean temperatures during June, July, August, and September increased by more than 45 degrees Celsius. Conversely, precipitation levels decreased in intensity during the winter and spring, with a decline in monthly mean rainfall (Yehia *et al*., 2023). The study found seasonal variations in the detection of UPEC isolates over the years. While there was a general trend of higher detection rates during colder months in 2020 and 2021, particularly in winter, there were exceptions with peaks occurring in summer months in 2022 and 2023 years and this agree with the findings from the Turkish study (Yolbas *et al*., 2013). The Iraqi study suggests that several factors beyond seasonality may contribute to the increased prevalence of UPEC during summer. These factors include: dietary changes like increased consumption of contaminated food and water and varying hygiene standards in different regions. These factors, in combination, likely create a more favorable environment for the transmission and spread of UPEC (Assafi *et al*., 2022). Overall, There's a general increase in resistance to most antibiotics over the four years, particularly for Ciprofloxacin [n=150,37%] Close to studying in Chile [n=9,22.5%] (Bacigalupo-Gorbea *et al*., 2023), Levofloxacin [n=104,25%] Compatible with an Iraqi study [n=28,38%] (Alfuraiji *et al*., 2022), Nalidixic acid [n=199,49%] not consistent with the Pakistani study [n=56,88%] (Iqbal *et al*., 2021), Trimethoprim-sulfamethoxazole [n=260,64%] It does not correspond to a Roman study [n=212,27%] (Miron *et al*., 2021) , Cefixime [n=195,48%] Relatively close to Iranian study[n=18,35%] (Ghasemi *et al*., 2020) , Ceftriaxone [n=250,61%] not compatible with the Pakistani study [n=118,88.7%] , Ceftazidime [n=153,37%] not appropriate with Turkish study[n=172,15.1%] (Samancı *et al*., 2020), Cefotaxime [n=258,63%] not trend with Vietnam study [n=19,38.8%] (Nguyen *et al*., 2022), and Ampicillin [n=230,56%] Somewhat similar to a Turkish study [n=1932,69.6%] (Samanci and PINARBAŞI, 2023). Amikacin [n=27,7%] does not agree with Indian study [n=23,31%] (Alfuraiji *et al*., 2022) and Nitrofurantoin[n=34,8%] dose not correspond with Pakistani study[n=74,35.3%] (Mir *et al*., 2022) . Chloramphenicol shows a decrease in resistance over time, indicating improved efficacy. Ciprofloxacin, Levofloxacin, Nalidixic acid, Trimethoprim-sulfamethoxazole show High and increasing resistance levels, limiting their effectiveness. Cefixime, Ceftriaxone, Ceftazidime, Cefotaxime, Ampicillin, Amoxicillin-clavulanate show High resistance rates, especially in later years, indicating limited utility. A significant increase in resistance to many antibiotics is observed between 2020 and 2023, highlighting the rapid evolution of bacterial resistance. The results of UPEC isolates based on sexes distribution shows Nitrofurantoin showed the highest susceptibility rates, particularly among females on the other hand high resistance rates were noted for Ciprofloxacin and Nalidixic acid, particularly in males. Overall, resistance and susceptibility patterns varied slightly between males and females, with males generally showing higher resistance rates and this result is conducted with Mexican study (Ramírez-Castillo *et al*., 2018) and Honduras study (Zúniga-Moya *et al*., 2016) The results show older age groups generally higher sensitivity. Infants <1 year often exhibit higher resistance levels. Nitrofurantoin and Amikacin show higher effectiveness across age groups. Trimethoprim-Sulfamethoxazole, Cefixime, Ceftriaxone, and Ampicillin show high resistance across all ages. Based on the search results, there are several key factors that contribute to higher resistance in infants compared to other age groups. The study reveals that the infant gut microbiome harbors a significantly higher abundance of antibiotic resistance genes (ARGs) compared to the adult gut microbiome. This increased abundance is closely linked to the presence and abundance of Escherichia coli, a major reservoir of ARGs. The infant gut microbiome undergoes substantial changes during the first year of life, influencing the overall abundance of ARGs. Notably, the study found a significantly higher relative abundance of ARGs at six weeks of age compared to one year of age. Many of the differentially abundant ARGs are associated with antibiotic efflux mechanisms, suggesting their role in conferring resistance to antibiotics (Lebeaux *et al*., 2021). Infants, with their immature immune systems, are more susceptible to infections compared to older age groups. This increased susceptibility often necessitates antibiotic use, potentially contributing to higher rates of antibiotic resistance development (Desai and Macrae, 2020). Among 409 isolate 96% of the isolates were resistant at least one antimicrobial agent, and 328 (80%) isolates were resistant at least 3 antimicrobials. MDR was defined as resistance to at least three antimicrobial classes as suggested by (Magiorakos *et al*., 2012). The current study shows more than

70% (n=287) of isolates detect MDR compared with Iraqi study (n=38,88%) (Al-Hasnawy *et al*., 2019) Bangladesh study (n= 416,96%) (Nobel *et al*., 2021) Taiwan study (n=58,37%) (Huang *et al*., 2018), South Kerala, Indian study (n=20,24%) (Jitendranath *et al*., 2015) Turkish study (n=81,14.5%) Chinese study (n=189,46%) (Huang *et al*., 2022) Russian study (n=31,29%) (Sarra *et al*., 2021) and South-East Gabon (n=59,44%) (Mouanga-Ndzime *et al*., 2023). Escherichia coli bacteria can develop resistance to antibiotics through several mechanisms Mutations in bacterial DNA can lead to resistance by altering target sites for antibiotics (Mazzariol *et al*., 2017) Transfer of resistance genes between bacteria through plasmids, transposons, or integrins (Khadgi *et al*., 2013).UPEC can expel antibiotics using efflux pumps, reducing drug concentration inside the cell (Yasufuku *et al*., 2011).Biofilms protect bacteria from antibiotics, making them harder to eradicate (Mittal *et al*., 2015). Excessive or inappropriate use of antibiotics accelerates resistance development.

5. Conclusion

The prevalence and trends of antibiotic resistance of UPEC in pediatric urinary tract infections (UTIs) were examined in this study. The empirical treatment of urinary tract infections (UTIs) has become more difficult in this region due to the increasing prevalence of multidrug-resistant (MDR) strains of Escherichia coli (E. coli). Continuous monitoring of MDR organisms and their resistance patterns is crucial to avoid treatment failures and limit the emergence of antibiotic resistance. With this knowledge, medical professionals in Iraq will be better equipped to choose the right antibiotics for UTI patients.

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Detoxification of Chlordiazepoxide Using Activated Charcoal Prepared from Olive Seeds

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Abstract

The study concerns the adsorption of chlordiazepoxide on the olive seeds-activated charcoal (OS-AC). It is carried out to investigate the possible use of OS-AC in the management of chlordiazepoxide intoxication. UV-visible spectrophotometry is applied to measure the concentration, and then it is used to study the adsorption isotherm and the factors influencing it, such as contact time, pH, temperature, ionic strength, and adsorbent dose. The results show that the best contact time is 1 min and 0.1 g dose of adsorbent olive seeds - -activated charcoal with no or small change concerning pH. The study of the adsorbent weight shows an increase in adsorption with an increase in olive seeds-activated charcoal weight. The removal of chlordiazepoxide by using olive seeds-activated charcoal is studied at different temperatures (10C°, 25C°and 50C°) to determine the adsorption isotherms and the thermodynamic functions. The experimental isotherm data are analyzed using Freundlich and Langmuir isotherm models. It is found that Langmuir's isotherm model fits the data very well for the drug chlordiazepoxide on olive seeds activated charcoal. According to Gile's classification, the shapes of the isotherms obtained from experimental data are comparable to the (H - Curve) type. Add the meaning of the abbreviation

السيطرة على سمية دواء الكلورديازيبوكسيد باستخدام الفحم المنشط المحضر من بذور الزيتون

زينب عبد الامير السلطان، رجوان عبد الجبار غزاي، سرى لطيف سلمان عبد الباري مهدي ماهود

الخلاصة

تتعلق الدراسة بامتزاز الكلورديازيبوكسيد على بذور الزيتون - الفحم المنشط. وقد أجريت هذه الدراسة للتحقيق في الاستخدام المحتمل لـ OS-AC في السيطرة على التسمم بالكلورديازيبوكسيد. يتم تطبيق مطيافية الأشعة فوق البنفسجية المرئية لقياس التركيز ومن ثم استخدامها لدراسة تساوي درجة حرارة الامتصاص والعوامل المؤثرة عليه مثل وقت التلامس والرقم الهيدروجيني ودرجة الحرارة والقوة الأيونية وجرعة المادة المازة. تظهر النتائج أن أفضل وقت تلامس هو 1 دقيقة وجرعة 0.1 جرام من المادة المازة لبذور الزيتون - الفحم المنشط مع عدم وجود تغيير أو تغيير بسيط للغاية فيما يتعلق بالرقم الهيدروجيني. تظهر دراسة وزن المادة المازة زيادة في الامتصاص مع زيادة وزن بذور الزيتون - الفحم المنشط. تتم دراسة إزالة الكلورديازيبوكسيد باستخدام بذور الزيتون - الفحم المنشط عند درجات حرارة مختلفة (10 درجة مئوية و25 درجة مئوية و50 درجة مئوية) لتحديد تساوي درجة حرارة الامتصاص والوظائف الديناميكية الحرارية. تم تحليل بيانات معادلة الحرارة التجريبية باستخدام نماذج معادلة الحرارة فراندلش ولانكَماير. وقد وجد أن نموذج معادلة الحرارة لانكَماير يناسب البيانات بشكل جيد للغاية لعقار الكلورديازيبوكسيد على الفحم المنشط لبذور الزيتون. وقد وجد أن أشكال معادلة الحرارة التي تم الحصول عليها من البيانات التجريبية قابلة للمقارنة بنوع (H-Curve) وفقًا لتصنيف جيلز.

1. Introduction

Chlordiazepoxide (CDPX) is 7-chloro-4-hydroxy-N-methyl-5-phenyl-3H1,4-benzodiazepine-2-imine Fig.1, having the molecular formula of $C_{16}H_{14}ClN_3O$ and a molecular weight of 299.758 g/mol, sold under the brand names of (Librium, Librax, Limbitrol) (Ford, 2015; Kang et al., 2011; M. Beale, 2011).

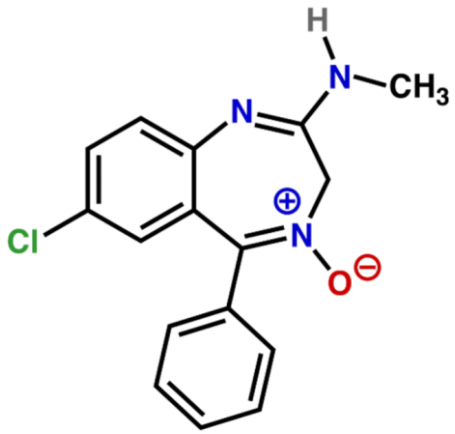

Figure1: Shows the Structure of Chlordiazepoxide

CDPX is used as an anticonvulsant and to treat symptoms of anxiety, another use is the treatment of irritable bowel syndrome (IBS) due to its muscular relaxing activity. The drug chlordiazepoxide (CDPX) has a complex metabolic pathway because it is biotransformed into several active metabolites (desmethylchlordiazepoxide, demoxepam, desmethyldiazepam, and oxazepam). Chlordiazepoxide (CDPX) clearance is reduced in the elderly and those with hepatic cirrhosis (Goodman & Gilman, 1966; Khonsary, 2023; Thompson Coon, 2002). Chlordiazepoxide is considered a safe drug because it is very rare to cause fatal effects when it is taken alone. However, in case of overdose, toxic effects appear like muscle weakness, CNS depression, ataxia and may cause respiratory depression, coma, which last for 24h, hypothermia and hypotension may occur. Chlordiazepoxide toxicity is increased if it is used in combination with other CNS-related drugs like tricyclic antidepressants or alcohol. In this study, the olive seeds activated charcoal is used to detoxification of Chlordiazepoxide. Detoxification can be defined as many interventions that manage acute intoxication, on the other hand clearing the body from acute accumulation of toxins and reduce the harmful or life-threatening effects that appear if the patient is left untreated (Goodman & Gilman, 1966; Khonsary, 2023; M. Beale, 2011; Thompson Coon, 2002). The toxic substances can be either as a byproduct from the normal human metabolic processes like carbon dioxide or urea and from the environment like pollutants, chemicals, which include drugs, and other harmful substances (M. A. Tadda et al., 2016, 2018). The activated charcoal adsorption process is one of the most commonly used techniques in the removal of trace organic compounds from the aqueous solution**.** One of the important properties of adsorbents is the high surface area to volume ratio and the activated charcoal is considered a good adsorbent for removal of organic compounds due to this ratio ranging from 500-1000 m^2/g (Adeniyi et al., 2023; M. Tadda et al., 2016). Activated charcoal is known as carbonic material with a wide internal surface area which has a highly developed porous structure that resulting from the processing of raw material at the high-temperature reaction. The appearance of activated charcoal is a fine, black powder that has no odor, no taste and it's nontoxic. It's produced through the slow combustion, or addition of acid, or addition of steam to a carbonic material like wood; peat; lignite, etc... The activated charcoal has a higher adsorption surface area due to a large number of pores.

The pores that are formed work through trapping liquid, solid or gaseous toxins and chemicals in GIT which prevent their absorption. These porous surfaces have negative electric charges and give rise to positive charges of chemicals and toxins in order to bond with them. So, they detoxify the system of the body and promote digestive functions by carrying the bounded toxins out of the body through feces (Abuelnoor et al., 2021; M. A. Tadda et al., 2018; Tan et al., 2017).

2. Materials & Methods

2.1. Materials

All the chemicals and instruments were presented in Table1 and Table2

Table1: Material Used in This Study

Materials / Instruments	Supplier/ Manufacture
Chlordiazepoxide	SDI
Hydrochloric acid HCl	HIMEDIA
Activated charcoal (olive seeds)	Prepared in lab
Potassium chloride KCl	HIMEDIA
sodium chloride NaCl	HIMEDIA
Magnesium chloride $MgCl_2$	HIMEDIA
Manganese(ll)chloride $MnCl_2$	HIMEDIA
Sodium hydroxide NaOH	HIMEDIA

Table2: The Instruments Used in This Study

Instruments	Manufacture
UV-1800 SHIMADZU with 1cm matched pair quartz cell and spectral bandwidth of 1nm.	SHIMADZU - Japan
Muffle oven	China
Sensitive balance.	Germany

2.2. Preparation of Olive Seeds Activated Charcoal

Previously collected, cleaned and dried olive seeds are placed in a closed crucible in a muffle oven, which its temperature is raised to 650 C^o and keep there for around 10 minutes. The resultant charcoal is left to be cooled and transferred to a mill to create a fine powder of activated charcoal.

2.3. Preparation of standard solutions

Standard solution of 100 µg/ml chlordiazepoxide is prepared by dissolving 0.010 gm of chlordiazepoxide in a 100 ml volumetric flask with 0.100 N HCl. Further working solutions are prepared by dilution (Ioannidou & Zabaniotou, 2007; Yogin Soodesh et al., 2024).

The solution of 0.100 N HCl is prepared by measuring 2.18 ml of concentrated HCl and completing the volume in a 250 ml volumetric flask to the mark with distilled water. The salts solutions *(0.02, 0.05, 0.07 M)* of NaCl, KCl, $MnCl_2$ and $MgCl_2$ are prepared by dissolving (0.058 - 0.204) g, (0.074 – 0.260) g, (0.120 – 0.440) g and (0.095 – 0.330) g of the compounds respectively in distilled water and the volume is completed to 50 ml in volumetric flasks.

2.4. Equilibrium time of adsorption system

To a series of 25 ml, volumetric flasks (1 ml) of the drug was transferred and completed with 0.1 N HCl to the mark. Olive seeds activated charcoal 0.1 gm are added to solutions and shaken for different times, which are filtered after that. The absorbance of these solutions are measured and recorded (Deng et al., 2011).

2.5. Adsorption isotherm

To determine the adsorption isotherm for the drug with olive seeds activated charcoal, different concentrations ranged from (4 - 8) ppm of CDPX are used and mixed with 0.1 g of adsorbents for 1 min, which are filtered then and their absorbance are measured and recorded.

3. Results & Discussion

3.1. Study of UV - Visible Spectrum

The UV – Visible spectrum of chlordiazepoxide in Fig.2 shows that high absorbance of the drug is at λ_{max} = 245 nm (Lennard, 2004; Sari et al., 2023).

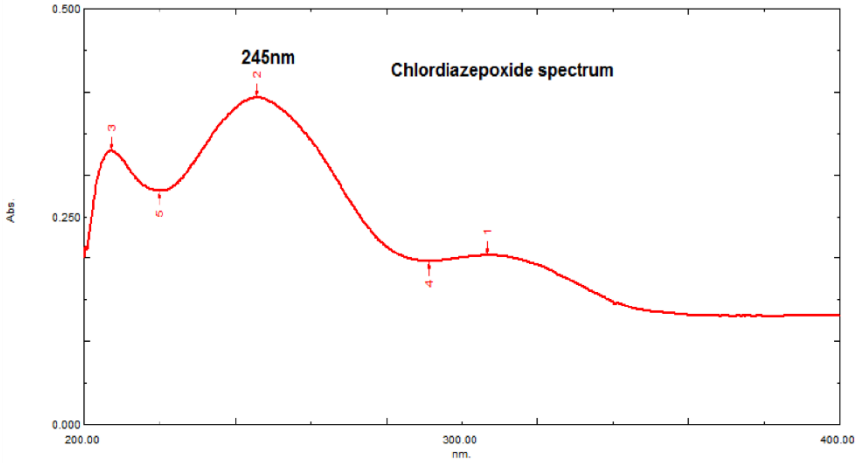

Figure2: UV-Visible Absorption Spectrum of Chlordiazepoxide, showing a prominent absorption peak at 245 nm, indicating the λmax (maximum absorbance wavelength) used for subsequent spectrophotometric measurements. The spectrum was recorded in the range of 200–400 nm using 0.1 N HCl as the solvent medium.

3.2. CDPX Calibration Curve

The calibration curve of CDPX is determined. Fig.3 shows a linear calibration curve at 245 nm in the range (0.5-15) ppm (Hegazy & Kabiel, 2010) .

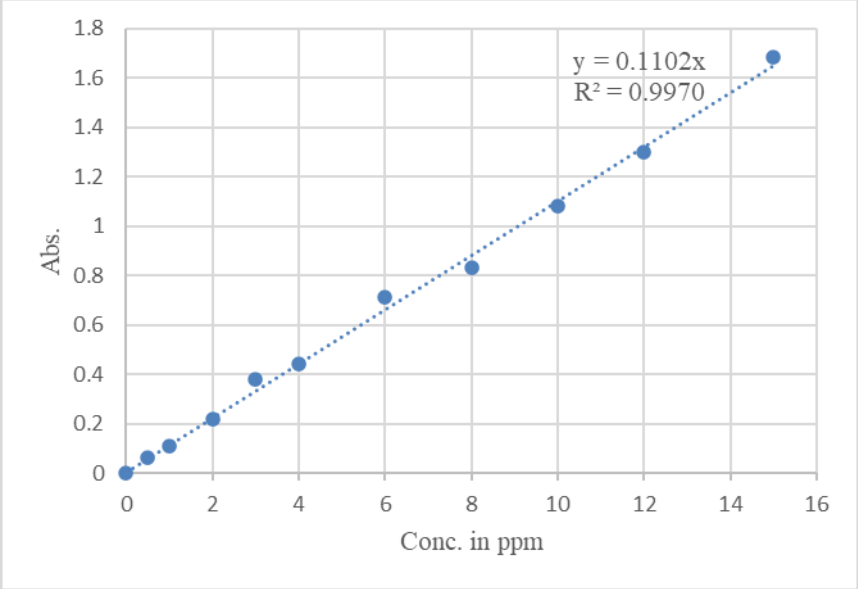

Figure3: Calibration Curve of Chlordiazepoxide Showing the Linear Relationship Between Absorbance and Concentration (Ppm) At 245 Nm. The regression equation is $y = 0.1102x$ with a high correlation coefficient ($R^2 = 0.9970$), indicating excellent linearity within the tested concentration range.

3.3. Effect of Charcoal Weight

The olive seeds activated charcoal (OS –AC) shows increased adsorption (a decrease in absorbance values with increased weight) with an increase in its amount (weight), and this is attributed to an increase in its adsorption capacity (its total effective surface area), with an increase in active sites that lead to increase in removal percentage (Re%) as shown in Fig.4 (Olalekan et al., 2013).

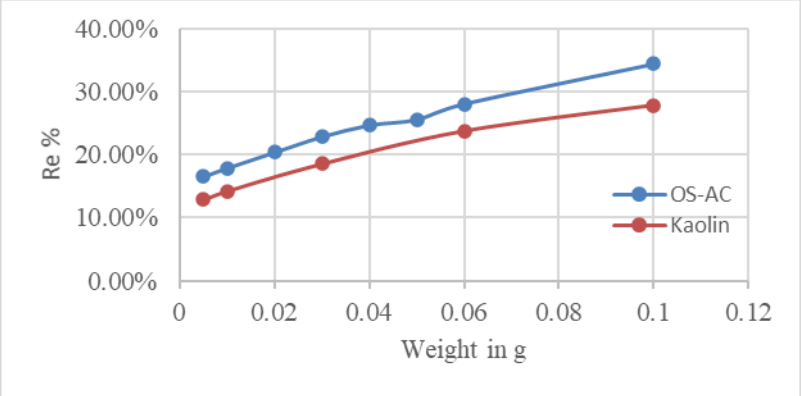

Figure4: Effect of Adsorbent Weight on The Removal Efficiency (Re%) of Chlordiazepoxide Using Olive Seed Activated Charcoal (OS-AC) And Kaolin.

As the weight of adsorbent increases, the removal efficiency also increases for both adsorbents, with OS-AC showing consistently higher performance compared to kaolin.

3.4. Effect of Time

The olive seeds activated charcoal shows the highest adsorption (drug removal) amount rapidly (at the 1 min. mark) which shows that it can be used for rapid drugs detoxification. The decline in the adsorption amount can be attributed to the breakage of weak physical bonds and only the chemical ones remain and observed with the constant removal amount after 13 min (Gale et al., 2021; Hassan et al., 2023).

3.5. Effect of Concentration of the Drug

Table3 refers to 0.1 gm of olive seeds activated charcoal shows the best and highest efficacy at a CDPX concentration of 4 ppm. (2 ppm is not used because it is too low for practical use) (Ehiomogue et al., 2022; Foo & Hameed, 2010).

Table3: Concentration of CDPX with and without OS-AC

Conc. in ppm	Abs. without charcoal	Abs. with charcoal	Re.%
2	0.260	0.119	45 %
4	0.472	0.266	40.65 %
6	0.703	0.531	19.69 %
2	0.840	0.764	14.3 %

3.6. Effect of Temperature

The adsorption amount increases with a decrease in a solution temperature and vice versa, which was expected as the adsorption process is an exothermic one and is improved by lowering the temperature of the solution as presented in Table4.

Table4: Effect of Temperature on Removal Percentage Re.%

Temp. in C°	Conc. in ppm	Abs.	Re.%
10	4	0.204	53.72 %
	6	0.496	24.98 %
	2	0.721	18.21 %
25	4	0.237	46.23 %
	6	0.532	19.54 %
	2	0.761	14.67 %
50	4	0.284	35.57 %
	6	0.559	15.45 %
	2	0.788	10.61 %

3.7. Effect of pH

The olive seeds activated charcoal shows constant adsorption amounts with different pH values ranging from 1 to 8, and this can be considered an excellent property because it means the olive seeds activated charcoal has a predictable and consistent adsorption amount regardless of the solution pH, so the stomach acidity will not affect the removal percent of OS- AC (Babel & Kurniawan, 2003; Ehiomogue et al., 2022; Mishra, 2022).

3.8. Effect Ionic Strength

Table5 refers to various univalent and bivalent salts in table (5) show very low adsorption amounts, which indicates that HCl is the best solution, in addition to the HCl found in the stomach, where the olive seed-activated charcoal is supposed to act (Kumar & Jena, 2016b, 2016a).

Table5: Effect of Ionic Strength in Removal Percentage Re.%

The concentration of salt	The removal percentage (Re.%)			
	NaCl	KCl	$MnCl_2$	$MgCl_2$
8	%9.7	%9.44	%4.4	%9.7
4	%6.384	%2.6	%4.294	%2.289
9	%9	%7.74	%6.44	%5..684

3.9. The Adsorption Isotherms

The experimental isotherms data were analyzed using Freundlich and Langmuir isotherm models as presented in Fig.5. and Fig.6 and Table6. The experimental isotherms data were analyzed by using Freundlich and Langmuir isotherm models. The data in the Table6 assert that Langmuir's isotherm model fits the data very well for the drug (CDPX) on olive seeds activated charcoal. The shapes of the isotherms obtained from experimental data that are found to be comparable to the (H-Curve) type according to Giles classification (Giles et al., 1960; Kumar & Jena, 2016b, 2016a; Mellouk et al., 2009).

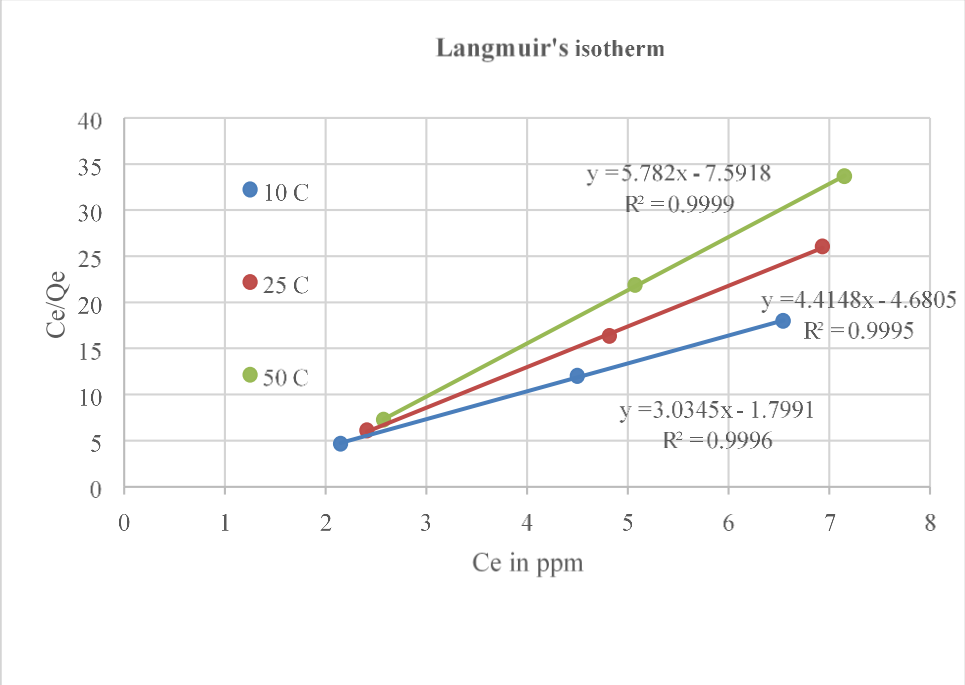

Figure5: Langmuir Isotherm Plots for The Adsorption of Chlordiazepoxide Onto Olive Seed Activated Charcoal (OS-AC) At Different Temperatures (10 °C, 25 °C, And 50 °C).

The linear relationship between Ce/Qe and Ce indicates the applicability of the Langmuir model. Increasing temperature leads to higher Ce/Qe values, suggesting reduced adsorption capacity at elevated temperatures. Each line corresponds to a different temperature with its respective regression equation and R^2 value indicating a good fit.

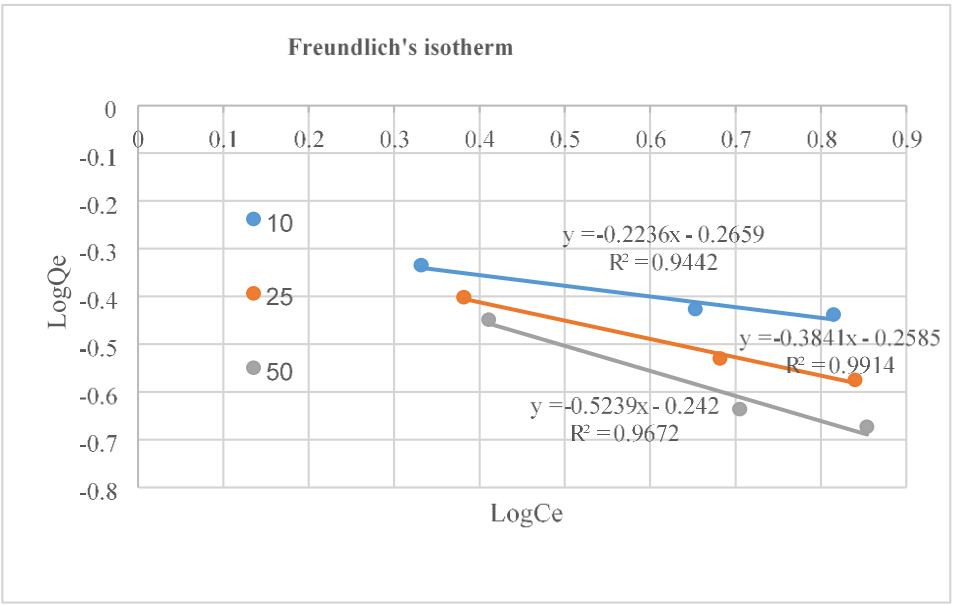

Figure6: Freundlich Isotherm Plots for The Adsorption of Chlordiazepoxide Onto Olive Seed Activated Charcoal (OS-AC) At Temperatures Of 10 °C, 25 °C, And 50 °C. The Linear Relationship Between Logce And Logqe Supports the Applicability of The Freundlich Model, Indicating Multilayer Adsorption on A Heterogeneous Surface.

The negative slopes represent the adsorption intensity (1/n), while the intercepts correspond to adsorption capacity (Kf). The correlation coefficients (R^2) indicate a good model fit across all tested temperatures, with adsorption intensity increasing slightly with temperature.

Table6: Results of Langmuir's and Freundlich's Equations

Temp. C^o	**Langmuir isotherm**				**Freundlich's isotherm**		
	a(mg/g)	b(mg/g)	(r^2)	RL	(kf)	Slope(n)	(r^2)
10	0.329	-1.623	0.9996	-0.182	–..8647	–4.4988	..7448
25	0.229	-0.348	0.9995	-0.348	–..8424	–8.6.34	..7754
50	0.173	-0.761	0.9999	-0.489	–..848	–5.7.29	..7698

4. Conclusion

This study demonstrates the potential of activated charcoal made from olive seeds (OS-AC) in effectively detoxifying chlordiazepoxide. The identified ideal adsorption parameters of neutral pH, 0.1g adsorbent dosage, and a one-minute contact duration, along with the accurate description of the adsorption process by Langmuir's isotherm model, inspire and motivate the future application of OS-AC in treating chlordiazepoxide intoxication, providing a new direction for professionals in the field of toxicology and pharmacology.

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Enhancing the Solubility of Class II Drug Via Nanosuspension: A Review

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Abstract

Poor aqueous solubility remains a significant challenge in drug development, particularly for biopharmaceutics classification system, a promising approach to enhance solubility and dissolution by reducing particle size and increasing surface area. This review explores the formulation and evaluation of nanosuspensions as an effective strategy to improve the bioavailability of poorly water-soluble drugs. Various techniques, including top-down and bottom-up methods, contribute to nanosuspension preparation, with high- pressure homogenization and media milling being the most widely used. Selection of stabilizers plays a crucial role in preventing aggregation and ensuring long-term stability. Characterization parameters such as particle size, zeta potential, drug content, and in vitro dissolution provide critical insights in to nanosuspension performance. Recent advancements in nanosuspension technology enable enhanced therapeutic efficacy, reduced dosing frequency, and improved patient compliance. Applications extend to oral, parenteral, and ophthalmic drug delivery, offering versatility in pharmaceutical formulations. Challenges related to physical stability, scalability, and regulatory considerations require further investigation to facilitate commercialization. Future research focuses on optimizing formulation techniques, exploring novel stabilizers, and integrating advanced analytical tools for better characterization. Nanosuspensions continue to demonstrate potential in overcoming solubility limitations and enhancing drug absorption, making them a valuable approach in pharmaceutical development.

تعزيز ذوبانية الأدوية من الصنف الثاني من خلال التعليق النانوية: مراجعة علمية

أنسام فلاح عباس، جمال علي عاشور، مريم حسين العيادي

ملخص

لا تزال الذوبانية الضعيفة في الماء تُشكل تحديًا كبيرًا في تطوير الأدوية، وخاصةً في نظام تصنيف المستحضرات الصيدلانية الحيوية، وهو نهج واعد لتعزيز الذوبانية والذوبان عن طريق تقليل حجم الجسيمات وزيادة مساحة السطح. تستكشف هذه المراجعة تركيب وتقييم المعلقات النانوية كاستراتيجية فعالة لتحسين التوافر الحيوي للأدوية ضعيفة الذوبان في الماء. تُسهم تقنيات مُختلفة، بما في ذلك الطرق التنازلية والتصاعدية، في تحضير المعلقات النانوية، ويُعدّ التجانس عالي الضغط وطحن الوسائط الأكثر استخدامًا. يلعب اختيار المُثبتات دورًا حاسمًا في منع التكتل وضمان الاستقرار طويل الأمد. تُوفر معايير التوصيف، مثل حجم الجسيمات، وجهد زيتا، ومحتوى الدواء، والذوبان في المختبر، رؤىً ثاقبة حول أداء المعلقات النانوية. تُمكّن التطورات الحديثة في تقنية المعلقات النانوية من تعزيز الفعالية العلاجية، وتقليل وتيرة الجرعات، وتحسين التزام المريض بالعلاج. تمتد التطبيقات إلى توصيل الأدوية عن طريق الفم والحقن والعين، مما يُتيح تنوعًا في التركيبات الصيدلانية. تتطلب التحديات المتعلقة بالاستقرار الفيزيائي وقابلية التوسع والاعتبارات التنظيمية مزيدًا من البحث لتسهيل التسويق التجاري. تركز الأبحاث المستقبلية على تحسين تقنيات الصياغة، واستكشاف مثبتات جديدة، ودمج أدوات تحليلية متقدمة لتحسين التوصيف. لا تزال المعلقات النانوية تُظهر إمكاناتها في التغلب على قيود الذوبان وتعزيز امتصاص الدواء، مما يجعلها نهجًا قيّمًا في تطوير الأدوية

1. Introduction

The formulation and evaluation of nanosuspension have been extensively explored as an advanced approach to enhance the solubility and bioavailability of biopharmaceutics classification system (BCS) class II drugs (Ahmed & Pirbal, 2023). These drugs have been characterized by low aqueous solubility and high permeability, resulting in dissolution-limited absorption, which has posed significant challenges in pharmaceutical development (Sadeghi et al., 2020). To overcome these limitations, various nanosuspension preparation techniques have been developed, including high-pressure homogenization, media milling, precipitation, and ultrasonication. Through these methods, drug particles have been reduced to nanometer scale, leading to substantial increase in surface area and an improvement in dissolution rate based on the principles of the noyes-whitney equation (Al-Mayahy et al., 2019). The stabilization of nanosized drug particles has been achieved by incorporating surfactants and stabilizers, which have played a crucial role in preventing aggregation and maintaining long -term stability (Al-Badry et al., 2023). Extensive research has been conducted to evaluate the impact of nanosuspensions on drug solubility, dissolution kinetics, and overall therapeutic performance. A significant enhancement in bioavailability has been observed, particularly in drugs with poor water solubility, allowing for better absorption and improved pharmacokinetic profiles (Sabri et al., 2020). Various characterization techniques have been employed, including particle size analysis, zeta potential measurement, differential scanning calorimetry, and x-ray diffraction, to assess the physicochemical properties of nanosuspensions (Pınar et al., 2023). The influence of formulation variables on particle size, stability, and drug release profile has been systematically studied to optimize nanosuspension formulations. Moreover, in vitro and in vivo studies have been conducted to establish the correlation between nanosuspension characteristics and their biopharmaceutical performance (Al-Badry et al., 2023; Pınar et al., 2023; Sabri et al., 2020). The sacksful application of nanosuspensions has been demonstrated in different routes of drug administration, including oral, parenteral, ophthalmic, and pulmonary delivery systems (Leone & Cavalli, 2015). The challenges associated with the physical and chemical stability of nanosuspensions have been addressed through advanced formulation strategies, ensuring their suitability for large scale production and clinical applications (Annu & Singhal, 2022). The regulatory aspects of nanosuspension have been carefully considered to ensure compliance with pharmaceutical guidelines and standards for safety and efficacy. Advances in nanosuspension technology have contributed to development of novel drug delivery systems capable of improving the therapeutic outcomes of poorly soluble drugs (Sahu et al., 2021). The combination of nanotechnology and pharmaceutical science has provided a promising platform for the formulation of effective nanosuspension based drug delivery systems (Rinoldi et al., 2021). Continuous advancements in formulation techniques and characterization methods have facilitated the optimization of nanosuspension formulations for various therapeutic applications (Tian et al., 2021). The integration of computational modeling and experimental approaches has further enhanced the understanding of nanosuspension behavior and performance (Elsebay et al., 2023). The potential of nanosuspensions in personalized medicine and targeted drug delivery has been recognized, paving the way for future innovations in pharmaceutical nanotechnology. The research on nanosuspensions has provided valuable insights into their formulation, evaluation and application in enhancing the solubility of BCS class II drugs (Elsebay et al., 2023; Rinoldi et al., 2021; Tian et al., 2021).

1.1.Preparation of Nanosuspension

The preparation of nanosuspensions involves the reduction of particle to the nanoscale to enhance their solubility, dissolution rate, and bioavailability (Guan et al., 2022). Various methods have been utilized for preparation of nanosuspensions, each aimed at achieving fine control over particle size, stability, and drug release characteristics (Aldeeb et al., 2024). The process generally begins with the selection of an appropriate drug that has low solubility but high permeability, making it suitable for formulation into a nanosuspension (Attia et al., 2021).

In the preparation process, the drug first dispersed into a suitable solvent or a mixture of solvents. This step is crucial for ensuring that the drug is well-dissolved or at least well-dispersed in the medium before particle size reduction is achieved using several techniques, with the most commonly used methods being high-pressure homogenization and media milling (Al Haj et al., 2008; Vinchhi et al., 2021).

1.2.High-Pressure Homogenization

high-pressure homogenization involves the forced passage of the drug suspension through a narrow gap at high pressure. As the suspension is forced through this gap, the drug particles are subjected to mechanical stress, shear forces and turbulence, leading to reduction in particle size to nanoscale. The process is repeated for several cycles to ensure that desired particle size is achieved as shown in Fig.1 (Bravo & Oliva, 2017; Kruszelnicka, 2022).

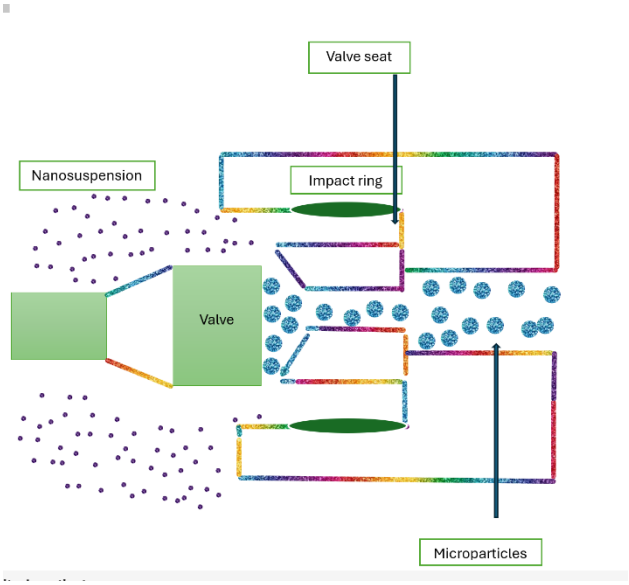

Figure1. Schematic Diagram of High-Pressure Homogenization for Nanosuspension Preparation

The drug suspension is forced through a narrow gap at high pressure via a valve system. Upon exiting the valve, particles collide with an impact ring and valve seat, generating intense shear forces and cavitation that reduce drug particles to the nanoscale. The result is a stable nanosuspension of smaller particles, improving solubility and bioavailability.

1.3.Media Milling

Media milling also known as wet milling, is another widely used method for producing nanosuspensions. In this process, a milling chamber is filled with the drug suspension along with milling media, such as beads made of ceramic or glass [23]. The suspension is agitated to create friction between the milling media and the drug particles, causing the particles to break down into smaller sizes. The milling process is monitored to ensure that the particles are reduced to required size, usually between 200 to 600 nanometers (Elsebay et al., 2023).

1.4.Precipitation Method

The precipitation method has widely used for nanosuspension preparation. In this technique, the drug was dissolved in a suitable solvent and then precipitated by adding a non-solvent under controlled conditions. Rapid mixing and stabilizer incorporation were essential to prevent particle growth and aggregation as shown in Fig.2 (Islam et al., 2022).

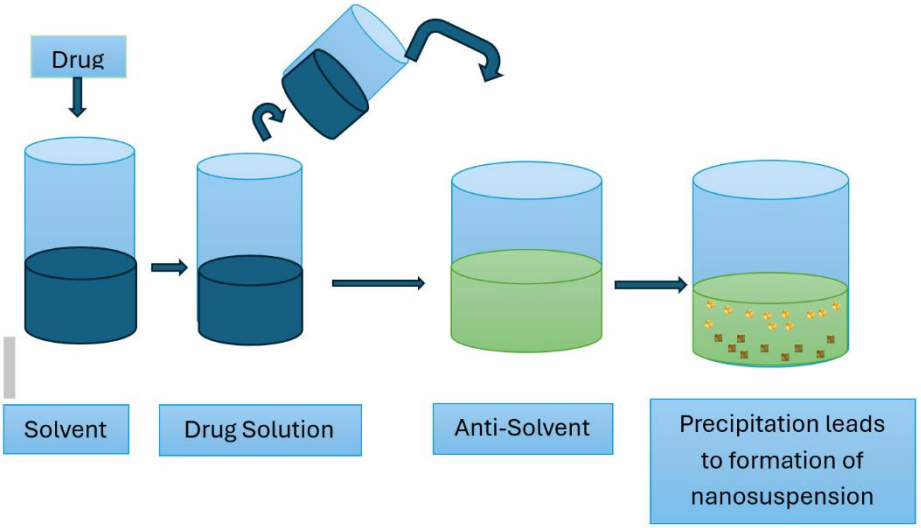

Figure2. Schematic Representation of Anti-Solvent Precipitation Method for Nanosuspension Preparation

The drug is first dissolved in a suitable solvent to form a drug solution. This solution is then rapidly added to an anti-solvent under controlled conditions, leading to precipitation of the drug particles due to reduced solubility. The resulting fine particles form a nanosuspension. This method is advantageous for thermolabile compounds and requires appropriate stabilizers to prevent particle aggregation.

1.5.Ultrasonication Method

Ultrasonication was used as a simple and effective method to break down drug particles into nanoscale sizes. High-frequency ultrasonic waves were applied to disrupt large aggregated, resulting in stable nanosuspensions with enhanced solubility as shown in Fig.3 (Guan et al., 2022).

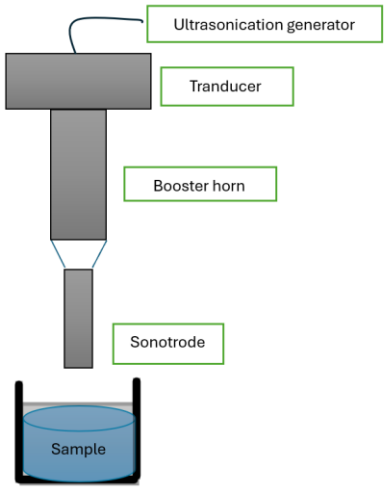

Figure3. Ultrasonication System Setup for Nanosuspension Formulatio

This method employs an ultrasonication generator connected to a transducer, booster horn, and sonotrode, which delivers high-frequency sound waves into the liquid sample. These waves generate cavitation forces that reduce the particle size of the drug to the nanometer range. Ultrasonication is particularly effective in breaking down coarse suspensions and enhancing drug solubility and stabilitv.

1.6.Supercritical Fluid Method

Supercritical fluid technology was explored as a novel approach for nanosuspension formulation. In this method, a supercritical fluid(e.g., carbon dioxide) was utilized to precipitate drug nanoparticles from solution. This process was recognized for its environmental benefits and ability to produce uniform particles (Cortés et al., 2021).

1.7.Stabilizer Used in Nanosuspension Formulation

Stabilizers play a crucial role in nanosuspension formulation as they prevent particle aggregation and ensured the stability of the system. Various types of stabilizers were employed, including surfactants, polymers, and lipids, each contributing to different stabilization mechanism (Guan et al., 2022; Tamang et al., 2022). Surfactants sch as polysorbates and sodium lauryl sulfate were used to reduce interfacial tension and provide steric hindrance, which minimize particle aggregation. Polymers like polyvinylpyrrolidone and hydroxypropyl methylcellulose were incorporated to enhance steric stabilizers such as lecithin were utilized to improve biocompatibility and enhance drug solubilization. The selection of suitable stabilizers depended on factors such as drug properties, intended route of administration, and desired formulation characteristics. A balance between hydrophilic and lipophilic properties was considered to ensure effective stabilization (Elmowafy et al., 2021; Jakubowska et al., 2022; Soroushnia et al., 2021; Tamang et al., 2022). Electrostatic stabilization was achieved through the use of ionic stabilizers that provided a surface charge, thereby preventing particle aggregation due to repulsive forces. the zeta potential of nanosuspension was measured to evaluate electrostatic stabilization, with values above 30 mv indicating good stability. The type and concentration of stabilizers were optimized to achieve minimal particle size and enhance the dispersion of nanosuspensions. Inadequate stabilization led to Ostwald ripening and sedimentation, which affected formulation

stability and drug bioavailability. Compatibility between stabilizer and drug was evaluated to avoid undesired interactions that could compromise drug efficacy (Bhalani et al., 2022; Chen et al., 2022; Jakubowska et al., 2021, 2022; Khan et al., 2022). The role of stabilizers in preventing crystallization and maintaining the amorphous state of the drug was also investigated. Several studies demonstrated that the appropriate selection of stabilizers significantly improved the dissolution rate and bioavailability of poorly water-soluble drugs. Stability studies were conducted to assess the impact of storage conditions on particle size, zeta potential, and drug content. The long-term effectiveness of stabilizers was evaluated to ensure the nanosuspension maintained its intended physicochemical properties. As research in nanosuspension technology advanced, noval stabilizers such as biopolymers and nanostructured materials were explored to enhance stability and drug delivery efficiency (Khan et al., 2022; Mahmood et al., 2023; Maleki et al., 2017; Rocha et al., 2023; Tupe et al., 2023).

1.8.Surfactant Used in Nanosuspension Formulation

Surfactants play a crucial role in the formation of nanosuspensions by stabilizing drug particles and preventing aggregation. The function by reducing the interfacial tension between the hydrophobic and drug particles and the aqueous dispersion medium, thereby enhancing the wettability and dispersibility of the drug (Cai et al., 2022). Non-ionic surfactants, such as polysorbates (tween 80, tween 20) and poloxamers (pluronic), contribute to steric stabilization by forming a protective layer around the nanoparticles, which prevents their aggregation through steric hindrance. These surfactants also improve the physical stability of the nanosuspension by minimizing Ostwald ripening and sedimentation (Aguirre-Ramírez et al., 2021; Tenorio-Garcia et al., 2022). Anionic surfactant, including sodium dodecyl sulfate (SDS), enhance electrostatic stabilization by imparting a negative charge to the drug particles. This charge generates repulsive forces between nanoparticles, which prevents their coalescence and maintains a uniform dispersion. Cationic surfactants such as cetyltrimethylammonium bromide (CTAB), provide similar electrostatic stabilization but with a positive surface charge, which can interact with negatively charged biomolecules or cell membranes, leading to potential bio adhesive properties. In some formulation, amphiphilic surfactants, including lecithin, offer dual stabilization mechanisms by combining electrostatic and steric stabilization. Lecithin molecules adsorb onto the particle surface, reducing surface energy and improving dispersion stability (Purohit et al., 2022).

1.9.Organic Solvent

Organic solvents were widely used in nanosuspension formulation to enhance the solubility and bioavailability of poorly water-soluble drugs various techniques were developed to prepare nanosuspensions using organic solvents ensuring controlled particle size and stability(Pulingam et al., 2022) . The solvent evaporation method was commonly employed where the drug was dissolved in a volatile organic solvent such as ethanol or acetone. This solution was then emulsified into aqueous phase containing stabilizers like PVP or Tween 80 after emulsification the organic solvent was evaporated under reduced pressure or continuous stirring leading to precipitation of drug nanoparticles which were stabilized by surfactants (Chatterjee, 2018; Kravanja et al., 2022; Thakkar & Misra, 2017).

Another widely adopted technique was the solvent precipitation method in which the drug was first dissolved in a water- miscible organic solvent upon rapid mixing with an anti-solvent usually water. The sudden supersaturation caused the immediate precipitation of nanosized drug particles the presence of surfactants and polymers prevented particle agglomeration and ensured nanosuspension stability(Bagheri et al., 2022; Farkas & Kramar, 2021) . Supercritical fluid technology was also explored where the drug was dissolved in supercritical fluid such as carbon dioxide upon controlled depressurization. The solubility of the drug in the supercritical fluid was reduced leading to nucleation and formation of nanosized particles. This technique was considered an advanced method due to its ability to produce pure nanoparticles without residual organic solvents (Bagheri et al., 2022; Farkas & Kramar, 2021; Karmakar, 2019).

2. Characterization and Evaluation of Nanosuspension

2.1.Particle Size and Polydispersity Index (PDI)

Dynamic light scattering (DLS) was employed to determine particle size distribution and PDI. A lower PDI value indicates a more uniform particle size distribution, which was critical for nanosuspension stability (Darabian et al., 2022; Karmakar, 2019).

2.2.Zeta Potential Measurement

Zeta potential analysis was conducted to assess the surface charge of nanoparticles. A high zeta potential value above 30 mv was indicative of good electrostatic stabilization, reducing the risk of aggregation (Shaikh et al., 2022).

2.3.Crystallinity and Morphology Analysis

Differential scanning calorimetry (DSC) and X-ray diffraction(XRD) were utilized to examine the crystalline state of the drug. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to visualize nanoparticles morphology (Aghrbi et al., 2021).

2.4.Saturated Solubility and Dissolution Studies

Saturated solubility tested were performed to evaluate the solubility enhancement achieved by nanosuspension formulation. In vitro dissolution studies were conducted to compare the drug release profiles of nanosuspensions and conventional formulations (Li et al., 2021).

3. Stability Studies

Long term and accelerated stability studies were conducted to assess the physical and chemical stability of nanosuspensions. changes in particle size, zeta potential, and drug content were monitored over time to ensure formulation robustness (Elshafeey & El-Dahmy, 2021; Sampathi et al., 2022).

4. Pharmacokinetic and Bioavailability Enhancement

Animal and human pharmacokinetic studies were performed to determine the bioavailability improvement achieved through nanosuspension administration [(67)]. Enhanced absorption, higher plasma drug concentrations, and prolonged circulation times were observed in various studies, confirming the effectiveness of nanosuspensions in improving drug performance (Guan et al., 2022).

5. Conclusion

Nanosuspensions have emerged as highly effective strategy for enhancing the solubility and bioavailability of BCS classII drugs. Various formulation techniques, including precipitation, high pressure homogenization, media milling, have been successfully employed to produce stable nanosuspensions. Comprehensive characterization and evaluation parameters were utilized to optimize formulation performance. Future advancements in nanosuspension technology are expected to further enhance drug delivery and therapeutic efficacy.

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Studying the Correlation Between Serum Hormone Levels in Infertile Women and the Results of IVF and Various Causes of Infertility

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Abstract

Background: Infertility is defined as the inability to achieve a clinical pregnancy following 12 months of consistent, unprotected sexual activity. Both male and female factors, or both, may contribute to infertility. Infertility is most often caused by ovulatory dysfunction, such as inadequate ovarian reserve (POR) and polycystic ovaries (PCO).

Methods: A cross sectional study includes 37 participants. The samples of blood were collected at cycle day two and detected the hormonal levels by MINI VIDAS system.

Results: The result of present study showed that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in women with female and combined factors with significantly different higher from male and unexplained factors with (p=0.000, p=0.000). While, the level of estradiol (E2), anti-Mullerian hormone (AMH) and Estradiol hormone (E2) at day of human chorionic gonadotropin (HCG) injection in women with male and unexplained factors with significantly different higher from female and combined factors (*P=0.007, P=0.000, P=0.003*) respectively. While, progesterone in women, there was no significant different between cause of infertility groups with p=0.467. In addition, Total oocyte number, Fertilization rate, Embryo Grade l(GI), Embryo Grade ll (GII) and transferred embryo of women with unexplained and male factor were significantly different from female and combined factor cases with (*P=0.056, P=0.037, P=0.001, p=0.059 and p=0.057*) respectively. Regarding the correlation this hormone with pregnancy outcomes, there is no statistical significance.

Conclusion: Serum FSH and LH levels were significantly associated with female and combined factor cases, whereas E2 day2, AMH, and E2 HCG were significantly associated with unexplained and male factor cases. In terms of progesterone, there was no significant difference between the causes of infertility groups. Furthermore, the total number of oocytes, fertilization rate, embryo grade l (GI), embryo grade ll (GII), and transferred embryo were all significantly associated with unexplained and male factor cases. There is no statistical significance to the correlation between these hormones and pregnancy outcomes.

دراسة العلاقة بين مستويات الهرمونات في مصل الدم لدى النساء المصابات بالعقم ونتائج الإخصاب خارج الجسم (IVF) وأسباب العقم المختلفة

آيات حاتم عبد الهاشم، مهند محسن أحمد، علي إبراهيم رحيم

الخلاصة

المقدمة

يُعرَّف العقم بأنه عدم القدرة على تحقيق حمل سريري بعد 12 شهرًا من النشاط الجنسي المنتظم غير المحمي. وقد تكون العوامل الذكرية أو الأنثوية أو كلاهما سببًا في العقم. وغالبًا ما يكون العقم ناتجًا عن خلل في الإباضة، مثل ضعف احتياطي المبيض (POR) أو متلازمة تكيس المبايض.(PCO)

العينات وطرق العمل

شملت الدراسة المقطعية 37 مشاركة. تم جمع عينات الدم في اليوم الثاني من الدورة الشهرية، وتم الكشف عن مستويات الهرمونات باستخدام نظام.MINI VIDAS

النتائج

أظهرت نتائج الدراسة أن هرموني التحفيز الجريبي (FSH) والهرمون اللوتيني (LH) لدى النساء اللواتي يعانين من أسباب أنثوية أو مشتركة للعقم كانا أعلى بشكل ملحوظ مقارنةً بمن لديهن أسباب ذكرية أو غير مفسرة (p=0.000لكلا الحالتين.(بينما كانت مستويات الإستراديول(E2) ، والهرمون المضاد لمولر(AMH) ، وهرمون الإستراديول في يوم حقن هرمون موجهة الغدد التناسلية المشيمية البشرية (HCG) أعلى بشكل ملحوظ في حالات العقم ذات الأسباب الذكرية وغير المفسرة مقارنة بالحالات ذات الأسباب الأنثوية أو المشتركة(P=0.007 ، P=0.000، P=0.003على التوالي.(أما بالنسبة لهرمون البروجستيرون، فلم يكن هناك فرق معنوي بين مجموعات أسباب العقم.(p=0.467) بالإضافة إلى ذلك، فإن العدد الكلي للبويضات، ونسبة التلقيح، وجودة الأجنة من الدرجة الأولى (GI) والدرجة الثانية(GII) ، وعدد الأجنة المنقولة كانت مرتبطة بشكل ملحوظ بحالات العقم غير المفسرة وذات الأسباب الذكرية مقارنة بالحالات الأنثوية أو المشتركة(P=0.056) ، P=0.037، P=0.001، p=0.059، p=0.057على التوالي.(أما فيما يتعلق بارتباط هذه الهرمونات بنتائج الحمل، فلم يكن هناك دلالة إحصائية.

الاستنتاج

ارتبطت مستويات هرموني FSH و LHفي الدم بشكل معنوي بحالات العقم ذات الأسباب الأنثوية أو المشتركة، بينما ارتبطت مستويات E2 في اليوم الثاني، وAMH، و E2في يوم حقن HCG بشكل معنوي بالحالات غير المفسرة أو ذات الأسباب الذكرية. لم يكن هناك فرق معنوي في مستويات البروجستيرون بين مجموعات أسباب العقم. كما أظهرت النتائج أن العدد الكلي للبويضات، ونسبة التلقيح، وجودة الأجنة GI) و(GII، وعدد الأجنة المنقولة كانت مرتبطة بشكل ملحوظ بالحالات غير المفسرة أو الذكرية. لم يُلاحظ وجود دلالة إحصائية لارتباط هذه الهرمونات بنتائج الحمل.

1. Introduction

Approximately 8–12% of couples worldwide experience infertility, with female factors accounting for over 50% of instances (Organization, 2023). Infertility rates are particularly high in Iraq, where they are caused by late marriages, consanguinity, and a lack of access to cutting-edge reproductive treatments (Al-Hilli et al., 2021). Male factor 30% and female factor 40% are the most frequent causes of infertility(El Adlani et al., 2021). The inability of a male spouse to conceive a child with a fertile female partner is known as male infertility(Shah et al., 2021). It is accounting for 40–50% of all infertility causes. Female infertility has many causes, ovulation disorders is one of this causes (Sala Uddin et al., 2018). Ovulation disorders frequently manifest as irregular periods (oligomenorrhea) or no periods at all (amenorrhea). Polycystic ovarian syndrome (PCOS) and primary ovarian insufficiency are the most common causes of female infertility, according to studies conducted globally(Deshpande and Gupta, 2019, Man et al., 2022). Ovulation, menstruation, embryo implantation, and pregnancy are among the primary events of female reproductive function that are linked to hormones and inflammatory systems. Pregnancy difficulties may be predisposed by hormonal abnormalities and a hyperinflammatory condition that disrupt the immune-endocrine cross-talk between the decidua and trophoblast, the endometrium, and the myometrium and cervix (Vannuccini et al., 2018). In reference to the intra-cytoplasmic sperm injection (ICSI), a mature egg is directly injected with a single healthy sperm. This kind of technology is known as assisted reproductive technology (ART)(Geng et al., 2020). In this study, Iraqi infertile and sub fertile women's serum levels of FSH, LH, E2, progesterone, and AMH will be correlated with fertility cases and ICSI results (live birth, clinical pregnancy). Our research could help develop tailored protocols to maximize the success of ART in this underprivileged area**.**

2. Patients and Methods

Based on the most prevalent causes of infertility, 37 patients were split up into four groups for this study. Islamic Fertility Center at Al-Kafeel Hospital in Karbala performed Intracytoplasmic Sperm Injection (ICSI). All patients were diagnosed by skilled gynecologists and embryologists after completing an antagonist program. The patients were between the ages of twenty and forty-one. On the second cycle day, five milliliters of the patients' blood were drawn into a gel tube, allowed to clot, and then centrifuged for five minutes at 3000 rpm to separate the serum. FSH, LH, prolactin, E2, AMH, E2 day of HCG injection, and B.HCG concentrations were measured using serum using the MINI VIDAS system.

3. Results

3.1. Demographics Characteristics According to the Reasons of Infertility

The patients were between the ages of 20 and 41. Women with male, female, and combination factors for infertility had mean ages of 29.6, 29.6, 32.4, and 31.6 years, respectively. The patient groups did not differ significantly (P = 0.131). In patients with unexplained infertility, the mean body max index (BMI) was 25.5, 27.5, 32.4, and 31.6 for male, female, and combination factor infertility, respectively. Regarding body max index, there was no significant difference between the patient groups (P=0.079). The mean values for the duration of infertility were 11.2, 10.2, 11, and 8.3 years for the male factor, female factor, and unexplained infertility, respectively. These differences were not statistically significant (P= 0.156). Regarding the infertility types, primary infertility had an unexplained infertility count of 3, whereas male, female, and combination causes had counts of 9, 4, and 6, respectively. Male,

female, and combination factor counts were 10, 1, and 2, respectively, but the unexplained infertility count for secondary infertility was 2. The types of infertility did not significantly differ among the patient groups (P = 0.121). as shown in Table1.

Table1: Lists Demographic Traits Categorized by the Cause for Infertility

Variables	Cause of infertility								P value
	Unexplained infertility (5)		Male factor (19)		Female factor (5)		Combined factor (8)		
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
Age (year)	29.6	4.09	29.6	5	32.4	5.6	31.6	5.06	0.131
BMI (Kg/m^2)	25.5	4.49	27.50	5.33	31.3	4.36	31.6	4.24	0.079
Duration (year)	11.2	2.94	10.2	5.58	11	4.18	8.3	4.83	0.156
	Count	%	Count	%	Count	%	count	%	
Primary Infertility	3	13.6	9	40.9	4	18.2	6	27.3	0.121
Secondary Infertility	**2**	13.5	10	66.7	1	6.7	2	13.3	
ANOVA test (LSD test), chi-square test, SD: standard deviation, infertility pri.: primary infertility, infertility sec.: secondary infertility.									

3.2. The Levels of Hormones in The Groups Under Study

The mean serum level of follicle stimulating hormone (FSH) in unexplained infertility cases was 4.62 mlU/ml, 4.45 mlU/ml in males, 7.64 mlU/ml in females, and 7.24 mlU/ml in combined factors. However, the female and combined factors differed significantly from the unexplained and male factors (P = 0.000). Furthermore, the mean serum luteinizing hormone (LH) level in the unexplained infertility group was 3.28 mlU/ml, 3.29 mlU/ml in the male factor group, 8.16 mlU/ml in the female factor group, and 6.26 mlU/ml in the combination factor group. However, the female factor caused a significant difference from the unexplained, male, and combination factor groups (P=0.000). In contrast, the mean progesterone levels in unexplained infertility were 0.78 ng/ml, 1.3 ng/ml for males, 0.93 ng/ml for females, and 0.98 ng/ml for combination factors. Serum progesterone levels did not significantly differ across patient groups (P=0.467). The mean levels of the hormone estradiol (E2) on cycle day two were 48.8 pg/ml for unexplained infertility, 45.66 pg/ml for males, 32.30 pg/ml for females, and 33.61 pg/ml for combined factors. However, there was a significant difference (p=0.005) between the unexplained factor and the male, female, and combined factor groups. The mean levels of anti-Mullerian hormone (AMH) were 2.91 ng/ml in cases of unexplained infertility, 3.30 ng/ml in male factor groups, 0.53 ng/ml in female factor groups, and 0.85 ng/ml in combination factor groups. Male causes, however, were significantly different from female, unexplained, and combination factors (P=0.000). On the day of the HCG injection, the mean levels of the hormone estradiol (E2) were 2185.04 pg/ml for unexplained infertility, 2703.85 pg/ml for males, and 1053.81 pg/ml for females, while the combined levels were 1365.00 pg/ml. However, there was a significant difference (P = 0.003) between the male factor and the unexplained, female, and combined factor groups see Table2.

Table2: Hormonal Levels Related to Infertility Reasons

Variables	Cause of infertility								P value
	Unexplained infertility (5)		Male factor (19)		Female factor (5)		Combined factor (8)		
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
FSH (mIU/ml)	4.62	0.80	4.45	1.29	7.64	0.75	7.24	2.00	**0.000**
LH (mIU/ml)	3.28	0.38	3.29	0.70	8.16	1.78	6.26	1.78	**0.000**
Progesteron (ng/ml)	0.78	0.19	1.3	1.98	0.93	0.27	0.98	0.35	0.467
E2 day 2 (pg/ml)	48.8	7.30	45.66	5.03	32.30	5.16	33.61	4.54	**0.005**
AMH (ng/ml)	2.91	0.97	3.30	0.64	0.53	0.20	0.85	0.40	**0.000**
E2 HCG (pg/ml)	2185.04	480.77	2703.85	389.97	1053.81	823.43	1365.00	475.25	**0.003**

Follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol hormone (E2) at day two of the cycle, anti-mullerian hormone (AMH), and human chorionic gonadotropin injection (HCG) are all examples of hormones. ANOVA (LSD) test.

3.3. Clinical Characteristics Associated with Infertility Etiology Include

Among the groups, there was no significant difference (P = 0.276) in the mean number of attempts at intracytoplasmic sperm injection (ICSI): 1.8 for unexplained infertility, 1.6 for male factor cases, 1.8 for female factor cases, and 1.5 for the combined factor group. The mean number of total oocytes was 13.40 for unexplained infertility, 13.94 for male factor cases, 10.20 for female factor cases, and 11.71 for the combined factor group. However, there was a significant difference between the male factor and unexplained infertility groups (P=0.056). The mean maturity rate was 88.46 in the group with unexplained infertility, 79.22 in cases with male factors, 70.86 in the group with female factors, and 80.71 in the group with combination factors. No significant difference was found (P=0.197). While, Fertilization rates were 86.7 in the group with unexplained infertility, 78.84 in cases with male factors, 70.66 in the group with female factors, and 64.24 in the group with combination factors. Nonetheless, unexplained factor there was a significant difference from the male, female, and combination factor groups (P=0.037). The mean embryo Grade I (GII) was 3.66 in the group with unexplained infertility, 3.57 in the group with male factors, 2.33 in the group with female factors, and 2.00 in the group with combination factors. However, there was a significant difference (P=0.001) between the male factor and the unexplained, female, and combined factor groups. Furthermore, the mean embryo Grade I (GII) was 3.66 in the group with unexplained infertility, 3.57 in the group with male factors, 2.33 in the group with female factors, and 2.00 in the group with combination factors. Nonetheless, unexplained and male factors there were a significant from the combined and female factor groups (P = 0.059). The mean of embryo Grade III (GIII) was approximately 3.00 in the group with unexplained infertility, 2.42 in cases

with male factors, 2.33 in the group with female factors, and 2.50 in the group with combination factors. However, there was no significant difference between the groups (P=0.488). Regarding transferred embryos, the mean was 3.20 in the group with unexplained infertility, 3.50 in cases with male factors, 2.40 in the group with female factors, and 2.83 in the group with combination factors. However, Male and unexplained factors differed significantly from the female and combined factor groups (p=0.057). The male factor infertility group had the highest conception rate, with 52.63% of the females achieving a successful pregnancy as indicated by a positive blood B HCG test. Conversely, the lowest conception rate (20%) occurred when a female factor contributed to infertility. as shown in Table3.

Table3: Mean of Clinical Traits Based on the Reasons for Infertility

Variables	Cause of infertility								P value
	Unexplained infertility (5)		Male factor (19)		Female factor (5)		Combined factor (8)		
	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD	
ICSI attempt	1.8	1.09	1.6	0.67	1.8	1.09	1.5	0.75	0.276
Total oocytes	13.40	6.87	13.94	5.86	10.20	7.85	11.71	4.99	0.056
Maturity rate	88.46	12.09	79.22	21.24	70.86	23.73	80.71	15.76	0.197
Fertilization rate	86.7	12.16	78.84	17.41	70.66	40.44	64.24	29.80	0.037
Embryo GI	3.66	2.88	4.07	2.01	1.66	0.57	2.00	0.81	0.001
Embryo GII	3.66	2.30	3.57	2.10	2.33	0.57	2.00	1.41	0.059
Embryo GIII	3.00	2.64	2.42	1.74	2.33	1.15	2.50	1.73	0.488
Transferred embryo	3.20	0.44	3.50	0.78	2.40	1.14	2.83	1.16	0.057
	Count	%	Count	%	Count	%	Count	%	
B HCG result -ve	3	60	9	47.37	4	80	6	75	0.121
B HCG result +ve	2	40	10	52.63	1	20	2	25	
ANOVA test (LSD test), chi-square test, GI: Grade I, B HCG: Beta-Human Chorionic Gonadotropins, and ICSI: Intracytoplasmic Sperm Injection									

3.4. Hormonal Analysis Using B HCG (Pregnancy Outcome)

The mean values of FSH, LH, Progesterone, E2 day 2, AMH, and E2 HCG in pregnant women were 4.86, 4.77, 0.90, 64.84, 3.45, and 2515.1, in that order. While, FSH, LH, Progesterone, E2 day 2, AMH, and E2 HCG mean levels in non-pregnant women were 7.39, 4.73, 0.75, 57.18, 5.39, and 2079, respectively. Table4 demonstrate that there was no significant difference among the B-HCG result groups (p=0.098, 0.488, 0.069, 0.295, 0.268, and 0.124).

Table4: Mean Variations in Hormonal Research Based on B HCG (Pregnancy Outcome)

Variables	B-HCG				P value
	Positive (pregnant)		Negation(pregnant)		
	Mean	SD	Mean	SD	
FSH (mIU/ml)	4.86	1.46	7.39	7.18	0.098
LH (mIU/ml)	4.77	1.75	4.73	2.27	0.488
Progesterone (ng/ml)	0.90	0.41	0.75	0.29	0.069
E2 day 2 (pg/ml)	64.84	25.29	57.18	26.84	0.295
AMH (ng/ml)	3.45	2.84	5.39	10.79	0.268
E2 HCG (pg/ml)	2515.1	986.1	2079	743.5	0.124

4. Discussion

The patients' ages ranged from 20 to 41 years, with the mean ages of women with unexplained infertility, male factor, female factor, and combined factor being 29.6, 29.6, 32.4, and 31.6 years, respectively. There were no significant differences between the patient groups in terms of age (P =0.131). This results were compatible with other research, such as the one completed by(Sarapik et al., 2012) who discovered that the mean age of the male and female factors was 32.6 and 34.8 years, respectively. A study by (Al-Musawy et al., 2018) whereby the mean age (27.7) revealed that there were no statistically significant variations in the age of the PCOS group between the patient and control groups. Although the research by (Bouet et al., 2020) in which the mean age was (36.68) and there was considerably lower in the control group compared with the POR group (p< 0.001). In addition, The mean of body max index (BMI) in patients with unexplained infertility, male factor, female and combined factor infertility (25.5, 27.5, 31.3 and 31.6 correspondingly). The BMIs of the various patient groups did not differ significantly (P=0.079). The current study's findings were connected to those of prior investigations, including the one conducted by (Spanou et al., 2018) whose BMI values were reported to be 23.4 for male factors and 23.6 for PCOS, respectively, with no significant correlation found (P > 0.05). Additionally, a study by (Mehta et al., 2013) The male factor's BMI mean was 23.97, although there were no noteworthy findings. The study by (Kudsy et al., 2016) where the PCOS group's BMI mean was 27.19, with no discernible change (P>0.05).The mean values for the duration of infertility were 11.2, 10.2, 11, and 8.3 years for the unexplained infertility, male factor, female factor, and combined factor infertility, respectively. These differences were not statistically significant (P= 0.156). It aligns with prior research, including that conducted by(Swadi et al., 2023) in which mean of infertility duration was (5) with no significant result. While in the study by (Vural et al., 2015) in which the mean of infertility duration was (7.6) with highly significantly association (P =0.01). Regarding the types of infertility, there was no significant differences about infertility types between patient groups with (P =0.121) in current study. Several studies such as the study by (Swadi et al., 2023). Their results showed no discernible difference between primary infertility (34), and subsequent infertility. regarding hormone levels in relation to the reasons of infertility in patient groups. Follicle stimulating hormone (FSH) was 4.62 mlU/ml in unexplained infertility and 4.45 mlU/ml in male factor cases, but it was 7.64 mlU/ml in females and 7.24 mlU/ml in combined factors. On the other hand, the unexplained and male factors were significantly different from the female and combined factors (P=0.000), which is in line with other research, including those conducted by(Sarapik et al., 2012) who reported the mean of FSH in male factor was (5.6), in PCOS group was (5.7), with no significant result, the levels of FSH was normal in these groups. The mean of follicle stimulating hormone (FSH) in female factor group was (7.64). Compared to male and unexplained causes, the female component was significantly different (P=0.000). (Rebar, 2007) demonstrated that the diagnosis of POR requires elevated FSH levels. The findings of the current study were in line with those of other investigations, including the study by (Barbakadze et al., 2015) who reported the FSH mean were 8.96 in the under-35-year-old group and 11.23 in the 35-40-year-old group. The relationship between age and FSH was positive, and the relayw25w2tionship between FSH level and age was highly significant (P<0.0001). The mean blood level of luteinizing hormone (LH) was 3.28 mlU/ml in unexplained factor group, 3.29 mlU/ml in the male factor group, 8.16 mlU/ml in the female factor group,

and 6.26 mlU/ml in combined factor group. However, the female factor group was significantly different from unexplained, male, and combination factor groups with(P=0.000).Other research by(Liu et al., 2019) who discovered that the LH mean was (3.2) and (3.8), respectively, with no statistically significant outcome (P = 0.20). The female factor group mean was greater than the male factor, which is in line with several research, including the one conducted by (Lisi et al., 2005) who discovered the endocrinological condition that is associated with hypersecretion of LH and ovulatory failure, which is linked to elevated LH levels. Also, the research by (Jain et al., 2022) revealed that mean of LH in PCO was (6.95). Additionally, the research by (Tsakos et al., 2014) It was discovered that ovarian stimulation may be accurately predicted by basal LH levels. However, a study conducted by (Liu et al., 2020) found that there was no significant difference in the mean LH levels between the control group (P=927) and those with inadequate ovarian reserve (P=5.64). In contrast, the mean progesterone levels in unexplained infertility was 0.78 ng/ml, 1.3 ng/ml in male factor, 0.93 ng/ml in female factor, and 0.98 ng/ml in combined factors. Serum progesterone levels did not significantly differ across patient groups (P=0.467). However, this study's findings differed from those of (Sahin et al., 2020) They discovered that the group with unexplained infertility had significantly lower serum progesterone levels than the fertile control group (p=0.02). Variations in sample size may be the cause of this discrepancy. The mean levels of the hormone estradiol (E2) at cycle day two were 48.8 pg/ml for unexplained infertility, 45.66 pg/ml for male factor, 32.30 pg/ml for female factor, and 33.61 pg/ml for combination factor. However, unexplained infertility differed significantly from the combined, male, and female factors groups (P=0.005). It aligns with prior research, including that conducted by(Zhang et al., 2019) They discovered that the mean levels of the hormone estradiol in PCOS cases were 51.97 and 53.8, respectively, with no discernible difference. Regarding female factor patients' levels of estradiol hormone (E2) on cycle day two, a number of investigations carried out by (Liu et al., 2020) and (Zhang et al., 2021) which showed that the mean levels of the hormone estradiol were 30.10 and 34.8, respectively. Anti-Mullerian hormone (AMH) mean levels in unexplained infertility were 2.91 ng/ml, 3.30 ng/ml in male factor group, 0.53 ng/ml in female factor group, and 0.85 ng/ml in combination factor group. Male causes, however, differed significantly from female, unexplained, and combination variables (P=0.000). It aligns with prior research, including that conducted by(Jain et al., 2022) They discovered that PCOS patients had an average AMH of 7.04. Another study carried out by (Liu et al., 2020) showed that the AMH mean for POR patients was 0.58 and the control was 2.56; there was a significant difference between the groups (P=0.001). Additionally, the research by (Zhang et al., 2021) The mean AMH in POR was shown to be (0.6) correlated with the age of the female; the advanced age group displayed lower AMH, and there was a significant difference between young and old POR patients. However, the research conducted by(Barbakadze et al., 2015) AMH was reported to be (2.5) in the group under 35 and (1.1) in the group between 35 and 40. Age-specific variations were better indicated by AMH values than by other variables. Estradiol hormone (E2) levels in unexplained infertility were around 2185.04 pg/ml on the day of HCG injection, 2703.85 pg/ml in males, 1053.81 pg/ml in females, and 1365.00 pg/ml in combined factors. The male factor, however, differed significantly from the female, unexplained, and combination factor groups (P=0.003). It resembles the research by(Kavrut et al., 2022) who disclosed that in POR instances, the mean E2 on HCG day was 684.66. According to the current study, the mean number of

intracytoplasmic sperm injection (ICSI) attempts was 1.8 in the group with unexplained infertility, 1.6 in cases with male factors, 1.8 in the group with female factors, and 1.5 in the group with combination factors. no significant variation between the groups (P=0.276). Females with unexplained infertility and male factors had mean total oocyte numbers of 13.40 and 13.94, respectively, whereas females with female or combined infertility had mean total egg counts of 10.20 and 11.71, respectively. The statistical significance of these differences was minor (p=0.056). A study carried out by (Swadi et al., 2023) They stated that the median number of oocytes in the male factor was 12, which was linked to excellent stimulation of FSH, AMH, and E2. Regarding the mean of maturity rate was 88.46 in the group with unexplained infertility, 79.22 in cases with male factors, 70.86 in the group with female factors, and 80.71 in the group with combination factors. However, no significant difference was found (P=0.197). This outcome contradicted a study of (Kamath et al., 2008) They discovered a strong correlation between PCOS etiology and maturity rate (P=0.006). Regarding the rate of fertilization, the current study's mean values for the unexplained, male, female, and combination factors were 86.7, 78.84, 70.66, and 64.24, respectively. The results of these differences were significant (p=0.037). This finding clarifies how the reason of infertility affects the rate of fertilization. Fertilization rates declined for women with female and mixed factors, whereas they increased for couples with unexplained infertility and male factors, according to the study. A study carried out by (Xu et al., 2022) According to their findings, ICSI greatly increases the rate of normal oocyte fertilization and the cycle's clinical pregnancy rate in male infertility. Additionally, it has a great safety profile and little effect on unfavorable pregnancy outcomes or obstetric and perinatal problems. In addition to the normal amor female side, which indicates good egg quality, the embryologist venally selects bept sperm for injection, demonstrating the ability of ICSI to overcome male factor causes. The mean embryo Gradel (GI) was 3.66 in the group with unexplained infertility, 4.07 in cases of male factors, 1.66 in the group with female factors, and 2.00 in the combined group. There was statistical significance (P=0.001) in these grades I differences. Furthermore, the mean embryo Grade I (GII) was 3.66 in the group with unexplained infertility, 3.57 in the group with male factors, 2.33 in the group with female factors, and 2.00 in the group with combination factors. However, there was a significant difference (P = 0.059) between the male and unexplained factor groups and the female and combined factor groups. The findings of the current study concurred with a study by (Sarapik et al., 2012) He stated that the male factor was (3.8), PCO was (2.9), and POR was (1.0) for high-quality embryos (embryo Grade I and ll). POR patients differed greatly from other patient populations (Sarapik et al., 2012). It would demonstrate the ability of a healthy oocyte source to fix a variety of sperm abnormalities in addition to the embryologist's selection of the most viable sperm. The mean of Embryo Grade III (Glll) was 3.00 in the group with unexplained infertility, 2.42 in the group with male factors, 2.33 in the group with female factors, and 2.50 in the group with combination factors. Nevertheless, there was no significant difference between the groups (P=0.488). This research runs counter to the study by (Lin et al., 2013) showed that poor embryo quality (Embryo Grade III) was associated with a decrease in AMH. The mean of transferred embryos was 3.20 in the group with unexplained infertility, 3.50 in instances with male factors, 2.40 in cases with female factors, and 2.83 in the group with combination factors. On the other hand, male and unexplained infertility factors differed significantly from female and combined factor causes (P=0.057). Regarding the female aspect in this study, fewer embryos are

acceptable for transfer because of the low quantity and poor quality of oocytes. This outcome is consistent with a study finding of (Opsahl et al., 2001) who showed the correlation between oocyte and embryo quality and quantity. Significant variations in follicle size may also be linked to disparities in follicular sensitivity to FSH and inadequate maturation. The quantity of viable oocytes and embryos may decline as a result of this event. The Beta-Human Chorionic Gonadotropins (B-HCG) test count, on the other hand. Following ICSI, the male factor infertility group had the highest pregnancy rate (52.63%). These findings suggest that whereas female factors have a poorer prognosis for ICSI outcomes, male factors have a good one. The idea that female variables are the most significant contributors to pregnancy following fertilization may help to explain these results. This outcome is in line with a study by (Ashrafi et al., 2013) who assessed the correlation between ICSI outcome and various infertility causes and reported varying ICSI success rates for various causes of infertility. Additionally, the mean levels of FSH, LH, progesterone, E2 day 2, AMH, and E2 HCG hormones in pregnant and non-pregnant women did not differ statistically significantly between the pregnancy result groups (p=0.098, p=0.488, P=0.069, P=0.295, P=0.268, and P=0.124), as indicated in table (3-6). This result is in line with numerous studies, including the study by (Bjercke et al., 2005, Bedaiwy et al., 2007, Al-Ghazali and Al-Jarrah, 2013) whose mentioned the same results of current study.

5. Conclusion

The current study concluded that there were significant differences in serum levels of FSH, LH, E2 D2, AMH, and E2 at HCG injection hormones between the various causes of infertility. Although there is a correlation between these hormones and the result of pregnancy, it is not statistically significant. To understand the role of these hormones in infertile women, more research with a larger sample size is required.

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Effect of CYP2D6*10 (100C > T) Polymorphisms on Clomiphene Citrate Response in Iraqi Women with PCOS

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Abstract

Background

Clomiphene citrate (CC) is a commonly prescribed drug to induce ovulation in women with PCOS, a hormonal disorder affecting women of reproductive age. The CYP2D6 is an enzyme vital in metabolizing CC to its active metabolite. Variations in this gene, such as CYP2D6*10, which reduces enzyme activity, can affect drug metabolism and potentially impact treatment outcomes.

Materials and Methods

The cohort study, conducted from September 2023 to April 2024, enrolled 80 women diagnosed with PCOS. All patients received 100mg/day of CC from cycle day 2 for at least 2 cycles. Whole blood was collected for hormonal assays and CYP2D6*10 genotyping by ARMS PCR. Furthermore, an ultrasound was performed to determine follicle size and endometrial thickness during the cycle.

Results

We found that the frequency of CYP2D6*10 genotypes is 66.3% (**CC**), 27.5% (**CT**), and 6.3% (**TT**). Women with the mutant allele exhibited significantly lower concentrations of the active metabolite and higher concentrations of the prodrug ($p < 0.05$). At the same time, the CT genotype showed a higher AMH level.

Conclusions

Our findings prove an association between the CYP2D6*10 genotype and CC metabolism. However, further research with a larger sample size to confirm these findings. Additionally, an assessment of AMH level may also help predict CC.

تأثير التغايرات الجينية لـ(100C>T) CYP2D6*10 على استجابة عقار الكلومفين ستريت في النساء اللواتي يعانون من متلازمة تكيس المبايض

مريم حسين الهر, اثير مجيد رشيد, حسن محمود ابو المعالي

الخلاصة

المقدمة

كلوميفين سيتريت هو دواء يستخدم عادة كالخط الأول لعلاج تحفيز الإباضة لدى النساء المصابات بمتلازمة تكيس المبايض، وهو اضطراب هرموني يؤثر على النساء في سن الإنجاب. يعد إنزيم CYP2D6 حيويًا في استقلاب سيترات الكلوميفين إلى مستقلبه النشط. يمكن أن تؤثر الاختلافات في هذا الجين، مثل **CYP2D6*10**، المعروفة بتقليل نشاط الإنزيم، على استقلاب الدواء وربما تؤثر على نتائج العلاج.

المواد وطريقة العمل

أجريت الدراسة الأترابية في الفترة من سبتمبر 2023 إلى أبريل 2024 وسجلت 80 امرأة مصابة بمتلازمة تكيس المبايض. تلقى جميع المرضى 100 ملغ يوميا من كلوميفين ستريت من اليوم الثاني للدورة و لمدة دورتين على الأقل. تم جمع الدم لعمل الفحوصات الهرمونية والتنميط الجيني لـ(**CYP2D6*10**) بواسطة ARMS PCR. علاوة على ذلك، تم إجراء الموجات فوق الصوتية لتحديد حجم جريب المبيض وسمك بطانة الرحم خلال فتره العلاج.

النتائج

لقد وجدنا أن تواتر اختلافات **CYP2D6*10** هو 66.3% (النوع السائد **CC**)، و27.5% (متغاير الزيجوت **CT**)، و6.3% (المتغاير الطافر **TT**). أظهرت النساء ذوات الأليل الطافر تركيزات أقل بكثير من المستقلب النشط وتركيزات أعلى من الدواء الأولي ($P < 0.05$). في حين لم يتم العثور على فروق ذات دلالة إحصائية في الهرمونات الإنجابية، ولكن كانت مستويات AMH أعلى في النمط الجيني CT.

الاستنتاج

تثبت النتائج التي توصلنا إليها وجود علاقة بين النمط الوراثي **CYP2D6 * 10** واستقلاب عقار كلوميفين ستريت. ومع ذلك، فإن إجراء المزيد من الأبحاث باستخدام حجم عينة أكبر يجب أن يؤكد هذه النتائج ويوضح الآثار السريرية. بالإضافة إلى ذلك، قد يساعد تقييم مستوى AMH أيضًا في التنبؤ باستجابة عقار كلوميفين.

1. Introduction

Polycystic ovarian syndrome (PCOS) is one of the most prevalent reproductive endocrine disorders in women. This condition is complicated by inadequate treatment regimens, delayed diagnosis, and diagnostic difficulties(Hoeger et al., 2020). Infertility is brought on by this syndrome(Patel, 2018). One of the oldest medications that is still the treatment of choice for inducing ovulation in patients with PCOS is clomiphene citrate (CC)(Bashir et al., 2021). CC is a selective estrogen receptor modulator (SERM) that exists as (Z)-clomiphene and (E)-clomiphene(Euler et al., 2022). Inducing estrogenic and anti-estrogenic effects, the medication binds specifically to estrogen receptors in the ovary, endometrium, cervix, and hypothalamus resulting in the inhibition of negative estrogenic feedback, thus increasing gonadotropins which increase the release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH)(Feh and Wadhwa, 2022). CYP2D6 is an enzyme that metabolizes E-clomiphene and converts it into even more potent metabolites called E-4-hydroxy-clomiphene (E-4-OH-CLO). About 8–54% of women are not responsive to clomiphene treatment, and characteristics like obesity and hyperandrogenemia influence response variability. Furthermore, studies have demonstrated the significance of the highly polymorphic CYP2D6 enzyme in (E)-Clomiphene bio-activation (Kovar et al., 2022). The frequency of the CYP2D6 allele differs widely between ethnic and ancestral groups. The CYP2D6*10 (rs1065852) 100C > T polymorphism leads to the substitution of proline to serine and causes an mRNA splicing defect, producing an IM phenotype(El Akil et al., 2022). This study aims to investigate CYP2D6*10 genotypes and their association with CC response and metabolism.

2. Patients and Methods

2.1. Patients

80 PCOS women who had been unable to naturally conceive and were diagnosed with PCOS according to the updated Rotterdam consensus (Al-Asadi, 2024) were recruited from the infertility outpatient clinic from the period of September 1, 2023, to April 1, 2024, at Gynecological and Obstetric Hospital in Kerbala province, Iraq. PCOS women take clomiphene citrate 100 mg/ dose for more than one cycle and obtain written consent from participants after explaining the study's purpose and requiring them to complete a designed questionnaire. Patients were excluded if they had started clomiphene citrate therapy concurrently with an insulin sensitizer agent, lipid-lowering agent, or any other ovulation induction therapy. Other reasons for exclusion include endocrine disorder, History of untreated thyroid, adrenal disorders, or pituitary dysfunction. Other causes of infertility like Male or tubal factors were also excluded.

2.2. Materials

Clomiphene citrate (Clomid) was acquired from a commercial pharmacy. FSH, LH, estradiol, prolactin (PRL), and anti-Müllerian hormone (AMH) kits were obtained from Roche in Germany. Materials for genetic analysis, including the gSYNC™ DNA Extraction Kit, DNA ladder marker, Master Mix, and primers were purchased from Geneaid (Taiwan), Bioneer (Korea), BioLabs (USA), and Macrogen (Korea), respectively.

2.3. Methods

2.3.1. Study Design

A cohort study was conducted from September 2023 to April 2024. Before starting medication, participants were enrolled on the second day of their menstrual cycle. Follow-up assessments were performed on the twelfth day of at least two consecutive menstrual cycles after drug administration. Data collected included age, BMI, menarche, family history of other diseases, Clomiphene dosage, hirsutism, and regularity of menstruation. A specialist doctor performed ultrasound examinations to measure follicle size and endometrial thickness and Hormone levels were also assessed on cycle day 12.

2.3.2. Blood Collection

Blood samples were collected from each participant on cycle day 2 (before clomiphene) and cycle day 12 (after clomiphene). Approximately 6 mL of blood was drawn into K3 EDTA tubes for genomic DNA extraction and Gel & Clot Activator Blood Collection Tubes for hormonal analysis. Blood samples were centrifuged at 5000 rpm for 10 minutes to obtain plasma for hormonal level measurement and to assess drug and metabolite concentrations.

2.3.3. Assessment of Reproductive Hormones

The levels of FSH, LH, estradiol (E2), and AMH in the blood were measured on the 2nd and 12th days of the menstrual cycle using a Cobas e 411 Analyzer from Roche, Germany.

2.3.4. Determination of Concentrations Of E-Clomiphene and Its Metabolite

E-Clomiphene (E-CLO) and its metabolite E-4-OH-CLO were measured at the zist Gene Baft Lab in Tehran, Iran, using a SCIEX 4500 QTrap LC/MS/MS apparatus. Standard samples were prepared by dissolving them in a solution of 5% acetic acid and acetonitrile. A standard solution containing 10 ppm was introduced into the LC/MS/MS apparatus, and an 80:20 ratio of acetonitrile to water was used as the mobile phase. To identify unknown metabolites, the device was scanned from mass 360 to 450. The interface gas temperature was set to 500°C. A standard curve was constructed using serial dilutions of standard samples and ImageJ software to quantify metabolite concentrations based on the peak area ratio(Ganchev et al., 2011).

2.3.5. Genotyping Analysis

DNA was isolated from 200 µL of peripheral whole blood Using gSYNC™ DNA Extraction Kit (Geneaid/Taiwan) according to standard protocol. Extracted DNA was stored at −20°C before analysis. Genetic variation was examined of the metabolizing enzymes CYP2D6, we performed amplification refractory mutation system-polymerase chain reactions (ARMS-PCR). We included primers to amplify CYP2D6*10 100C>T (rs1065852) which was designed in (Hinrichs et al., 2007). Table1 provides information about the intended set of primers, including product sizes.

Table1: Sequence of the Primers for the Variant CYP2D6*10 with Product Size

SNPs	Primer sequence (5 ’->3 ’)	Product size
2D6*10 F out	GGG GCA AGA ACC TCT GGA GC	505 bp
2D6*10 R out	CTG GTC CAG CCT GTG GTT TC	
2D6*10 R WT	AGT GGC AGG GGG CCT GGA GG	351 bp
2D6*10 F*10	ACG CTG GGC TGC ACG CTT CT	192 bp
F out= outer forward primer, R out= outer reverse primer, R WT= reverse wildtype primer, F*10= Mutant primer		

Following several iterations of optimization, the PCR mixture contained 8 µL of OneTaq Quick-load 2X Master Mix (New England BioLabs/ USA), 2µL of extracted DNA, 1 µl from 2D6*10 F out primer, and 1 µl from 2D6*10 R out primer, and for 2D6*10 R WT and 2D6*10 F*10 primers, 1 µl of each were added to separate PCR tube as the volume completed to 20 µl with 7 µL of ddH2Othe. Final optimized thermal cycle conditions for this variation were as follows: 5 min at 95 °C of initial denaturation, followed by 35 PCR cycles of 30 sec at 95 °C (denaturation), 30 sec at 63 °C (annealing temperature), and 1 min at 72 °C (extension) then the final extension flowed 10 min at 72 °C. PCR products were separated based on 2% agarose gel at 45 V for 1 h and visualized by ethidium bromide under a UV illuminator. The 505bp represents the internal control while 351bp is an indication of a wildtype allele and 192bp is an indication of a mutant allele.

2.4. Statistical Analysis

Statistical analyses were conducted using SPSS 26. To determine whether the data are normally distributed, we conduct normality tests (Shapiro-Wilk test). The Wilcoxon Signed-Rank Test was utilized to compare study variables before and after treatment during menstruation. The Kruskal-Wallis test was employed to analyze differences among CYP2D6*10 genotypes (CC, CT, and TT). This non-parametric test is suitable for analyzing groups when the normality is unmet. Chi-square tests were used to assess categorical results. A P-value < 0.05 was considered statistical significance. It is worth mentioning that small sample sizes can introduce increased variability, reduce statistical power, and hinder the detection of true differences between groups.

3. Results

The study examined hormonal changes with endometrial and follicle development during treatment in the menstrual cycle. Table2 displays that estradiol (E2), LH, and the LH/FSH ratio significantly increased (P<0.05), while FSH remained relatively stable (P>0.05). Additionally, there were significant increases in follicle size and endometrial thickness at P=0.001.

Table2: Study Variables Before and After Treatment (Data Present as Median +IQR)

Parameter	Median +IQR (before treatment)	Median +IQR (after treatment)	*P*-value	Conclusion
E2	35.6+29.3	75.65+80.63	0.001	Significant
FSH	5.93+4.075	5.75+2.657	0.127	Not significant
LH	7. 85+5.76	14.95+7.485	< 0.001	Significant
LH/FSH ratio	1.3+1.27	2.3+2.0	0.001	Significant
Follicle size	6.0+2.0	16.0+8.75	0.001	Significant
ET	4.5+1.50	8.0+3.0	0.001	Significant
E2: Estradiol, FSH: follicle stimulating hormone, LH: luteinizing hormone, ET: endometrial thickness				

3.1. Distribution of CYP2D6*10

ARMS PCR was utilized to identify the CYP2D6*10 variation. The gel imaging results showed gel band patterns in Fig.1.

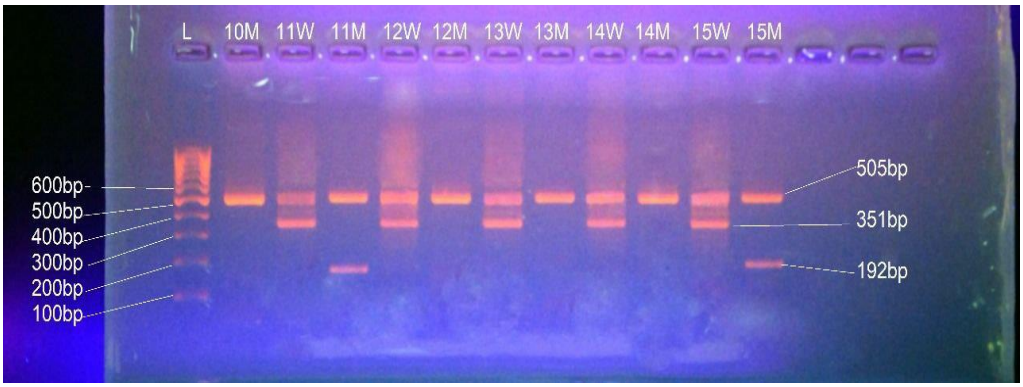

Figure 1: Image of CYP2D6*10 (100 C>T) Genotyping Using ARMS-PCR
L refers to DNA Ladder; M Indicates the Mutant Allele and W Indicates the Wildtype Allele.

It was found that the Frequency of the CYP2D6*10 allele in PCOS women carrying homozygous genotype **(CC)** is about 53(66.3%) and heterozygous genotype **(CT)** is 22(27.5%), while homozygous for the minor allele **(TT)** are 5(6.3%) as shown in the Fig.2

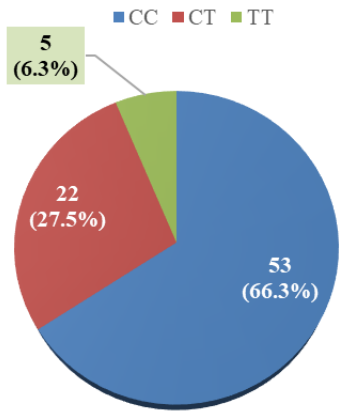

Figure2: Allele Genotypes Frequency of CYP2D6*10 for All Studied Women (N=80)
CC Represents Wildtype, CT Represents Heterozygous Carrier and TT Represents Homozygous Mutant.

3.2. Demographic Data

Demographic data including age, BMI, menarche, and marital duration were assessed in PCOS women. TT genotype showed older age (30y), earlier menarche (11) and higher BMI (72.2) but no significant differences were observed

compared to other genotype regarding these demographic factors (P > 0.05). Hirsutism and menstrual irregularity were assessed using the Chi-square test. Results for both hirsutism and menstrual irregularity were relatively similar (P > 0.05). as presented in Table3.

Table3: Demographic Data of PCOS Women Categorize According to Genetic Polymorphism (Data Present as Median + IQR and No (%))

Variables		Alleles of CYP2D6*10 100C > T			*P* – value
		CC (n=53)	CT (n=22)	TT (n=5)	
Age (y)		27+10.5	25+6.5	30+12.5	0.771
BMI (Kg/m^2)		26.2+6.1	27.3+2.1	27.2+7.6	0.907
Menarche (y)		12+1.5	12.5+1	11+4	0.278
Duration of Marriage (y)		6+4	4.5+1	3+10	0.928
Hirsutism	**No (n=40)**	26(49.1%)	11 (50%)	3 (60%)	0.896
	Yes (n=40)	27 (50.9%)	11 (50%)	2 (40%)	
Menstrual Regularity	**No (n=31)**	19 (35.8%)	9 (70.83%)	3 (60%)	0.554
	Yes (n=49)	34 (64.2%)	13 (29.1%)	2 (40%)	

3.3. Effects of CYP2D6*10 Genotypes on Hormonal Levels

The research examined the impact of CYP2D6*10 genotypes on reproductive hormones. Although there were variations in LH, LH/FSH ratio, and estrogen levels, none of these variances were statistically significant (P > 0.05). However, AMH levels exhibited a noteworthy increase in the CT genotype (3.5) compared to CC and TT at (P=0.002), as indicated in Table4.

Table4: CYP2D6*10 Genotypes Effect of Reproductive Hormones (Data Present as Median + IQR)

Variables	Alleles of CYP2D6*10 100C > T			Kruskal-Wallis H	P-vale
	CC (n=53)	CT (n=22)	TT (n=5)		
AMH (ng/ml)	2.5+1.8	3.5+2.1	3.1+1.5	12.108	0.002
FSH (mIU /ml)	5.9+3.4	5.4+4.7	5+2.3	0.77	0.68
LH (mIU /ml)	15+8.6	15.1+8.1	11.1+3.4	3.614	0.164
LH/FSH ratio	2.2+2.3	3.1+2	1.8+1	3.353	0.187
E2 (pg/ml)	75+88.3	64.2+68.4	125.4+127.2	3.562	0.168
AMH: Anti-Müllerian hormone, FSH: Follicle stimulating hormone, LH: Luteinizing hormone, E2: Estradiol					

3.4. Effect of CYP2D6*10 Genetic Polymorphism on Ultrasound

The impact of CYP2D6*10 genotypes on follicle size and endometrial thickness was investigated, and the findings are shown in Table 5. The median follicle sizes and endometrial thickness were similar across all genotypes, and no statistically significant differences were observed (P > 0.05).

Table5: Influence of CYP2D6*10 Polymorphisms on Ultrasound (Data Present as Median + IQR)

Variables	Alleles of CYP2D6*10 100C > T			Kruskal-Wallis H	P-value
	CC (n=53)	CT (n=22)	TT (n=5)		
Follicle Size (mm)	16+9	16.5+9.6	18+7	0.481	0.786
ET (mm)	8+3	8+1.5	8.5+3.3	0.705	0.703
ET= Endometrial Thickness					

3.5. Effects of CYP2D6*10 Polymorphism on E-Clomiphene Citrate And E-4-Hydroxyclomiphene Levels

As concentrations of drug (E-CLO) and its active metabolite E-4-hydroxy-clomiphene (4-OH-CLO) were measured at cycle day 12 to analyze the effect across different CYP2D6*10 genotypes, it had been observed that E-CLO levels were significantly higher in the TT genotype compared to CT and CC genotypes at $P<0.05$ and E-4-OH-CLO levels also differed significantly with CT and CC genotypes having higher levels than the TT genotype ($P<0.05$). These findings suggest that CYP2D6*10 genotypes influence the metabolism of E-clomiphene. Table6.

Table6: Effect of CYP2D6*10 Polymorphisms on Drug and Its Metabolite (Data Present as Median + IQR)

Variables	Alleles of CYP2D6*10 100C > T			Kruskal-Wallis H	*P* – value
	CC (n=53)	CT (n=22)	TT (n=5)		
E-CLO (nM)	3.4+0.5	3.4+0.5	3.9+0.4	10.786	0.005
E-4-OH-CLO (nM)	4.4+0.7	4.2+0.5	2.8+0.5	14.451	0.001

4. Discussion

Clomiphene citrate (CC) is a drug used for ovulation induction, especially for PCOS. It contains a mixture of unequal isomers known as E-clomiphene and Z-clomiphene (Rostami-Hodjegan et al., 2004).CC is a non-steroidal that demonstrates estrogenic agonist and estrogenic antagonist properties (Gadalla et al., 2018). CC demonstrates varying efficacy in inducing ovulation, with success rates ranging from 73% at 50 to 150 mg doses. Despite these overall positive outcomes, individual responses to CC can differ. While factors predicting resistance to CC are currently unclear, the inconsistent findings across studies hinder the accurate prediction of ovulation induction failure(Kane, 2021). CYP2D6 is important in metabolizing clomiphene as it acts primarily on E-clomiphene and converts it into active metabolites (Ghobadi et al., 2008). Previous research has indicated that the CYP2D6*10 (100C>T, rs1065852) polymorphism is related to an IM status which carries a higher risk of side effects or non-reaction to pro-drugs. The **(C)** allele represents the wild-type, while the **(T)** allele is a variant(Orengo-Mercado et al., 2013). The CYP2D6*10 allele differs among populations and ethnic groups, some Asian populations have a higher frequency of the gene than do those with European ancestry(Gaedigk et al., 2017). Since our study provides useful data on the CYP2D6*10 genotype frequency in Iraqi PCOS women, direct comparisons with other populations are limited due to the lack of published data from many countries. Our finding reveals a high frequency of the wild-type **CC** genotype, with a lower frequency of the **CT** and even lower mutant **TT** genotypes. This suggests that many PCOS women have normal CYP2D6 enzyme activity. Previous studies of Korean PCOS women have identified allele*10 as the most frequent allele(Ji et al., 2016) A similar study involving random volunteers in Korea also reported comparable findings(Kim et al., 2018). On the other hand, The CYP2D6*10 allele frequency varies across different populations in the Middle East and North Africa. Iran had the highest frequency of this allele at 20.4%, followed by Jordan and Turkey with frequencies of 14.8% and 13.14%, respectively. The lowest frequencies were observed in the United Arab Emirates, Saudi Arabia, and Syria, ranging from 3.3%, 3%, and 2.94% respectively(Khalaj et al., 2019). While it was reported in Iraq at a frequency of 13.4%. Arabs exhibited a higher frequency of normal metabolizers (NMs) compared to Europeans, East Asians, and Americans, with rates of 70.53% in Arabs, 51.05% in Europeans, 51.91% in East Asians, and 63.6% in Americans(Alali et al., 2022).

The study focused on women with PCOS and prolonged infertility, aiming to understand the potential role of genetic factors in this condition. All participants had a similar age of menarche, suggesting that the CYP2D6 polymorphisms studied do not influence menarche onset. Obesity is commonly associated with PCOS (Messinis et al., 2015), the study found no significant differences in BMI among the genotypes, indicating that obesity in PCOS is not linked to CYP2D6*10 variations and it has been proposed that women with a higher BMI may require larger doses of Clomiphene due to their potential resistance to the medication (Ghobadi et al., 2009). Hirsutism is a frequent occurrence in women with PCOS(Oliveira and Comim, 2024) half of the participated PCOS women had it. Irregular periods are also one of the most common features of PCOS resulting from elevation in free testosterone caused by obesity(Mari et al., 2023) Both show no relation with CYP2D6*10 polymorphism. In conclusion, this study did not find a significant association between the CYP2D6*10 genotype and demographic characteristics in PCOS women.

This study demonstrates the effectiveness of CC therapy in inducing ovulation and preparing the reproductive system for pregnancy in women with PCOS. Our study suggests that the CYP2D6*10 genotype may influence AMH levels in women with PCOS. Women with the **CT** genotype, carrying one variant allele, exhibited significantly higher AMH levels than those with the **CC** (wild-type) or **TT** (mutant) genotypes. AMH is a biomarker of ovarian reserve and can affect follicular development and response to ovulation induction therapies(Peluso et al., 2014). Higher AMH levels have been associated with reduced sensitivity to FSH and increased resistance to CC treatment(Garg and Tal, 2016). Therefore, the observed association between CYP2D6*10 genotype and AMH levels may partially explain the variability in response to CC therapy among PCOS patients. The significant increases in LH, LH/FSH ratio, follicle size, and endometrial thickness observed after treatment are consistent with the known mechanisms of action of CC. The increase in E2 levels is likely a secondary effect of the increased LH, as LH stimulates ovarian follicle development and estrogen production(Holesh et al., 2017). The significant increase in LH levels is a key factor in inducing ovulation. The increase in follicle size is essential for the development of a mature egg that can be released during ovulation and thickened endometrium is necessary for the implantation of a fertilized egg (Rachmawati et al., 2023). Overall, these findings highlight the effectiveness of CC therapy in PCOS. Although no significant differences were found in these reproductive hormones (FSH, LH, LH/FSH ratio, and E2) between the three genotypes. Furthermore, the results of this study indicate that the CYP2D6*10 genotype may not notably affect follicle size or endometrial thickness in women with PCOS undergoing CC therapy. Although there were minor differences in these parameters among the genotypes, they did not show statistical significance. Several factors, including sample size, other genetic polymorphisms, or factors influencing follicle development and endometrial thickness in PCOS, could contribute to the absence of a clear association between the CYP2D6*10 genotype and ultrasound parameters.

Lastly, Previous studies have demonstrated that the CYP2D6*10 allele is associated with a reduced-function phenotype, leading to decreased enzyme activity (Kane, 2021), and according to the proposed theory, individuals with an intermediate metabolizer (IM) phenotype were expected to have lower levels of active drug metabolites. Another study reported a direct correlation between the amount of CYP2D6 present and the extent of E-clomiphene metabolism (Ghobadi et al., 2008). Our study implies that the CYP2D6*10 genotype can influence the metabolism of CC in women with PCOS. Patients with the **TT** genotype, carrying two variant alleles, have high levels of the parent drug (E-CLO) and lower levels of the active metabolite (E-4-OH-CLO). These findings align with a Korean study that

identified significant differences in parent drug concentration between the wild-type and mutant genotypes. Additionally, the elimination half-life and total drug exposure of E-4-OH-CLO were significantly longer in individuals with the mutant genotype compared to those with other genetic variations (Kim et al., 2018). Similarly, another study identified a correlation between CYP2D6*10 genotypes and plasma concentrations. Plasma levels of the active metabolites were 8 times higher than the (PM) women, who had parent drug concentrations (E-CLO) 6 times higher(Mürdter et al., 2012).

This study has some limitations, such as the relatively small sample size, particularly for the TT genotype, which may have limited the statistical power to detect significant differences in reproductive hormones.

5. Conclusion

In conclusion, the metabolite concentrations were significantly changed across the genotypes although the reproductive hormones and ultrasound were not, and thus these findings highlight the complexity of genetic influences on these different parameters and suggest that other genetic, environmental, or lifestyle factors may be more relevant in understanding variations in these traits. Further research could explore additional genetic markers or larger populations to confirm these observations.

6. Ethical Approval

The research was conducted in Kerbala city and was reviewed and approved by the Training and Human Development Center/research committee in Kerbala with approval number 2023180. All patients provided informed consent before they participated in the study.

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The Impact of the Rs3806596; T>C Genetic Polymorphism of UDP Enzyme on the Response to Deferasirox Drug Among Iraqi Thalassemia Patients

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Abstract

Background: thalassemia is an autosomal recessive hereditary condition, Patients with beta thalassemia need ongoing blood transfusions and iron chelating treatment due to the body's inability to excrete iron. Clinical observations of transfusion dependent thalassemia (TDT) patients in the local community show that many of them continue to have elevated blood ferritin levels even after receiving treatment with the iron chelator Deferasirox (DFX). One of the primary UGT1 enzyme families that metabolizes DFX is the UDP-glucuronosyltransferase 1A3 (UGT1A3) gene. This study examines the impact of the UGT1A3 gene's rs3806596; T>C single nucleotide polymorphism (SNP) on Iraqi TDT patients' clinical response to DFX chelation treatment.

Methodology: Cross-sectional study included 96 TDT patients of Iraqi male and female.They were administered with 30–40 mg/kg of oral DFX daily for at least 3 months, and they ranged in age from 8 to 39. Serum erythroferrone, liver and kidney function tests, and serum ferritin were evaluated. The method of allele-specific polymerase chain reaction was used to identify the rs3806596; T>C SNP.

Results: The genotype distribution of rs3806596; T>C SNP among Iraqi population was 36.5%, 18.5% and 44.8%, for TT, TC and CC respectively. The frequency of the wild (T) allele is 0.46, compared to 0.54 for the mutant (C) allele. The mutant group (CC) of rs3806596; T>C SNP showed no significant in the three different groups.

Conclusion: There is no significant association between rs3806596; T>C SNP of the UGT1A3 gene and the therapeutic response to DFX in our samples of Iraqi population for TDT patients.

تأثير التعدد الشكلي الجيني للطفرة Rs3806596; T>C لإنزيم UDP على استجابته لدواء ديفيراسيروكس بين مرضى الثلاسيميا العراقيين

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الخلاصة

الثلاسيميا هي حالة وراثية متنحية تؤثر على إنتاج الهيموجلوبين، ويحتاج مرضى الثلاسيميا بيتا إلى عمليات نقل دم مستمرة، كما أن العلاج بالاستخلاب بالحديد ضروري بسبب عدم قدرة الجسم على طرح الحديد بشكل طبيعي. ومع ذلك، تُظهر العلامات السريرية لمرضى الثلاسيميا المعتمدين على نقل الدم (TDT) في المجتمع المحلي أن العديد منهم يستمرون في الحصول على مستويات مرتفعة من الفيريتين في الدم حتى بعد تلقي العلاج باستخدام عقار ديفيراسيروكس (DFX). إحدى عائلات إنزيم UGT1 الأساسية التي تستقلب عقار ديفيراسيروكس هي جين UDP-glucuronosyltransferase 1A3 (UGT1A3). تتبين هذه الدراسة تأثير تعدد أشكال النوكليوتيدات المفردة (SNP) لجين UGT1A3 على الاستجابة السريرية لمرضى الثلاسيميا العراقيين المعتمدين على نقل الدم لعلاج الاستخلاب بعقار ديفيراسيروكس.

العينات وطرق العمل

شملت هذه الدراسة المقطعية ستة وتسعين مريضًا مصابين بالثلاسيميا المعتمدين على نقل الدم (TDT) من الذكور والإناث العراقيين، تم إعطاؤهم جرعة 30–40 مجم / كجم من DFX عن طريق الفم يوميًا لمدة ثلاثة أشهر على الأقل، وتراوحت أعمارهم بين 8 إلى 39 عامًا. تم تقييم مصل إريثروفيرون ومصل الفيريتين واجراء اختبارات لوظائف الكبد والكلى. تم استخدام طريقة تفاعلات الكوثرة المتسلسلة النوعية للألليل لتحديد تأثير التعدد الشكلي الجيني للطفرة rs3806596; T>C.

النتائج

اثبت ان توزيع النمط الجيني لـrs3806596; T>C بين السكان العراقيين هو 36.5٪ و 18.5٪ و 44.8٪، لـ TT و TC و CC على التوالي. حيث كان معدل الأليل السائد (T)هو (0.46)، مقارنة بـ (0.54) للأليل المتنحي (T). لم تظهر المجموعة المتنحية (CC) من rs3806596؛ T>C SNP أي تأثير أو أهمية بين المجموعات الثلاث المختلفة.

الاستنتاج

لا يوجد تأثير واضح بين rs3806596؛ T>C SNP لجين UGT1A3 والاستجابة العلاجية لـ DFX في عيناتنا من السكان العراقيين لمرضى TDT.

1. Introduction

Beta-thalassemia is an autosomal recessive hereditary condition that affects the β-chain of hemoglobin production. In the Eastern Mediterranean area, including Iraq, the disease is prevalent (Yahya, 1996, Hamamy and Al-Allawi, 2013). The β-thalassemia mutations cause a range of clinical symptoms, starts from thalassemia minor in individuals who are heterozygous for it to β-thalassemia major (β-TM) in those who are homozygous or compound heterozygous , β-TM phenotype is aserious condition linked to several problems with a lifelong need for blood transfusions, stunted growth, diabetes mellitus, liver illness, hypothyroidism, hypoparathyroidism and hypogonadism are among the most common consequences (Galanello and Origa, 2010). With a significantly higher quality of life and fewer problems in developed compared to developing nations, the frequency of these issues vary somewhat throughout β-TM cohorts and are related to the effectiveness of care and follow-up given to these patients (Mikael and Al-Allawi, 2018). Patients should usually start iron chelation treatment when their ferritin levels above 1000 ng/ml or after ten to twenty times blood transfusions (Cappellini et al., 2013). Excessive serum iron can increase the morbidity and death if it is not treated appropriately (Tanaka, 2014). Chelation therapy with parenteral deferoxamine has been the standard treatment for iron overload, but patient compliance is often low due to the discomfort and demands of its administration regimen. Therefore, significant emphasis has been placed on creating oral chelating agents. Deferiprone has been available in Asia for nearly twenty years and has shown promising results for cardiac iron removal and long-term effectiveness. However, its serious side effects, such as agranulocytosis and neutropenia, have hindered its widespread use in clinical settings. Deferasirox (DFX) is a new oral chelator that effectively eliminates iron from the heart and liver over the course of a 24-hour dosage period, according to preclinical and clinical studies. The best way to treat patients with severe thalassemia is the accessibility of oral iron chelators, which remain significant obstacles in many Asian nations (Viprakasit et al., 2009). In more than 70 country worldwide, including the US in 2005 and the EU in 2006, DFX has been approved as a first-line therapy for thalassemia major (Cappellini et al., 2013). DFX is a tridentate ligand that is achiral and exhibits a strong affinity and selectivity for ferric iron (Fe3+) (Nick, 2007). Only over 8% of DFX and its metabolites are removed by the kidneys; the majority, roughly 84%, is removed in the feces. Uridine glucuronosyltransferase (UGT) is the primary enzyme responsible for the drug's substantial metabolism by glucuronidation (Allegra et al., 2017). DFX at dosages of 10 to 40 mg/kg was found to reduce liver iron, lower serum ferritin and increase iron excretion in thalassemia patients (Cappellini et al., 2013). Prolonged DFX treatment may have serious side effects, such as bone marrow suppression, gastrointestinal bleeding, liver and kidney failure (Cusato et al., 2015). The essential metabolic route for DFX is glutaronidation, which is primarily carried out by UDP glucuronosyltransferase family 1 member A1 (UGT1A1) and, to a lesser degree, by UDP glucuronosyltransferase family 1 member A3 (UGT1A3)(Agent, 2021). Through a process known as glutaronidation, phase 2 conjugation routes enhance the clearance of a variety of substances by increasing their solubility in water (Nagar and Blanchard, 2006).The UGT1A3 gene produces a member of the UDP-glucuronosyltransferase (UGT) family, which is involved in the glucuronidation process, which is a vital part of the metabolism of many endogenous and exogenous substances. The detoxification and removal of medications, hormones, and other chemicals from the body depend heavily on this enzyme activity and expression. Individuals' susceptibility to specific diseases and how they react to drugs may be impacted by variations in the UGT1A3 gene (Mullapudi et al., 2021), which certainly affect the drug serum concentration resulting in variation in drug response. Although the liver is where UGT1A3 is mostly expressed, it is also present in the bile ducts, the stomach

and the intestines (Sabolovic et al., 2000). The single UGT1 gene on chromosome 2q37 encodes UGT1A3. All eight additional functional UGT isoforms expressed from the UGT1A locus share four of the five exons that make up the UGT1A3 mRNA, which encodes the C-terminal half of the molecule(Caillier et al., 2007).

The rs3806596; T>C single nucleotide polymorphism (SNP) lies inside the UGT1A3 gene's main promoter region, specifically located. A previous research has shown that polymorphisms in the promoter region of UGT1A3 are linked to differences in drug responses and the risk of various diseases (Mullapudi et al., 2021). This study aimed to investigate the impact of the rs3806596; T>C SNP in the UGT1A3 gene on the therapeutic response to DFX.

2. Materials and Methods

2.1. Study Subjects

The Scientific and Ethical Committee of Karbala University College of Pharmacy examined and approved this cross-sectional study. (Reference number: 2023HU7).

Between November 2023 and April 2024, 96 individuals with iron excess beta-thalassemia major (specifically transfusion-dependent thalassemia, or TDT) were selected from a total of 650 patients at Karbala Teaching Hospital for Children, a thalassemia specialty center. The patients were between the ages of 8 and 39. Prior to being recruited for the study, each participant provided written informed consent that included a detailed explanation of the study's goals. The participants were also given a specially designed questionnaire to complete. The subjects had been receiving DFX chelation medication as monotherapy for a minimum of three months. The highest dose of DFX that is advised by the Thalassemia International Federation is 30–40 mg/kg/day; this is the same dose that was chosen for this study. Alcohol users, patients with sickle cell anemia, patients with liver illness and patients with thalassemias other than beta-thalassemia were excluded from the study. Patients having any medical or surgical problems that may substantially impact a drug's absorption, metabolism, or excretion were also not allowed to participate in this study.

Using a disposable syringe, five milliliters of blood were extracted from each participant's vein(Miran et al., 2024). A plain tube devoid of anticoagulants was filled with 2.5 milliliters of blood, which was allowed to coagulate for around half an hour. The serum required to assess the amounts of human erythroferrone (ERFE) and other biomarkers was extracted from the blood samples by centrifuging them at a speed of 4000 x g. Before being utilized, the sera samples were kept at -20 °C. The second portion of the blood sample (2.5 ml) was transferred into evacuated ethylenediaminetetraacetic acid (EDTA) tubes, for other hematological testing and DNA extraction.

2.2. Genetic Analysis

Following the manufacturer's instructions, the genomic DNA was extracted from the whole blood samples using the Geneaid DNA Extraction/Genomic DNA Purification Prep Mini Kit (Taiwan). An allele-specific polymerase chain reaction (PCR) method was used to find the rs3806596; T>C (SNP). The lyophilized primers were developed with the Primer-BLAST software and provided by Macrogen-Korea(Kadhim and Gaaib, 2024). Each primer was dissolved in a predetermined volume of nuclease-free water to create a stock solution with a concentration of 100 pmol/µL. The diluted (10 pmol/µL) working solutions were made by mixing 90 µL of nuclease-free water with 10 µL of each forward and reverse primer stock solution. The working solutions were stored at -20 °C until they were utilized. The primer sequences are as follows: The forward primer is 5′-ATCCTGGTGCGAAAAACGA-3′, the first reverse primer is 5′ CCTGCTACATTTGCTTTCTTCA-3′, and the second reverse primer is 5′-CCTGCTACATTTGCTTTCTTCG-3′. 12 µL of master mix (Promega, United States

of America), 2 µL (100 ng/µL) of the extracted DNA, 1 µL (10 pmol/µL) of the forward primer, 1 µL (10 pmol/µL) of the reverse primer, and 9 µL of nuclease-free water made up the 25 µL PCR reaction mixture. The following programming conditions were used for the PCR amplification: a 3-minute initial denaturation step at 95 °C, followed by 30 cycles of amplification that included 30 seconds of denaturation at 95 °C, 50 seconds of annealing at 64 °C, and 40 seconds of extension at 72 °C. The last extension phase was carried out at 72 °C for five minutes. Electrophoresis was performed on a 2% (w/v) agarose gel to confirm the amplification and identify the PCR products.

2.3. Biochemical Analysis

Red blood cell (RBC) and hemoglobin (Hb) levels in patients are precisely measured with the Swelab Alfa plus Basic Hematology Analyzer, which provides crucial information for evaluating blood health and identifying any medical disorders. A Sunlong Biotech kit was used to assess human ERFE levels using an enzyme-linked immunosorbent assay (ELISA) sandwich technique. Human Humareader HS and Human Combiwash from Germany were used to process the test. While the Mindray BS 240 system was used to perform the liver and kidney function tests, the Cobas e411 analyzer from Germany was used to detect the levels of serum ferritin (SF).

2.4. Statistical Analysis

Each participant received a unique identifying number, and a data sheet was used to record their responses. Multiple entries were made for verification in order to minimize mistakes. The Statistics Package for the Social Sciences, 22nd edition (SPSS Inc., Chicago, US), was used to analyze the study data.

The Chi-square value was obtained by using the goodness of fit test to determine if Hardy-Weinberg equilibrium applied to allele distribution. For two groups, the T-test was used to determine the means of the hematological and biochemical parameters; for three or more groups, a one-way ANOVA was used. A p-value of less than 0.05 was deemed to indicate a meaningful change. (AL-Safar et al., 2024)

3. Results

This study included ninty-six Iraqi patients with major β-thalassemia, 42 of whom were male and 54 of whom were female. The rs3806596; T>C SNP was found using allele-specific PCR. Whether T or C alleles were present affected the PCR amplification. The T column of the agarose gel showed a single 519 bp PCR band for the wild type (TT). Two PCR bands, each measuring 519 bp in size were seen in the T and C columns for the heterozygous mutant type (TC). Only the C column showed a single 519 bp PCR band for the homozygous mutant type (CC). Fig.1 illustrates the outcomes.

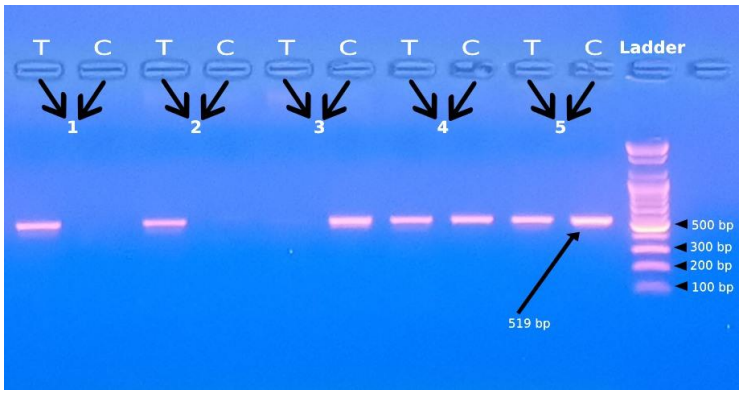

Figure1: Agarose Gel Electrophoresis Employing Allele-Specific PCR to Detect the Rs3806596; T>C SNP. Wild type (TT) is represented by lanes 1 and 2, heterozygous mutant type (TC) by lane 4 and 5 and homozygous mutant type (CC) by lane 3.

The sex distribution and other demographic properties of the study participants are demonstrated in Table1. According to the patient demographics, the participants' ages ranged from 8 to 39 years old, with around 67% of them being under 19 and just two of them being older than 30. About 82.3% of the participants were urban residency while only 17.7% were rural. Based on marital relativity or paternal consanguinity, the research groups were split into two sex distribution and other demographic characteristics. The rs3806596; T>C SNP genotype distribution revealed that the wild type (TT) allele is making up around 36.5% of individuals, whereas the mutant (CC) allele only makes up 44.8% of the research participants Table2. The patients were divided into three groups according to their genotype. Notably, the analysis reveals no significant differences were observed in the hematological and biochemical parameters Table3.

Table 1: Demographic Characteristics of the Study Subjects

Character		Frequency (%)
Sex	Male	42(43.4)
	Female	54(56.3)
Age	8-18 years old	64 (66.6)
	19-39 years old	32 (33.3)
Residency	Urban	79(82.3)
	Rural	17(17.7)
Marital relativity	Yes	85(88.5)
	No	11(11.5)
Blood group	A	32(33.3)
	B	22(22.9)
	AB	9(9.4)
	O	33(34.4)

Table2: The Genotype Distribution of rs3806596; T>C SNP Among the Study Subjects

Genotype	Frequency (%)	Chi squared	P value
TT	35(36.5)	37.19	0.0001
TC	18(18.8)		
CC	43(44.8)		
Allele	Frequency		
T	0.46		
C	0.54		

Table 3: The Association Between Rs3806596; T>C SNP And the Hematological and Biochemical Parameters

Parameter	TT (n=35) Mean ±SD	TC (n=18) Mean ±SD	CC (n=43) Mean ±SD	P value
Age (year)	17.11±1.11	17.05±1.53	15.02±1.09	0.34
BMI (kg/m^2)	18.12±0.63	18.84±0.69	18.31±0.50	0.76
Dose (mg/Kg/day)	34.02±0.53	33.72±0.86	33.76±0.61	0.93
SF (ng/ml)	3432.34±376.84	3060.88±365.47	3324.97±323.68	0.72
AST (U/L)	38.34±2.43	39.91±5.66	40.89±5.63	0.72
ALT (U/L)	34.78±4.53	32.13±6.01	39.81±6.77	0.53
S. creatinine (mg/Dl)	0.36±0.03	0.36±0.03	0.29±0.01	0.13
Blood urea (mg/dL)	26.73±1.33	26.56±1.72	25.76±1.51	0.87
TSB (mg/dL)	1.74±0.15	1.75±0.14	2.25±0.18	0.06
ERFE (ng/L)	39.42±2.82	53.00±8.16	43.72±4.58	0.18
RBC (Million/mm3)	3.06±0.09	2.98±0.15	2.88±0.07	0.35
HB (g/ dL)	8.10±0.18	8.20±0.24	7.90±0.14	0.51
Blood TF (Days)	21.97±2.78	18.27±0.89	19.02±0.83	0.37

4. Discussion

This study looked at how the rs3806596; T>C SNP affected the therapeutic response to the DFX medication in TDT patients from Iraq. People with thalassemic syndrome may get iron overload as a result of frequent blood transfusions. Since the body lacks the ability to remove excess iron, an iron-chelation therapy, such as a DFX medication, must be taken (Chaudhary and Pullarkat, 2013, Naderi et al., 2013). The symptoms of thalassemia range from basic chronic tiredness and arthropathy to cardiomyopathy, diabetes, and liver cirrhosis, and many individuals are unresponsive to DFX. Pharmacogenetic and demographic researches are crucial for determining the optimal therapeutic response and more precisely identifying potential causes of treatment failure.

TDT is positively impacted by consanguinity, especially in nations where marriages between cousins are widespread (64.6% of the Iraqi population) (Yahyaa et al., 2019). Consanguinity seems to have a significant role in the extent of the problem in Iraq; about 88.5% of the patients in this research were the children of first- and second-cousin marriages. Similar findings (88%) were observed by other previous studies (Al-Haj, 1992), (Adaay et al., 2011), whereas Awad reported a lower result (41.6%) in his study (Awad, 1999). A, B, AB, and O were the four groups into which the research participants were assigned based on the ABO blood group classification. The blood group O had the largest percentage among TDT patients (34.4%), followed by the A blood group (33.3%), while the blood group AB had the lowest rate (9.4%). These findings were consistent with earlier research on the Iraqi population (Marbut et al., 2018, Adaay et al., 2011). According to the current study, the patients' BMI is around 18 kg/m^2, and several previous studies show that over half of β-thalassemia patients are underweight (Salih and Al-Mosawy, 2013, Soliman et al., 2023, Hammod et al., 2018). Our findings are mostly explained by the fact that the majority of participants (66.6%) were under the age of 18 (Table 1). Thalassemia has historically been associated with a 30% to 60% risk of short height and developmental failure in children. However, the risk of short stature has considerably lowered with current adherence to modern transfusion and iron chelation regimens, as well as cautious treatment to prevent iron chelator overdose, potentially enhancing endocrine development in children with TDT (Farmakis et al., 2022). Based on their genotype, the research participants were split into three groups: TT, TC, and CC groups. This study showed that 36.5% of the patients carry the wild type (TT), 18.8% carry TC and 44.8% carry the CC genotype, this means that the prevalence of rs3806596; T>C SNP in the study subjects is 63.6%. The Thalassemia International Federation Guidelines 2021 for (TDT) state that DFX should be taken at a dose of 10–40 mg/kg per day (Babu and Panachiyil, 2022). Only patients who received 30–40 mg/kg/day of DFX as their daily dose were included in this study in order to identify the cases that were not responsive to chelation treatment, even at high doses, and to investigate any possible association with genetic polymorphism analysis. Hematological and biochemical indicators did not significantly differ between patient groups. Serum ferritin (SF) is a crucial indicator of excessive iron (Farmakis et al., 2022). It is a simple and reasonably priced test for assessing iron overload. The TDT Guidelines 2021 state that its levels are used to determine if DFX treatment is being received appropriately or insufficiently. They are connected with the total body iron burden (Chirnomas et al., 2009, Brittenham et al., 1993) .Since DFX is metabolized by the liver glucuronidation, mainly by UGT1A1,UGT1A3 as well as bilirubin (Waldmeier et al., 2010) genetic polymorphism of rs3806596; T>C SNP show no significant results or impact on gene expression in our study of Iraqi population as the following previous study (Cao et al., 2020), while in other study there was a significant result for this SNP in certain different population (Cusato et al., 2016). A glycoprotein hormone called erythroferrone functions as a "responsive erythroid" regulation element for erythropoietin (Saad et al., 2021).

Because of inefficient erythropoiesis, ERFE is elevated in thalassemia and plays a critical role in controlling the release of stored iron (Kautz et al., 2014b, Kautz and Nemeth, 2014). It also mediates the suppression of the iron-regulatory hormone hepcidin, which promotes iron intake and mobilization from storage, and is essential for the recovery of hemorrhage-induced anemia (Kautz et al., 2014a). No significant results has been proved for this study, this confirm that the rs3806596; T>C SNP has no impact on the UGT1A3 gene in DFX users. Nephrotoxicity from deferasirox may show up as a little increase in blood creatinine levels. However, there is currently no clearly defined threshold value for determining nephrotoxicity,(Cappellini et al., 2011, Cappellini et al., 2006, Cappellini et al., 2010). A reduction in glomerular filtration rate, either acute or chronic, is one of the nephrotoxic DFX side effects. Nephrotoxicity's molecular mechanisms are still poorly understood, despite the fact that it is frequently reversible and/or non-progressive (Díaz-García et al., 2014). In the present study serum creatinine showed normal values.

5. Conclusion

There is no significant association between rs3806596; T>C SNP in the UGT1A3 gene and the hematological and biochemical parameters in Iraqi thalassemia patients treated with deferasirox.

6. Conflicts of Interest

The authors declare no conflict of interests

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Demographic and Clinical Assessment of Warfarin Treatment in Thromboembolic Disease

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Abstract

Introduction: Warfarin is a widely prescribed oral anticoagulant used to prevent thromboembolic events. However, its therapeutic response varies significantly among individuals due to clinical and demographic factors. Personalized warfarin dosing is essential to minimize complications such as bleeding or clotting. This study aims to investigate the clinical and demographic factors that may influence the required weekly dose of warfarin in patients receiving anticoagulation therapy.

Materials and Methods: A cross-sectional study was conducted involving patients on warfarin therapy. Key variables collected included age, sex, body weight, INR (International Normalized Ratio), and platelet count. Data were analyzed to identify correlations between these variables and the weekly warfarin dose.

Results: Statistical analysis revealed that both body weight and age significantly influenced warfarin dose requirements. Higher body weight was associated with a higher therapeutic dose, whereas older age was associated with a lower dose requirement. No significant associations were found between warfarin dose and sex or platelet count.

Conclusion: Age and body weight are two critical demographic factors that should be considered when determining the optimal warfarin dose. Adjusting dosage based on these parameters may enhance therapeutic outcomes and reduce the risk of adverse events, supporting a more individualized approach to anticoagulation therapy.

التقييم الديموغرافي والسريري لعلاج الوارفارين في الأمراض الخثارية الصمامية

علاء علي محمد، آمال عمران موسى، أبو المعالي حسن محمود

الملخص

المقدمة

يُعد الوارفارين من مضادات التخثر الفموية الشائعة الاستخدام للوقاية من الحوادث الخثارية الصمامية. ومع ذلك، فإن الاستجابة العلاجية له تختلف بشكل كبير بين الأفراد بسبب عوامل سريرية وديموغرافية. إن التحديد الشخصي لجرعة الوارفارين يُعد أمراً ضرورياً للحد من المضاعفات مثل النزيف أو التجلط. تهدف هذه الدراسة إلى فحص العوامل السريرية والديموغرافية التي قد تؤثر على الجرعة الأسبوعية اللازمة من الوارفارين لدى المرضى الخاضعين للعلاج بمضادات التخثر.

المواد والطُرق

أُجريت دراسة مقطعية شملت مرضى يتلقون علاجاً بالوارفارين. تم جمع بيانات أساسية شملت: العمر، الجنس، وزن الجسم، النسبة المعيارية الدولية(INR) ، وعدد الصفائح الدموية. وتم تحليل البيانات لتحديد العلاقة بين هذه المتغيرات والجرعة الأسبوعية من الوارفارين.

النتائج

أظهرت التحليلات الإحصائية أن كلاً من وزن الجسم والعمر يؤثران بشكل كبير على متطلبات جرعة الوارفارين. إذ ارتبط الوزن الأعلى بزيادة في الجرعة المطلوبة، في حين كان التقدم في السن مرتبطًا بانخفاض الحاجة إلى الجرعة. ولم تُظهر التحليلات وجود علاقة ذات دلالة إحصائية بين الجرعة والجنس أو عدد الصفائح الدموية.

الاستنتاج

يُعد كل من العمر ووزن الجسم عاملين ديموغرافيين حاسمين يجب أخذهما بعين الاعتبار عند تحديد الجرعة المثلى للوارفارين. إن تعديل الجرعة استنادًا إلى هذه العوامل قد يُحسّن النتائج العلاجية ويُقلل من خطر حدوث المضاعفات، مما يدعم التوجه نحو نهج علاجي فردي أكثر فعالية في معالجة حالات التخثر المزمنة.

1. Introduction

Warfarin is one of the most frequently used drugs for blood clotting diseases. This drug is particularly very important in patients who have an increased risk for clot formation, say, patients who are in atrial fibrillation (AF), deep vein thrombosis (DVT), pulmonary emboli (PE), or patients fitted with certain kinds of prosthetic heart valves. Embolic phenomena for these patient categories are very dangerous, and when not controlled tend to lead to a cerebrovascular accident, pulmonary embolism, and sometimes death. Warfarin pharmacology is also tied to its mode of action – in this case, factors II, VII, IX and X that are necessary in blood coagulation are inhibited through the control of vitamin K synthesis. Warfarin lowers the body's ability to clot and, hence, again offers protection against the tendency to clot. Nevertheless, Warfarin therapy comes with immense challenges, stemming from the narrow therapeutic index as well as to variations in the individual's responses to the drug, making the titration of the dosage a critical component of the therapy(Holford, 1986, Wittkowsky, 2003). Managing warfarin dosing, however, has its own difficulties due to its low therapeutic index. In situations of under-anticoagulation, patients may be at an increased risk of thromboembolic events, on the other hand, over-anticoagulation leads to the risk of severe bleeding episodes like GI or intracranial hemorrhage, which can be fatal. Monitoring of warfarin therapy has been done through the use of the International Normalized Ratio (INR), a ratio which represents the time taken by blood to clot in an individual (Tang et al., 2003). INR is used by clinicians to titrate warfarin dosage, and for most thromboembolic conditions, a value of 2.0 to 3.0 is the typical target. But some conditions might even specify target ranges depending on patient's comorbidities and risk factors. As such, it is critical to keep the patient's INR, or to be more specific, the time his or her blood can be expected to be free from clotting, within the target range in order to avoid both over and under-pescribed and administered doses of the drug. This clearly highlights the case for individualized dosing of warfarin therapy (Wigle et al., 2013). To achieve the desired effect of allowing the blood to coagulate after aminocaproic acid is injected, blood loss is ideally controlled from an initial approximate dose based on the age weight body structure and health of the patient. However, even that rate can fluctuate, as the inter-individual responding to warfarin is high, and such parameters alone do not address it automatically (McNicol et al., 1961). This dynamic is caused by different demographic, clinical, as well as genetic traits that affect the pharmacodynamics and pharmacokinetics of warfarin. The most critical key demographic for warfarin response variations are age, sex and body weight combined with liver and renal functions along with medication. For example, there is a common practice within the drug prescribing include patients with liver or renal insufficiency who also show lower doses of warfarin effective (Limdi et al., 2010). Another biographical characteristic that importantly influences individual response to warfarin therapy is the weight of a patient. Elderly patients usually require less doses, because of changes in hepatic and renal function associated with aging resulting in reduced clearance of the drug. Consequently, older patients become more prone to situations of over-anticoagulation and are likely to suffer higher cases of bleeding tendency if the doses are not properly controlled)Garcia et al., 2005(. Weight likewise, helps to determine the pharmacokinetics of warfarin, because it is known that the more the body mass the larger the therapeutic dose is bound to be. This is explained by the need for a higher volume of distribution and circulation to achieve the required concentration of the anticoagulative material. Medical literature data aims to prescribe a lower warfarin dose to an average person, whereas these aims are unreasonable for a significant number of patients who are aimed at reducing the risk of either thrombotic or hemorrhagic events (Gong et al., 2011). Albeit demographic characteristics, genetic factors also contribute in influencing the warfarin metabolism. It is well-

established that warfarin metabolism is influenced by genetic variants of CYP2C9 and VKORC1 and those some degree of certain alleles may affect the enzyme activity. For instance, polymorphisms in the CYP2C9 gene that encodes the warfarin metabolic enzyme can decrease the rate of warfarin elimination which results in increased risks of prolonged anticoagulation and bleeding. Similarly, VKORC1 polymorphisms, position of the drug target receptor which can therefore influence the level of drug sensitivity (Johnson et al., 2017, Johnson et al., 2011). It had been shown by authors such as Schwarz et al. (2008) that if accompanied by genotypic data, there would be warfarin dose adjustments with a reduced risk of complications and enable therapeutic levels of INR to be achieved more quickly. But genetic tests are not present in every clinical practice setting, and this specifies the need for other predictive factors which are not genetic that can be useful in staging the doses (Schwarz et al., 2008). Although considerable work has been done regarding warfarin dosing, there still exist many questions pertaining among others how these doses should be adjusted to suit different patients (Horton and Bushwick, 1999). For instance, few if any studies exist that have investigated the individual impact of demographic and clinical factors on the need for warfarin maintenance dose in practice. Most of the studies carried out to determine the factors affecting warfarin dose maintenance have either been too narrow in terms of the study populations or the number of variables studied. This study seeks to fill these gaps by focusing on these relationships in a multicenter database of warfarin dosages that has a large number of patients. The aim of the research is to answer the questions on the reasons for the large variability of doses and therefore make a significant contribution in understanding the personalized approach to warfarin treatment (Wadelius et al., 2009). This study aims to help healthcare practitioners initiate therapy by providing them with dose adjustment predictors based on the data obtained from the dose requirements found. Ultimately, the objective is to deliver more efficient and safer therapy to patients taking warfarin using a more personalized approach considering multiple demographics, clinical and genetic characteristics, all of them are intricately connected. In this respect, this research contributes to the further development of such personalized protocols for oral anticoagulation that meet the requirements of various patients on long-term warfarin therapy.

2. Patients & Methods

This cross-sectional study was conducted to evaluate the influence of demographic and clinical factors on warfarin dose requirements in patients receiving oral anticoagulation therapy. A total of 97 patients undergoing warfarin treatment at [insert hospital/clinic name if applicable] were enrolled.

2.1. Inclusion Criteria

Included adult patients (≥18 years old) who had been on a stable warfarin dose for at least four weeks and had a recent INR measurement within the therapeutic range. Patients with known hepatic dysfunction, renal failure, malignancy, or those taking medications known to strongly interact with warfarin were excluded.

For each participant, the following data were collected:

- Demographic variables: age, sex, and body weight (kg)
- Clinical parameters: weekly warfarin dose (mg), International Normalized Ratio (INR), and platelet count ($\times 10^3/\mu l$).

Blood samples were obtained under standard clinical procedures, and INR and platelet counts were measured using automated laboratory analyzers. The mean weekly dose of warfarin was calculated based on the patients' stable dosing regimen.

2.2. Statistical Analysis

The data was processed in IBM® SPSS® Statistics software, Version 24. Descriptive statistics such as mean and standard deviation, were calculated for every variable. Pearson's and Spearman's correlation coefficients were computed to examine the association between the weekly warfarin dose and other relevant study variables after normality testing so that both linear and non-linear associations could be explored in detail. Simple linear regression analysis was further employed to determine the strength of the relationship between the independent variables with statistically significant correlation and the weekly average warfarin dose. Other analyses included independent-sample t-tests of the differences in weekly dose requirements between the genders and between over and under 50 years of age. In this regard, a P-value cut-off of 0.05 was used.

3. Results

3.1. Descriptive Statistics

Age, weight, INR, and platelet count are summarized as follows: The sample had an average age of 49.6 years, with an age range that allowed analysis across different life stages. The average body weight of 77.28 kg, with significant interindividual variability (± 16.87 kg), highlights potential dosing adjustments based on body mass. Mean INR was 2.61, a value maintained within the therapeutic range for most patients, indicating effective anticoagulation. The average platelet count was 237.64 $x10^3/\mu L$, a value generally within the normal range, ensuring no underlying thrombocytopenia or platelet abnormalities that might complicate interpretation Table1.

Table1. Baseline Demographic and Clinical Characteristics of the Study Participants (N = 97)

Parameters	N (%)
N	97
Sex	
Male	38 (39.2)
Female	59 (60.8)
Mean ± SD	
Age (years)	49.6 ± 10.99
Weight (kg)	77.82 ± 17
INR	2.61 ± 0.52
PLT (x103 /µl)	237.64 ± 77.17
Warfarin Weekly dose (mg)	31.63 ± 7.66

3.2. Correlation Analysis

The study found that age was negatively correlated and weight was positively correlated with the weekly warfarin dose ($r = -0.328$, $P = 0.001$) and ($r = 0.491$, $P < 0.0001$) respectively. This means that older patients did not need higher doses and patients with higher body weight needed higher doses. Adjustments explained above are necessary ensuring that therapeutic anticoagulation levels are met so that older patients are on lower doses and patients on the other extreme of the spectrum are on higher doses. On the contrary, the weekly warfarin dose and INR ($r = 0.049$, $P = 0.62$) as well as PLT ($r = -0.005$, $P = 0.9$) do not have a significant association and therefore these variables could be viewed as having no relation with dosing requirements in the patient group for this study.

In the same breath, sex (r = 0.046, P = 0.65) seemed to have no strong relationship either so that men and women patients needed similar doses when the other factors were taken into account Table2.

Table2: Correlation Between Weekly Warfarin Dose and Clinical/Demographic Variables

Variable	Age	Weight	INR	PLT	Sex
Correlation coefficient (r)	-0.328**	0.491**	0.049	-0.005	0.046
P-value	0.001	<0.0001	0.62	0.90	0.65
**Correlation is significant at the 0.01 level, *. Correlation is significant at the 0.05 level.					

3.3. Regression Analysis

For the study of the impact of the parameters – age, weight, sex, INR and PLT, on the weekly selected dose of warfarin, a broader perspective was taken by simple linear regression regressions analysis Models was run.

Age: Age negatively correlated with the warfarin dose with a coefficient B = -0.228, P = 0.001thus 8.9% of the variance in warfarin dose is explained by age ($R^2 = 0.11$). This could probably be linked to decreased drug metabolism which is age-related or increased sensitivity of older individuals to the anticoagulation effects, thereby needing lower doses. Weight: Was found to be the most positive predictor towards the increase of the warfarin dose (B = 0.49, P <0.0001), explaining 18.2% weight-determined variance ($R^2 = 0.24$). This relationship reinforces the argument on drug dosing as the bigger body mass relative to the individual means more drug will be distributed and metabolized.

Sex, INR, and PLT: These variables showed no statistically significant influence on the weekly dose of warfarin, where sex had a coefficient B = 0.8 P = 0.6 and INR and PLT values were not P = 0.6 and P = 0.96 creating a situation in which their level of predictive power was diminished for this instance of dosing Table3.

Table3: Linear Regression Analysis of Predictors for Weekly Warfarin Dose

Predictor	Coefficient (B)	P-value	R^2
Age (years)	-0.228	0.001	0.11
Weight (kg)	0.490	<0.0001	0.24
Sex	0.800	0.600	0.003
INR	-0.720	0.600	0.0025
PLT (×10³/µl)	-0.00046	0.960	0.00002
The table shows unstandardized regression coefficients (B), corresponding p-values, and the coefficient of determination (R^2) for each predictor. Bolded variables (age and weight) are statistically significant predictors of weekly warfarin dose ($p < 0.05$).			

3.4. Differences Due to Gender

The average weekly dose for males was 32.11 ± 7.86 mg while for females it was 31.31 ± 7.57 mg leading to a mean difference of -0.8mg (P = 0.61). This difference, although present, was not statistically significant, and it can be therefore concluded that sex does have much effect on variability in weekly dose Table4.

Table4: Comparison of Weekly Warfarin Dose Between Male and Female Patients

Variable	Female (n = 59)	Male (n = 38)	Mean Difference	P-value
Warfarin Weekly Dose (mg)	31.31 ± 7.57	32.11 ± 7.86	-0.80	0.61
Values are presented as mean ± standard deviation. No statistically significant difference was observed in warfarin dose between male and female patients ($p > 0.05$)				

3.5. Age Group Comparisons

A significant difference was noted between patients aged 50≥ years (mean dose 33.26 ± 13.53 mg) and those 50< years (mean dose 27.19 ± 13.68 mg), with a mean difference of 6.06 mg (P = 0.036). This suggests that age may influence dose adjustments, especially in older patients who might require more cautious dose escalation to avoid excessive anticoagulation Table5 and Fig.1.

Table5: Comparison of Weekly Warfarin Dose Between Age Groups

Variable	Age ≥ 50 (n = 58)	Age < 50 (n = 39)	Mean Difference	P-value
Warfarin Weekly Dose (mg)	33.34 ± 8.36	29.07 ± 5.67	4.26	0.007
Values are presented as mean ± standard deviation. A statistically significant higher warfarin dose was observed in patients aged ≥ 50 years compared to those < 50 years (p < 0.01)				

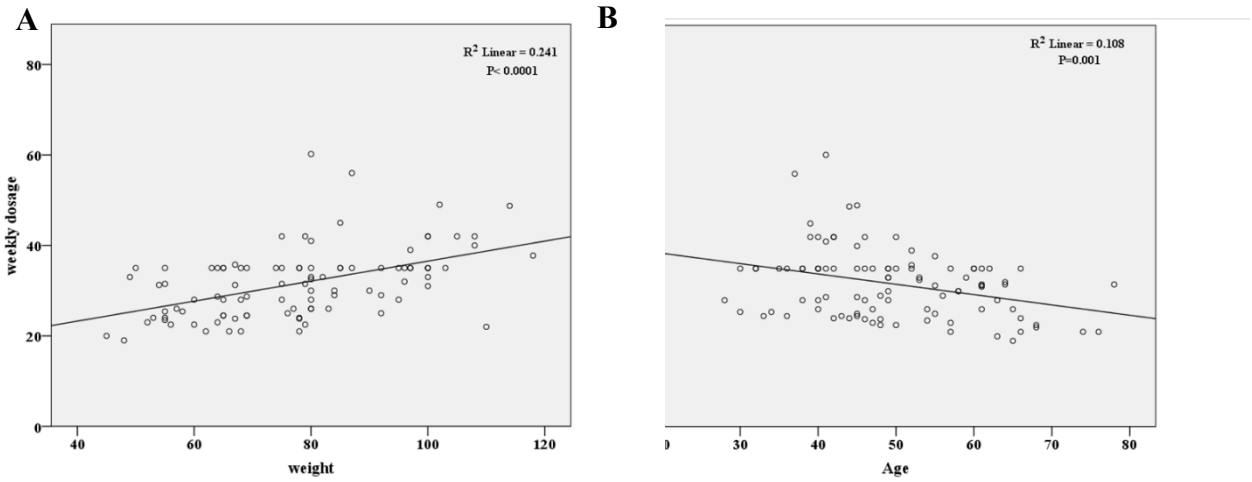

Figure1: Correlation Between Body Weight and Weekly Warfarin Dose

A) The scatter plot demonstrates a positive linear relationship between **body weight** and **weekly warfarin dose**. The regression line shows a moderate positive trend, with **R^2 = 0.241** and **p < 0.0001**, indicating a statistically significant correlation. Heavier patients tend to require higher weekly doses of warfarin. **B) Correlation Between Age and Weekly Warfarin Dose**. This scatter plot illustrates a negative linear correlation between **age** and **weekly warfarin dose**. The regression analysis yielded an **R^2 = 0.108** with a **p-value = 0.001**, suggesting a statistically significant inverse relationship. Older patients generally require lower warfarin doses.

4. Discussion

The results of this investigation bring to light the vicious underpinnings involved in the attainment in the warfarin dose that is deemed therapeutic, with demographic variables particularly body mass and age coming out as major factors. Among these, regarding the effective weight of the patient, the body weight was the underlying factor most correlating with the requirements for weekly doses of warfarin. This relationship can be explained by the fact that body weight has a bearing on the volume of distribution and metabolic clearance of warfarin, which implies that as a person's body mass increases, there is increased demand for the drug in order to achieve a therapeutic effect. This observation is in line with earlier studies that have most of the time emphasized the importance of weight adjusted dosing particularly in achieving and sustaining INR targets so as to prevent an increased risk of adverse effects like bleeding or thrombotic complications. For instance, a systematic review by Garcia et al. (2005) also expanded on the

fact that body mass is the primary determinant when it comes to the pharmacokinetics interactions with warfarin, which exerts pharmacodynamics effects on the target cells or tissue in the patient. When such individuals are not weight-adjusted, under anti-coagulation will occur in individuals with greater body mass putting them at risk of clot prevention, and vice versa for individuals with lower mass who will be at more risk of bleeding (Boonyawat et al., 2017, Pan et al., 2016, Miao et al., 2007, Boriani et al., 2019, Yu et al., 1996). In addition, the pharmacokinetic models such as the one established by the International Warfarin Pharmacogenetics Consortium (2009), also point towards the need for weight adjustments while determining the doses. These models have pointed out that the volume of distribution of warfarin and the clearance rates are dependent on the body mass of an individual, which ultimately determines the pharmacokinetics of the drug. Consequently, such dosing strategies have also been associated with a low incidence of dose related adverse effects, thereby improving the safety and the efficacy of the drug. Therefore, weight-based dose modifiers are now central to the improvement of the algorithms used for dosing warfarin, allowing better management of the therapeutics target (Consortium, 2009, Mueller et al., 2014, Röshammar et al., 2021, Anderson et al., 2012). It has been reported that older patients require less warfarin than younger patients to reach therapeutic INR levels. This phenomenon is consistent with numerous studies that highlight age-related physiological changes, such as diminished hepatic and renal functions which reduces the metabolic clearance for warfarin (Kimmel et al., 2008; Hylek et al., 1997). It has also been shown that the activity of cytochrome 450 isoenzymes particularly CYP2C9, are related to age, meaning an increase in age leads to an increase in half-life as well as heightened sensitivity to warfarin (Wang et al, 2016). Age-related factors also lead to an increased risk of patients being over anti-coagulated, hence requires alterations in dosages and also gradual changes in the dosage so as to attain the therapeutic ranges (McCarthy et al., 2012). Based on clinical indications, this explains why more than 60 patients are advised by medical practitioners to reduce their starting doses of warfarin which is also consistent with the results demonstrating the need for individualized anti-coagulation therapy (Wang et al, 2016; Kimmel et al, 2008)(Hylek et al., 1996, Kimmel et al., 2008). Our study showed no statistically significant association of weekly warfarin dose with either INR or platelet (PLT) levels. The authors may find this finding a bit odd as INR is the primary parameter employed in assessing the safety and effectiveness of warfarin therapy. Yet this finding is not surprising since the INR should not be viewed as the dosage maintenance target but rather as a versatile metric that informs dose adjustment decisions in real-time. In instances where patients are routinely monitored for their INR levels and subsequently adjusted their doses, the variability in dose has to be constant in order to manage the patient's INR to maintain it at the target level. Hence, without constant revision of the dose for various reasons, including fluctuation of INR measurement taken into consideration, the average weekly dose would have no indication relation to the INR. In this regard, other studies support this hypothesis by stating that while INR is very important in making real-time dose changes, it is not a single independent suitor for determining the predictor of maintenance dose. For example, other studies have reported that INRs at baseline levels are not good pieces of information in determining dosage requirements for treatment over a prolonged period. Rather a personalized approach taking into account demographics and clinical mess should be utilized for best outcomes advanced by a retrospective cohort study (Gupta et al., 2015, McMillin et al., 2011). Additionally, the findings are in accordance with previous studies concerning the correlation between PLT levels and bleeding events, which suggest that platelet count does not alter warfarin's pharmacokinetics or pharmacodynamics.

Research such as that by Hylek et al (1997) has shown that while platelet count may be routinely taken into account for the purposes of investigating bleeding risk in patients receiving anticoagulants, it is scarcely predictive of the warfarin dose required. It is however more plausible that one includes platelet count when determining one's risk of bleeding rather than the amount of warfarin, in this regard it becomes important to distinguish factors affecting the safe range of the dose from factors affecting the adverse effects of the drug, for example factors such as body weight and age. In this regard, bleeding risk and dosing requirements are in fact self-evident; in practice, the dosing schedule is formed mainly by demographic and physiological factors, and only in lesser proportions by platelets or INR among other indicators (Hylek et al., 1998, Proietti et al., 2019, Lazo-Langner et al., 2009). When all these aspects are considered, there comes out the need to individualize warfarin dosing based on the patient's characteristics such as age and weight as well as monitor INR level closely for dose modification. Physicians can reduce the risks of thrombosis and bleeding by starting the treatment with a specific dose and adjusting it periodically as the clinical situation warrants. There is potential in the future for combining pharmacogenetics with demographics to optimize warfarin dosing guidelines. For example, genetic variations in the CYP2C9 and VKORC1 warfarin metabolism pathway genes might be helpful to develop better dosing models that do not have to rely on trial and error depending on clinically adjusted dosing requirements such pharmacogenetics would allow a patient-centered approach to warfarin dosing (Absher et al., 2002, Daly, 2009, Kangelaris et al., 2009).

5. Conclusion

This study recognizes the importance of dose individualization factors: body weight and age for most of the ageing population on warfarin therapy. For warfarin to be effective and safe, there is need to adhere to weight-based capacity since it largely affects distribution and clearance of the drug. In addition, older patients are expected to be on lower doses since older age is associated with increased risk of bleeding due to physiological changes. Thus, future conclusions may be more definitive by using a larger sample of patients with different pathology and the presence of such parameters as: age, sex, and polymorphisms CYP2C9, VKORC1, etc. Measures of these demographic traits, the genetic, and clinical characteristics aim at evaluation of blood thinning medications may result in better individual dosing predictions and increase efficacy and reduce risks of warfarin use.

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Frequency of Relapse in Nephrotic Syndrome Children Treated with Prednisolone

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Abstract

Background: Relapsing-remitting idiopathic nephrotic syndrome is a childhood disorder that presents with a 20-30% lifetime occurrence rate of a single episode, while the remaining individuals experience relapses.

Objective: Investigate the correlation between demographic and biochemical factors and the occurrence of relapses in children who are responsive to prednisolone.

Patients and Methods: A cross-sectional analysis was conducted at the Kerbala Teaching Hospital for Children in Iraq, spanning from August 1st to November 2023. There was a correlation between relapse and factors such as gender, age, albumin serum level, total cholesterol level and proteinuria.

Results: Out of the 80 children diagnosed with steroid-sensitive nephrotic syndrome, 15 (18.8%) didn't experience any relapses, 44 (55%) had infrequent relapses and 21 (26.2%) had frequent relapses. The patients' ages ranged from one to sixteen years. The male population accounted for 54% of the total, and the female population accounted for 26%. There were no significant differences in age, gender and blood cholesterol levels between the different groups (p values 0.224, 0.488 & 0.319, respectively). A strong positive relationship was found between low levels of serum albumin, proteinuria and recurring relapses (with p-values of 0.016 and 0.042, respectively).

Conclusion: There was a significant correlation between relapses and reduced levels of serum albumin and proteinuria.

تكرار الانتكاس في الأطفال الذين يعانون من متلازمة التناذر الكلوي المعالجين بالبريدنيزولون

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الخلاصة

المقدمة

متلازمة التناذر الكلوي المتكررة والمستمرة مجهولة السبب هي اضطراب يحدث في مرحلة الطفولة ويتميز بحدوث نوبة واحدة بنسبة 20-30% طوال العمر، بينما يعاني الأفراد الآخرون من انتكاسات متكررة.

الهدف

دراسة العلاقة بين العوامل الديموغرافية والكيميائية الحيوية و حدوث الانتكاسات لدى الأطفال الذين يستجيبون للبريدنيزولون.

المرضى وطرق العمل

أجريت هذه الدراسة المقطعية في مستشفى كربلاء التعليمي للأطفال في العراق، من 1 أغسطس إلى نوفمبر 2023. تم العثور على علاقة بين الانتكاسات وعوامل مثل الجنس، العمر، مستوى الألبومين في المصل، مستوى الكوليسترول الكلي والبيلة البروتينية.

النتائج

من بين 80 طفلًا تم تشخيصهم بمتلازمة التناذر الكلوي المستجيبة للأدوية الستيرويدية ، لم يعاني 15 (18.8%) من أي انتكاسات، وكان 44 (55%) يعانون من انتكاسات غير متكررة، في حين كان 21 (26.2%) يعانون من انتكاسات متكررة. تراوحت أعمار المرضى من سنة إلى 16 سنة. شكل الذكور 54% من إجمالي الحالات، بينما شكلت الإناث 26%. لم تكن هناك اختلافات ذات دلالة إحصائية في العمر والجنس ومستويات الكوليسترول في الدم بين المجموعات المختلفة (القيم الاحتمالية 0.224، 0.488 و 0.319 على التوالي).
وجدت علاقة إيجابية قوية بين انخفاض مستويات الألبومين في المصل والبيلة البروتينية والانتكاسات المتكررة . (بقيم احتمالية 0.016 و 0.042 على التوالي)

الاستنتاج

كانت هناك علاقة ذات دلالة إحصائية بين الانتكاسات وانخفاض مستويات الألبومين في المصل والبيلة البروتينية .

1. Introduction

Nephrotic syndrome is a prevalent kidney disease among children, distinguished by the presence of heavy proteinuria (>40 mg/m^2/hr; 1 g/m^2 daily) and hypoalbuminemia (serum albumin <3 g/dL). It is Characterized by edema and heavy proteinuria (Sinha et al., 2021). The annual incidence and prevalence of nephrotic syndrome are two to seven new cases and sixteen new cases per one hundred thousand children, respectively. The incidence of nephrotic syndrome in the adult population is three new cases per one hundred thousand adults annually (Politano et al., 2020). Nephrotic syndrome is predominantly idiopathic, with minimal change disease accounting for approximately 80-90% of these cases, steroid therapy is effective in approximately 80% of cases involving minimal change disease; however, 70–85% of these patients will experience a relapse, and 50% will develop relapses that occur Frequently, the most prevalent age at presentation is 2 years, although the age range afflicted is typically 1 to 10 years (Hodgin et al., 2022). Approximately 80-90% of children who are prescribed Steroid-Sensitive Nephrotic Syndrome (SSNS) undergo one or more subsequent relapses, which may manifest as frequent or infrequent relapses or steroid dependence (Ali et al., 2022). The International Study for Kidney Disease in Children (ISKDC) initially estimated a relapse rate of 60%. A small percentage of individuals (approximately 15%) are dependent on steroids, whereas 25%–40% of those who respond to prednisolone experience infrequent relapses. When it comes to relapses, relapse therapy has conventionally included using prednisone daily until remission, followed by alternating days for the next four weeks; treatment is then discontinued or tapered over the course of four to eight weeks (Shanta et al., 2023). Relapse is associated with a considerable number of complications, elicits anxiety in patients, and places a considerable financial burden. Furthermore, the prognosis for the progression of the disease becomes challenging once the initial recurrence has been treated (Akter et al., 2020). To study the potential correlated factors that contribute to the increased incidence of relapse in children with nephrotic syndrome and the relative frequency of relapse in this patient population.

2. Patients and Methods

This observational cross-sectional study was conducted at Kerbala Teaching Hospital for Children from August to November 2023. After approval by the scientific and ethical committee of College of Pharmacy, University of Kerbala. Informed consent was taken from each participant (or relative) before starting the study. A total of 80 patients, spanning in age from 1 to 16 years, were included in this study and were found to have cases of SSNS. Patients with congenitally acquired NS, incomplete data, steroid-resistant NS, or ages below 1 or exceeding 16 years were excluded from the study. Based on the following criteria, NS was diagnosed: Albustix >2+ (for older children with collected 24-hour urine or non-toilet-trained children), heavy proteinuria exceeding 40 mg/h/m^2 (for non-toilet-trained children or complex collections of 24-hour urine), hypoalbuminemia below 2.5 g/dL or edema, and hyperlipidemia with total cholesterol ranging from 170 to 200 mg/d. The protocol established by the International Society for Kidney Disease in Children (ISKDC) guided the clinical approach at the pediatric nephrology unit. Following the initial four weeks of Prednisone treatment at a rate of 60 mg/m^2 divided daily, the dosage was decreased to 40 mg/m^2 divided daily to accommodate the duration of the attack. After this, four weeks of alternate-day therapy were administered prior to its tapering. Relapse was controlled with prednisone administered at a dose of 40 mg/m^2

on alternate days for a duration of four weeks. Following the collection of urine, prednisone was administered at a daily dose of 60 mg/m^2 for three days (Shanta et al., 2023, Ali et al., 2016). The definitions that followed were incorporated: Relapsed is defined as the presence of proteinuria over 40 mg/h/m^2 or 50 mg/kg/day, or a positive result on Albustix >2+ for three consecutive days following a period of remission. Frequent relapse was defined as the occurrence of 2 or more relapses within 6 months of the initial response, or 4 or more relapses within a period of 1 year. Infrequent relapse was defined as the occurrence of 1 recurrence within 6 months, or 1-3 relapses within 12 months. Remission is characterized by proteinuria levels below 4 mg/h/m^2 or the absence of protein on a protein stick test for 3 consecutive days. Steroid therapy can lead to complete remission, known as SSNS. Steroid-resistant nephrotic syndrome (SRNS) refers to patients who do not achieve remission after an 8-week treatment with corticosteroids (Niaudet and Boyer, 2009, Shaker et al.)

2.1. Data Collection

At the time of enrollment, the participants' demographic and clinical information was collected on a pre-established document. Age, gender, proteinuria (as detected by a urine dipstick in the wee hours of the morning), S. albumin, and S. cholesterol and relapse incidence were recorder. Following routine blood testing, blood samples were collected in the blood collection area of the hematology laboratory.

2.2. Statistical Analysis

Software Package for the Social Sciences (SPSS 26) will be utilized to conduct the statistical analysis. The numerical data were descriptively represented by the mean and standard deviation of the mean (Mean ± STD), whereas the non-numerical data were presented in the form of percentages and numbers. The assessment of data normality was performed utilizing the Shapiro–Wilk test. Age and other numerical data that follow a normal distribution will be analyzed using the independent sample T-test and the one-way ANOVA-post-hoc LSD test, respectively. Albumin, cholesterol, and protein urea, which have non-normal distributions numerically, will be analyzed utilizing nonparametric tests and legacy dialogs, specifically the Kruskal Wallis test. The Chi-square test will be employed to analyze the non-numerical data. P values that are smaller than 0.05 will be deemed to be statistically significant.

3. Results

This study involved 80 children who had been diagnosed with SSNS. We identified three subgroups of SSNS based on age, gender, and biochemical findings: NO relapse, FR (frequent relapse) and IFR (infrequent relapse). The relapse rate for childhood INS is detailed in Table1. 15 patients (18.8%) did not experience a relapse, while 44 (55%) had IFR and 21 patients (26.2%) had FR.

Table1: Distribution of Patients According to Frequency of Relapse

		N	Percentage
Relapse	**NO**	15	18.8%
	Infrequent	44	55.0%
	Frequent	21	26.2%
Total		80	100.0%

The following data was obtained from the demographic data of the study group: Children ranged in age from one to sixteen years at the time of the study. In terms of gender-based distribution of NS, it was observed that males constituted the plurality, comprising 54 of the total patients, whereas females constituted 26.

As shown in Table2, age differences between groups weren't statistically significant (p=0.224). Regarding gender, no statistically significant distinction was found between groups (p=0.488). Male participants comprised 11 (73.3%) in the no relapse group, 31 (70.5%) in the IFR group, and 12 (57.1%) in the FR group; females accounted for 4 (26.7%), 13 (29.5%), and 9 (42.9%), respectively.

Table2: Demographic Data of Children with Nephrotic Syndrome

Variables		**Relapse of nephrotic syndrome**			***P* – value**
		No (n=15)	**Infrequent (n=44)**	**Frequent (n=21)**	
Age (y)		7.07 ± 4.85	8.66 ± 3.22	9.1 ± 3.28	0.224
Gender	**Male**	11 (73.3%)	31 (70.5%)	12 (57.1%)	0.488
	Female	4 (26.7%)	13 (29.5%)	9 (42.9%)	
Data Present as Mean ± SD and No (%)					

As shown in Table3, there were statistically significant variations between groups in serum albumin (g/ml) and proteinuria (mg/ml) (p=0.016 and p=0.042, respectively). There was no significant correlation observed between relapse and level of cholesterol (mg/mL) (p=0.319). Fig.1 and Fig.2 presents data showing significant diminishment of serum albumin levels in all categories of relapse: frequent (mean = 2.85 ± 1.25), infrequent (mean = 3.35 ± 1.09), and no (mean = 3.99 ± 0.97). In contrast, Fig.3 indicate that proteinuria is greatest in the category of frequent relapse (244.76 ± 340.07), followed by infrequent relapse (107.73 ± 259.02), and finally, no relapse (23 ± 78.66).

Table 3: Correlation Between Biochemical Findings and Relapses in Patients with Nephrotic Syndrome

Variables	**Relapse of Nephrotic Syndrome**			***P* -value**
	No (n=15)	**Infrequent (n=44)**	**Frequent (n=21)**	
S.albumin (g/ml)	3.99 ± 0.97	3.35 ± 1.09	2.85 ± 1.25*	0.016
S.cholesterol (mg/ml)	243.71 ± 133.68	233.01 ± 128.69	276.53 ± 129.75	0.319
Proteinuria (mg/ml)	23 ± 78.66	107.73 ± 259.02	244.76 ± 340.07*#	0.042
(Data present as mean ± STD), Significant effect (P < 0.05) compared to no relapse group, Significant effect (P < 0.05) compared to infrequent group.				

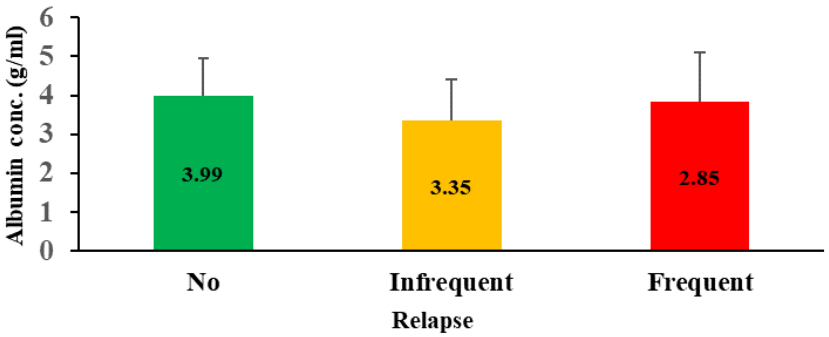

Figure1: Comparison of Serum Albumin Concentration Across Relapse Frequencies

The bar chart illustrates the mean serum albumin concentration (g/ml) among three patient groups based on relapse frequency: **No relapse** (green): 3.99 g/ml, **Infrequent relapse** (yellow): 3.35 g/ml, **Frequent relapse** (red): 2.85 g/ml. A decreasing trend in albumin levels is observed with increasing relapse frequency. Error bars represent the standard deviation.

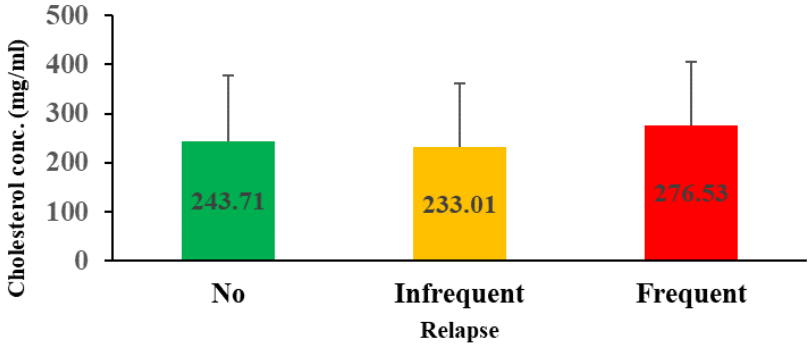

Figure2: Comparison of Serum Cholesterol Concentration Across Relapse Frequencies

The bar chart shows the mean serum cholesterol levels (mg/ml) in patients grouped by relapse frequency: **No relapse** (green): 243.71 mg/ml, **Infrequent relapse** (yellow): 233.01 mg/ml, **Frequent relapse** (red): 276.53 mg/ml. An increase in cholesterol concentration is observed in the **frequent relapse** group compared to the other groups. Error bars represent the standard deviation.

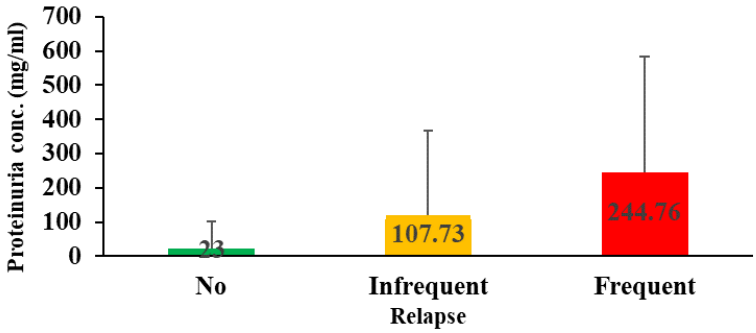

Figure3: Comparison of Proteinuria Concentration Across Relapse Frequencies

The bar chart displays the mean proteinuria concentration (mg/ml) among patients categorized by relapse frequency: **No relapse** (green): 23 mg/ml, **Infrequent relapse** (yellow): 107.73 mg/ml, **Frequent relapse** (red): 244.76 mg/ml. A marked increase in proteinuria levels is observed with increasing relapse frequency. Error bars indicate standard deviation.

4. Discussion

Nephrotic syndrome is a recurring and chronic kidney illness that has a higher occurrence rate compared to other kidney disorders. The very varied incidence of relapses is linked to several risk factors. Male patients predominated over female patients, a finding consistent with previous research (Ali et al., 2022, Ali et al., 2016, Shaker et al., Noer, 2005).(Rahi et al., 2009) found no statistically significant correlation between gender and relapse frequency, similar to the findings of the present study. The statistical insignificance of the variation in FR and IFR by gender indicates that sex is an inadequate predictor variable. The present findings diverge from those of prior research conducted by (Akter et al., 2020, Hiraoka et al., 1995). FR is negatively correlated with gender, according to a number of investigations (Constantinescu et al., 2000, Fujinaga et al., 2011, Sinha et al., 2012). (Andersen et al., 2010) found that male gender remained associated with an increased risk of steroid dependence and FR, irrespective of the length of the steroid course. In another study, male gender was identified as a predictor of FR (Noer, 2005). Consistent with prior investigations carried out by (Ali et al., 2016, Constantinescu et al., 2000, Fujinaga et al., 2011, Takeda et al., 2001), our results do not suggest any correlation between age at presentation and subsequent relapses among patients diagnosed with NS. On the contrary,(Andersen et al., 2010, Sarker et al., 2012) reported a remarkable correlation between age at presentation and the subsequent rise in the frequency of relapses. According to an Indian study, individuals diagnosed with FR exhibited a younger age at disease onset, and the incidence of relapses decreased as age increased (Sinha et al., 2012). The observed inconsistency could be attributed to variations in idiopathic NS patterns among different ethnic groups and racial disparities in the study populations of different research studies (Andersen et al., 2010). Following this, 15 patients (18.8%) did not experience a relapse, 44 patients (55%) had fewer than two relapses (IFRNS), and 21 patients (26.2%) had more than two relapses (FRNS). This observation differs significantly from the results reported by (Shanta et al., 2023, Chwat et al., 2014), in which the most prevalent subgroup was frequently relapsing nephrotic syndrome. Conversely, numerous other researchers, including (Karim, 1999) and (Gulati et al., 1997), documented infrequently relapsing nephrotic syndrome as the most prevalent subgroup. The biochemical results indicated a statistically significant correlation between low serum albumin levels and frequent relapses. The outcome bears resemblance to Prior research that has documented a correlation between relapse and deficient serum albumin levels ($\leq$ 15 g/l) (Shanta et al., 2023, Takeda et al., 1996). The levels of serum albumin and proteinuria showed significant differences between the FR and IFR groups ($p=0.016$ & $p=0.042$, respectively), while there was no significant difference in serum cholesterol levels ($p=0.319$). Our research findings demonstrate that a decreased blood albumin level is really a significant risk factor for recurrent relapse of nephrotic syndrome.

5. Conclusion

This study indicates that infrequent relapse was recorded more frequently than frequent relapse in children with steroid sensitive nephrotic syndrome with an abundance of males. A notable correlation was observed between the frequency of relapse and hypoalbuminemia and proteinuria ($p=0.016$ and $p=0.042$). These results will facilitate the timely detection of infants with frequent relapse allowing for appropriate treatment.

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Evaluation of Cytomegalovirus Infection and Interleukin-33 Levels in Women with Recurrent Pregnancy Loss

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Abstract

Background

Recurrent pregnancy loss determine by the "American Society for Reproductive Medicine" and the "European Society of Human Reproduction and Embryology", is defined as two or three clinically identifiable failed pregnancies before twenty to twenty-four weeks of gestation, as confirmed by histopathologic examination or ultrasound. The cytomegalovirus is a member of the Herpesviridae family's Betaherpesvirinae subfamily. It is a common virus that is known to cause congenital infections in infants and in people with weakened immune systems as pregnant. Interleukin 33 (IL-33) a cytokine, is a member of the Interleukin 1 family. bind to the special ST2 receptor.

The aim of study

To determine the unbalanced inflammatory factors such as interleukins 33 (IL-33) and cytomegalovirus infections can be an important factor in recurrent pregnancy loss.

Methods & materials

This study's case-control methodology included 60 Recurent pregnancy loss as case group and 60 healthy controls with successful delivery. IL-33 measure & CMV IgM and IgG from serum blood samples using ELISA technic method.

Results

The obtained results showed that measure of IL-33 in the control was significantly more than case ($p = 0.001$). cytomegalovirus antibodies in the control were significantly more than case (IgM) ($p = 0.01848$). & (IgG) ($p = 0.00001$) , BMI and Age non-significant to link with RPL .

Conclusion

Generally, we showed that the BMI & Age no important role but IL- 33 level and cytomegalovirus infections play an important role in RPL.

تقييم الإصابة بفيروس السيتوميجالو (CMV) ومستويات الإنترلوكين-33 لدى النساء المصابات بالإجهاض المتكرر

محمد كاظم مطشر , تغريد فاضل المحبوبي , احمد موسى عيسى

الملخص

المقدمة

تُعرّف الإجهاضات المتكررة، وفقًا للجمعية الأمريكية للطب التناسلي والجمعية الأوروبية للتكاثر البشري وعلم الأجنة، بأنها فشل حدوث حمل سريري يمكن التحقق منه مرتين أو ثلاث مرات قبل بلوغ فترة الحمل من 20 إلى 24 أسبوعًا، وذلك كما تؤكده الفحوصات النسيجية أو التصوير بالأمواج فوق الصوتية. يُعد الفيروس المضخم للخلايا (Cytomegalovirus) من فيروسات عائلة الهربس (Herpesviridae) وتحديدًا من فصيلة الـBetaherpesvirinae ، وهو فيروس شائع ومعروف بتسببه في إصابات خلقية لدى الأجنة، بالإضافة إلى تأثيره في الأشخاص ذوي المناعة الضعيفة، مثل النساء الحوامل. يُعتبر الإنترلوكين 33 (IL-33) من السيتوكينات التابعة لعائلة الإنترلوكين 1، ويرتبط بالمستقبِل النوعي.ST2

هدف الدراسة

تهدف هذه الدراسة إلى تحديد ما إذا كانت العوامل الالتهابية غير المتوازنة مثل الإنترلوكين 33 (IL-33) والعدوى بفيروس المضخم للخلايا (CMV) تلعب دورًا مهمًا في حدوث الإجهاضات المتكررة.

العينات وطرق العمل

أُستخدم في هذه الدراسة تصميم الحالة–الشاهد، حيث شملت 60 امرأة تعاني من الإجهاض المتكرر كمجموعة الحالات، و60 امرأة ذوات حمل ناجح كمجموعة ضابطة. تم قياس مستويات IL-33 والأجسام المضادة IgM و IgGلفيروس CMV في عينات الدم المصلية باستخدام تقنية الإليزا.(ELISA)

النتائج

أظهرت النتائج أن مستوى IL-33 كان أعلى بشكل ملحوظ في مجموعة الضوابط مقارنةً بمجموعة الحالات .($p = 0.001$) كما كانت الأجسام المضادة لفيروس CMV (IgM و IgG)أعلى بشكل ملحوظ لدى المجموعة الضابطة مقارنةً بمجموعة الحالات، حيث بلغت القيم الاحتمالية ($p = 0.01848$) و ($p = 0.00001$)على التوالي. في المقابل، لم يظهر كل من مؤشر كتلة الجسم (BMI) والعمر ارتباطًا ذا دلالة إحصائية مع حالات الإجهاض المتكرر.

الاستنتاج

بشكل عام، تُظهر نتائج هذه الدراسة أن كلًّا من العمر ومؤشر كتلة الجسم لا يلعبان دورًا مهمًا في حدوث الإجهاضات المتكررة، في حين أن انخفاض مستويات IL-33 والعدوى بفيروس CMV يُحتمل أن يكون لهما دور بارز في هذه الظاهرة.

1. Introduction

A multifactorial event known as recurrent pregnancy loss (RPL) occurs when two or three consecutive abortions occur before 20 weeks of gestation. One to five percent of women of reproductive age suffer from RPL because several causes such as a severe reproductive issue (Turesheva et al., 2023). Haematological, anatomical, chromosomal ، genetic, and endocrinological variables all contribute to the pathogenicity of RPL, a diverse disorder. Environmental variables of RPL also include exposure to ethylene oxide and lead. Additionally, immunological and infectious factors. also, by incorrect medication use (Pei et al., 2019; Turesheva et al., 2023). Interleukin 33 (IL-33) Known as IL-1F1-IL-1F11, it is a new cytokine that is a member of the 11-member IL-1 family. IL-33 is liganded by the orphan receptor T1/ST2 (IL-1RL1) (Cayrol & Girard, 2018).

A healthy pregnancy is linked to the balance of Th1 & Th2 cytokines. Furthermore, the physiological development of the human foetus is influenced by the decrease of Th1 cytokines throughout pregnancy (Ahmadi et al., 2017; Wang et al., 2020). Human cytokine production is regulated by genetic background. The immune system and inflammatory processes are both significantly regulated by this cytokine, endothelial cells produce IL-33, which is crucial for Th2 and mast cell activation. Additionally, it belongs to the IL-1 family and is essential for immunological responses as well as a number of physiological and pathological processes, including tissue homeostasis, autoimmune disorders, and cancer (Cayrol, 2022; Cayrol & Girard, 2022; Molofsky et al., 2015). Human cytomegalovirus It is Part of the Herpesviridae family's Betaherpesvirinae, Direct or indirect contact with bodily fluids, including saliva, urine, cervical or vaginal secretions, semen, breast milk, or blood, is the main way that the infection is spread. A pregnancy can be greatly impacted by the cytomegalovirus (CMV), which may result in unfavourable results, such as abortion. Significant inflammation and cellular damage can result from placental cell infection by the cytomegalovirus (CMV). Reduced effectiveness in the transport of vital nutrients and oxygen from the mother to the fetus results from this infection's disruption of the placenta's normal design and function. A miscarriage is more likely as a result of the ensuing placental insufficiency, which can seriously impair fatal growth and development. Further raising the possibility of spontaneous abortion is the possibility that CMV-induced placental damage may hinder the generation of vital hormones required to sustain pregnancy. (Lindholm & O'Keefe, 2019). It is a prevalent virus that can cause serious infections in adults with compromised immune systems and congenital illnesses in neonates (Dantoft et al., 2017; Fulkerson et al., 2021; Lynn et al., 2023; Nogalski et al., 2014). In this study, we looked into the relationship between the IL-33 levels & CMV infection in recurrent pregnancy loss in women from Iraq.

2. Materials and Methods

2.1. Patients & Sample Collection

60 case with RPL served as the case group in this case-control research, while 60 healthy controls gave birth without incident. Women aged 20 to 42 who had at least two consecutive abortions prior to Twenty weeks of pregnancy and were diagnosed with RPL made up the case group. Additionally, as healthy controls, women who had at least two successful pregnancies and were free of autoimmune disorders, endocrinopathies, steroid therapy, or inflammation were chosen. Interviews and questionnaires were used to gather data from the case and control groups on clinical traits and lifestyle.,. The women who were chosen for this study were all from Karbala city. As required by ethical standards, all study participants were informed about the study and the consent form.

Leave the blood undisturbed at room temperature to allow it to clot after the whole blood has been collected. Usually, this takes twenty minutes. then Centrifuging (Thermolas Scientific, C-12000) at 2,000–3,000 rpm for twenty minutes to remove the clot. Use the BioTek 50 TS ELISA washer and the BioTek 800 TS ELISA reader.

and Sun Long Biotech Co., LTD's Human Interleukin 33 (IL-33) ELISA Kit , Standard diluents (150µl) are added to dilute the standard (270 pg/ml) to 180 pg/ml, 120 pg/ml, 60 pg/ml, 30 pg/ml, and 15 pg/ml. Sandwich-ELISA mode is the technique used with this ELISA kit Using spectrophotometry, the optical density (OD) is determined at a wavelength of 450 nm and Camp medica CMV IgG \ Romania , Camp medica CMV IgM \ Romania Using spectrophotometry, the optical density (OD) is determined at a wavelength of 450 nm.

2.2. Statistical Analysis

The statistical package for the social sciences (SPSS) software (version 21.0) was used to do the statistical analysis of the collected data. The relationship between the examined IL-33 and RPL risk was examined using logistic regression. $p < 0.001$ was established as the threshold for statistical significance.

3. Results

3.1. Distribution of Age and BMI in Patients and Control

mean age of case as mentioned in Table 1 was less than the mean age of control (29.267 years vs 29.683 years, P. value = 0.74842) There are non-significant statistical differences between patients & control group, Table1. mean BMI of control more than mean age of case (24.218 vs 23.588) non-significant difference in BMI between the two groups (P. value =0.43023). Table1, Fig.1

Table1: Distribution and Characteristics of Patients and Control According to the Study Subjects

Parameters	Groups	Mean	Std. Deviation	P. value
Age	Control	29.683	7.203	0.74842
	Patients	29.267	6.994	
BMI	Control	24.218	4.990	0.43023
	Patients	23.588	3.617	
Independent T-Test and Mann-Whitney U test have been utilized to conduct a comparative analysis between two groups on the same continuous variable.*. The mean difference is significant at the 0.05 level." **. The mean difference is significant at the 0.01 level."				

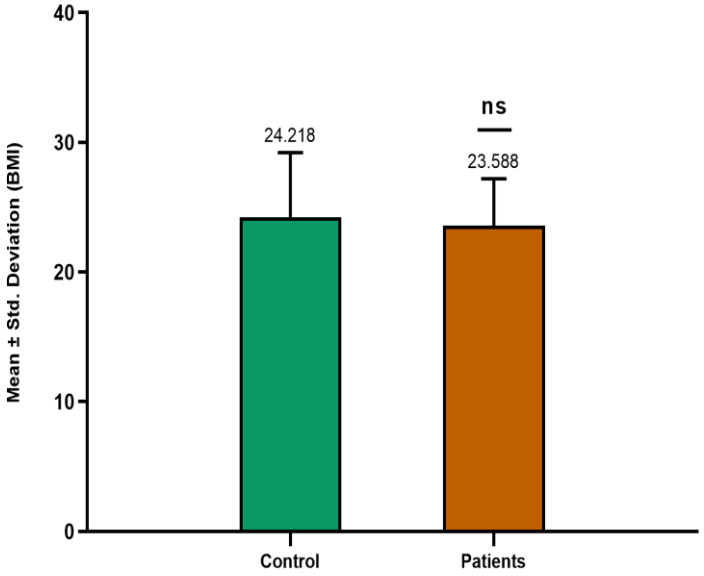

Figure1: Comparison of BMI Between Male and Female Patients

3.2. Measurement of CMV IgM & CMV IgG in Patients and Control by ELISA

IgM CMV mean in patient more than control (0.378 vs 0.212) significant p= 0.01848*. IgG CMV mean in patient more than control (1.232 vs 0. 717) significant p= 0.00001**. by this results the CMV IgM & IgG important risk factor in RPL. Table2, Fig.2.

Table2: The Comparison Between Research Parameters in Patients and their Controls

Parameters	Groups	Mean	Std. Deviation	P. value
CMV IgM	Control	0.212	0.135	0.01848*
	Patients	0.378	0.115	
CMV IgG	Control	0.717	0.108	0.00001**
	Patients	1.232	0.169	
Independent T-Test and Mann-Whitney U test have been utilized to conduct a comparative analysis between two groups on the same continuous variable. *. The mean difference is significant at the 0.05 level, **. The mean difference is significant at the 0.01 level				

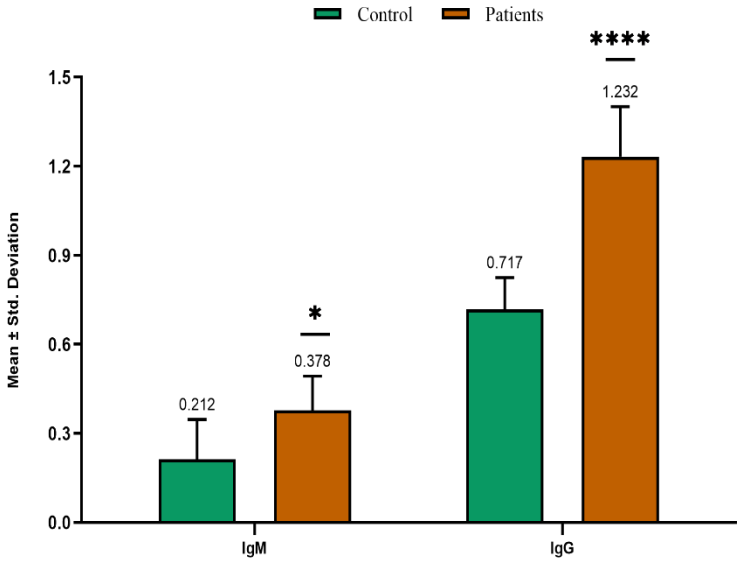

Figure2: Mean Cytomegalovirus (CMV) IgM and IgG Antibody Levels in Patients Versus Controls

The bar chart compares the mean ± SD titres of CMV-specific antibodies between patient and control groups. Patients show a modest but significant elevation in IgM (0.378 ± SD) relative to controls (0.212 ± SD; $p < 0.05$). A pronounced increase is observed for IgG, with patients (1.232 ± SD) far exceeding controls (0.717 ± SD; **** $p < 0.0001$). Error bars represent standard deviation; asterisks denote statistical significance (**** = $p < 0.0001$, *

3.3. Measurement of IL-33 in Patients and Control by ELISA

IL-33 mean in patient less than control (6.782vs 8.781) significant p= 0.00129**. by this results the IL-33 important protective factor in RPL Table3, Fig.3.

Table3: The Comparison Between Research Marker (IL-33) in Patients and Their Controls

Parameters	Groups	Mean	Std. Deviation	P. value
IL-33	Control	8.781	4.363	0.00129**
	Patients	6.782	1.534	
Independent T-Test and Mann-Whitney U test have been utilized to conduct a comparative analysis between two groups on the same continuous variable. *The mean difference is significant at the 0.05 level. **The mean difference is significant at the 0.01 level				

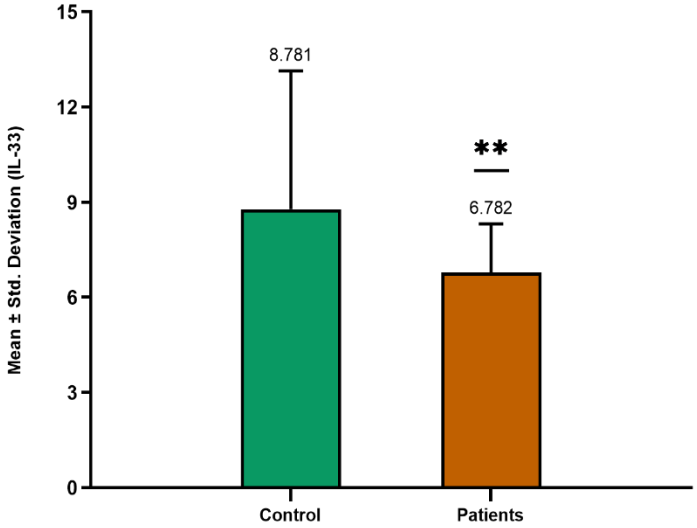

Figure3: Comparison of Serum IL-33 Levels Between Patients and Controls
The bar chart illustrates the mean concentration of interleukin-33 (IL-33) in patient and control groups. Patients exhibit significantly higher IL-33 levels (118.1 ± SD) compared to controls (88.4 ± SD), with the difference reaching statistical significance (p<0.01). Error bars represent standard deviation; asterisks indicate statistical significance.

4. Discussion

According to some research, pregnant women who have experienced a miscarriage show significantly lower levels of interleukin 33, making it an important indicator of the success of the pregnancy because human endometrial stromal cells (HESCs) must activate the IL-33/ST2 pathway in order for a pregnancy to be successful but another study found the IL-33 increase in abortion more than in healthy deliver (Salker et al., 2012).

The same applies to cytomegalovirus infection. Previous research has shown that this viral infection causes problems for both the fetus and the pregnant woman, including recurrent miscarriages (Kareem et al., 2022),

Both in vivo and in a multicellular ex vivo model, CMV infection alters the placental immunological milieu, indicating that CMV-induced cytokine modulation may be a cause or aggravating factor of placental and fatal harm (D'Antonio et al., 2023; Kareem et al., 2022; Njue et al., 2021). CMV was capable of invading several placental cells in vitro. Destroying the trophoblastic progenitor stem cell (syncytium and cytotrophoblast precursor), which decreases the number of mature cells, the extra villous trophoblast cells (floating cytotrophoblast), which invades the uterine vascular wall and is in charge of remodelling the circulation during pregnancy, would have a negative impact on the pregnancy because it would decrease maternal blood circulation in the placenta, which would lessen fatal growth restriction or even miscarriage. Additionally, CMV possesses immunomodulatory qualities that change the host immunological response and disrupts important autoregulatory mechanisms in the cytotrophoblast, which would change trophoblast migration. Preterm labour, fatal development

limitation, and miscarriage could result from these changes. Development restriction in embryos unaffected by CMV whose mothers have been diagnosed with the disease can also be explained by the fact that CMV raises tumour necrosis factor- alpha levels in vitro, which causes increased trophoblast death (D'Antonio et al., 2023; Hamilton et al., 2012; Le-Trilling et al., 2023; Zischke et al., 2017), As gestational age increases, the risk of fatal infection rises, most likely as a result of cytotrophoblast differentiation. With a preference for the reticuloendothelial cells & central nervous system (CNS), the virus finally replicates in the tubular epithelium of the fatal kidney after passing through the placenta, the first organ to become infected. Placental infection, maternal viremia, and fatal dispersion through the hematogenous route are the likely sequence of events (lasting 7 to 8 weeks) that result in fatal infection (Fisher et al., 2022; Shahar-Nissan et al., 2020; Zischke et al., 2017). mean BMI of control more than mean age of case this result agrees with study that found non-significant between BMI and RPL and disagree with study that found increase BMI in patient more than control. The inconsistent results across studies may be due to differences in study design and demographic factors; sample sizes may vary significantly between cross-sectional and longitudinal research, which affects statistical power and generalizability; some studies consider three or more losses, while others consider two or more; and population variations also play a role; factors such as genetic background, access to healthcare, and diet can have a significant impact on results and may explain why BMI is considered a risk factor in some contexts but not in others(Eapen et al., 2021) .mean age of case as mentioned in Table 1 was less than the mean age of control There are non-significant statistical differences between patients and control group , this result agree with another study the age non-significant with recurrent abortion. but disagree with study found the age play role in abortion. Variations in study design and demographic variables may be the cause of the inconsistent results among studies. Sample sizes might differ significantly between cross-sectional and longitudinal research, which impacts generalizability and statistical power. Furthermore, whereas some research calls for three or more losses, others take into account two or more. Population variations also come into play; variables like genetic background, access to healthcare, and diet can have a big impact on results and could be the reason why age is seen as a risk factor in some contexts but not in others (Eapen et al., 2021; Yue et al., 2016).

5. Conclusions

In general, our research showed that among women in Karbala City, IL-33 level and cytomegalovirus infectious was linked to RPL risk. But the BMI and Age non-significant to link with RPL. Therefore, more research on various regions with higher sample sizes is advised to identify the impacts of BMI, RPL, IL-33, cytomegalovirus on RPL in order to better understand the link with RPL.

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Evaluation of Plasma Galanin Hormone Levels in Iraqi Women Patients with Endocrine Thyroid Disorder

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Abstract

Background: Thyroid hormones play a vital role in regulatory processes, including metabolism, energy balance, and neuroendocrine function. Galanin, a neuropeptide expressed throughout many organs and tissues in the body, including the brain and peripheral tissues, is implicated in hormonal regulation and metabolic processes. Evidence suggests a potential relationship between galanin and thyroid function, particularly in hormonal disorders such as hypothyroidism or hyperthyroidism.

Aim of the study: Assess whether galanin acts as a biomarker or elucidates the pathogenesis of thyroid dysfunction by comparing galanin levels in women recently diagnosed with hypothyroidism, hyperthyroidism, and normal thyroid function.

Subjects and Method: We performed enzyme-linked immunosorbent assay (ELISA) to measure serum galanin concentrations in a case-control study comprising (n=60) diagnosed with thyroid dysfunction, including (n=30) with hypothyroidism and (n=30) with hyperthyroidism, compared with apparently healthy control subjects (n=30). Demographic information was obtained from all participants, including age and BMI. in addition, biochemical parameters were also estimated in all participants, including thyroid function test, lipid profiles and liver enzymes

Results: significantly serum concentration of galanin hormone was increased sharply in patient with hyperthyroid comparing with hypothyroid group (p-value<0.001) and with control subject (p-value<0.001). Furthermore, are slightly correlated with LDL-C.

Conclusion: These results suggest a possible regulating function of galanin in thyroid hormone imbalance and its potential involvement with the pathogenesis of thyroid dysfunction.

تقييم مستويات هرمون الجالانين في بلازما النساء العراقيات المصابات باضطرابات الغدة الدرقية الهرمونية

فرقد البيضاني

الخلاصة

المقدمة

تلعب هرمونات الغدة الدرقية دورًا حيويًا في العمليات التنظيمية، بما في ذلك التمثيل الغذائي، وتوازن الطاقة، ووظيفة الغدد الصماء العصبية. هرمون الجالانين، وهو ببتيد عصبي يُعبَّر عنه في العديد من أعضاء وأنسجة الجسم، بما في ذلك الدماغ والأنسجة الطرفية، له دور في التنظيم الهرموني والعمليات الأيضية. تشير الأدلة إلى وجود علاقة محتملة بين هرمون الجالانين ووظيفة الغدة الدرقية، وخاصةً في الاضطرابات الهرمونية مثل قصور أو فرط نشاط الغدة الدرقية.

هدف الدراسة

تقييم ما إذا كان هرمون الجالانين يعمل كمؤشر حيوي أو يُوضح مسببات خلل وظيفة الغدة الدرقية، وذلك بمقارنة مستويات الجالانين لدى النساء اللواتي شُخِّصْنَ مؤخرًا بقصور أو فرط نشاط الغدة الدرقية، ووظائف الغدة الدرقية الطبيعية.

الموضوع والطريقة

أجرينا اختبار الممتز المناعي المرتبط بالإنزيم لقياس تركيزات هرمون الجالانين في المصل في دراسة حالة وشاهد شملت (ن = 60) مريضًا تم تشخيص إصابتهم بخلل في الغدة الدرقية، منهم (ن = 30) مصابون بقصور الغدة الدرقية و(ن = 30) مصابون بفرط نشاط الغدة الدرقية، مقارنةً بأفراد سليمين ظاهريًا (ن = 30). تم الحصول على المعلومات الديموغرافية من جميع المشاركين، بما في ذلك العمر ومؤشر كتلة الجسم. بالإضافة إلى ذلك، تم تقدير المعايير الكيميائية الحيوية لجميع المشاركين، بما في ذلك اختبار وظائف الغدة الدرقية، ومستويات الدهون، وإنزيمات الكبد.

النتيجة

زاد تركيز هرمون الجالانين في المصل بشكل ملحوظ لدى المرضى الذين يعانون من فرط نشاط الغدة الدرقية مقارنةً بمجموعة قصور الغدة الدرقية (القيمة الاحتمالية <0.001) ومجموعة الضبط (القيمة الاحتمالية <0.001).

الخلاصة

تشير هذه النتائج إلى وظيفة تنظيمية محتملة لهرمون للجالانين في اختلال توازن هرمون الغدة الدرقية وتورطه المحتمل في التسبب في خلل نشاط الغدة الدرقية.

1. Introduction

In 1983, Viktor Mutt's lab and the Karolinska Institute of Science in Stockholm, Sweden, discovered a neuropeptide called galanin(Crawley, 1995). Galanin peptide, found from the porcine origin digestive system, has been proven to have the capacity to influence the contraction of smooth muscles and metabolism of glucose across species(Abot et al., 2018). Galanin neuropeptide consist of length sequence about 30 peptide amino acids residue with molecular weight 3400 Da in humans. In the structure of galanin the C terminal is Amidation; amid group is NH2 instead of hydroxyl group OH, which is very important in the biological activity of this neuropeptide (Zhu et al., 2022)

Galanin has been demonstrated to be integral to various physiological process including cognition (Zhu et al., 2022)nutrition (Marcos and Coveñas, 2021)and a sensory perception (Fonseca-Rodrigues et al., 2022). Importantly, it involved in the regulation of various anterior pituitary hormones (Falkenstetter et al., 2020). The relationship of galanin to the thyroid gland is somewhat complicated, including indirect and direct effects on thyroid function via the central nervous system and hormonal homeostasis. According to several research, Galanin modulates the release of the thyroid hormones thyroxine and triiodothyronine by influencing the gland's interaction with nerve impulses and the surrounding hormone system, which controls the gland's function. For instance, it has an impact on energy balance, metabolism, and other gland processes (Can et al., 2024a). Galanin regulates thyroid gland function by acting on hormone-regulating centers in the brain because it contains three neuroreceptors GALR1, GALR2 and GALR3 with different signaling pathways (Šípková et al., 2017),which are found in regions of the brain that control hormonal balance and thyroid function (Šípková et al., 2017). In additional, this neuropeptide involved in the mechanism of managing the stress response, and as is known stress and tension have a significant impact on the gland's levels (Radhika and Rekha, 2024). Some investigations have confirmed that Galanin has an effect on appetite management and metabolic activity, which is related to the actions of these hormones in regulating metabolism. Galanin's function is summarized by its influence on nerve tissues that maintain hormonal homeostasis (Fang et al., 2015). The disturbance in the balance of these processes lead to changes in hormonal homeostasis in respond primary to the changes in work of central nerve system. For these reasons, this study is to investigate any possible correlation between thyroid function and serum galanin levels in women. The study aims to evaluate whether galanin reflects a biomarker or helps to explain the pathophysiology of thyroid malfunction by comparing galanin concentrations in women newly diagnosed with hypothyroidism, hyperthyroidism, and normal thyroid.

2. Subjects and Methods

2.1. Subject

The research design is a (case-control) study, conducted with (n=30) controls and (n=60) patients; comprising (n=30) hypothyroid and (n=30) hyperthyroid individuals. age ranged between (20-65) years female. The selection of the patients depends on several criteria's; for the patient should be newly diagnosed in hyperthyroidism or hypothyroidism. The practical side of the study was performed at Imam Hassan Al-Mujtaba Center for Diabetes and Endocrinology were randomly selected from the patients attending the Endocrinology Consultation Unit. Questionnaires have been designed to gather information from both the control and case groups, including patients diagnosed with thyroid dysfunction. The

medical history of each female patient was recorded, including age and any prior disorders. Furthermore, those with chronic liver illness, malignancies, those aged 65 and older or under 20, as well as patients with cardiovascular disorders, peripheral vascular disease, stroke, infections, and emergency cases were excluded. Furthermore, women who are presently pregnant or intend to become pregnant in the near future have also been excluded from this study. The local ethics committee approved the study, and all participants provided written informed permission prior to their involvement.

2.2. Study Parameters

2.2.1.Clinical Assessment

This study involved measuring the height and body weight of all participants to compute the Body Mass Index (BMI) using the formula: weight (kg) divided by height (m^2) according to standardized equations; BMI = Weight (Kg) / Height (cm2) ("Quetelet's index (W/H2) as a measure of fatness. - Abstract - Europe PMC," n.d.) In this investigation, TSH, Free T3 and Free T4 was measured to diagnosed the thyroid disfunction, with lipid profile and Galanin concentration.

2.2.2.Blood Collection Data

Blood samples are taken from all subjects after a 10- to 12-hour fast. The sampled volume of blood is 5 milliliters, extracted with disposable syringes while seated. The collected blood was preserved in clean, sterile containers. The samples were collected between 8:00 and 12:30 a.m. Blood was permitted to coagulate at 37°C for 10-15 minutes prior to centrifugation at 2000xg for approximately 10-15 minutes. Subsequently, the serum is divided into two components and stored at -20°C. The serum taken from patients and controls was used to measure the following parameters: TSH, free (T4 and T3), lipid profile and liver enzymes (ALT, AST).

2.2.3.Estimation of Plasma Serum Galanin Concentration Levels

The concentration levels determined using a kit called Quick Step Human galanin, GAL ELISA Kit (enzyme-linked immunosorbent assay). However, according to the instruction's manual (SunlongBiotech,China). The assay range of detection 3 - 200 pg/mL and the sensitivity 0.6 pg/mL, with an intra-assay coefficient of variance (CV) less than < 10% and inter -assay coefficient of variance (CV) less than < 12%.

2.3. Statistical Analysis

Statistical Analysis for Social Science software (SPSS 24 IBM, Armonk, USA) is used to explain results as mean ± SD. To calculate the degree of variability and variations in mean values of variables between control, apparently healthy, and patient groups. The data was examined using the analysis of variance (one-way ANOVA) test. The differences between the control and patient groups were compared using the t-test. Also, calculate. Correlations between all of the researched variables were evaluated using Pearson's correlation coefficient (r), and linear regression analyses were utilized to evaluate data. The ROC analysis was additionally used to determine the sensitivities and specificity of this study biomarker.

3. Result

3.1. Comparison of Plasma Clinical Biomarkers and Demographic Data Among Control, Hypothyroid, and Hyperthyroid Groups

The clinical characteristics including 90; patients (n = 60), this comprises (n=30) patients with hypothyroid and 30 patients with hyperthyroid (females; mean ± SD age, 52.70 ± 11.567 years) and

(n=30) control (n=30) (female; mean ± SD age, 50.12 ± 11.533years). According to the presented data, their testing parameters are seen in Table1, Table2, Table3 and Table4.

Table1: Clinical Characteristics of Demographic Information

Parameters	Control (n=30)	Hypo - TR (n= 30)	Hyper - TR (n=30)
Age (year)	48.89 ± 10.033	50.12 ± 11.533	52.7 ± 11.567
BMI (kg/m²)	26.21± 4.26	29.76± 3.38 ^{c***}	27.87± 4.31 ^{b*}
b: significant vs. hypothyroidism group, **c**: significant vs. hyperthyroidism group, *p < 0.05, **p < 0.001			

Table2: Clinical Characteristics of Thyroid Function Test Information and Study Biomarker

Parameters	Control (n=30)	Hypo - TR (n= 30)	Hyper - TR (n=30)
TSH(µIU/ml)	2.08 ± 0.40	6.95 ± 1.83 $^{a**, c***}$	0.29 ± 0.20 ^{b***}
Free T3(Pg/dL)	3.00 ± 0.58	2.12 ± 0.55 $^{a***, c***}$	6.54 ± 1.05 ^{b***}
Free T4(ng/dL)	1.18 ± 0.29	0.57 ± 0.28 $^{a***, c***}$	3.43 ± 0.81^{b***}
Galanin (pg/ml)	176.85 ± 89.52	412.41 ± 261.41$^{a***, c*}$	280.15 ± 183.03 ^{b*}
a: significant vs. control group, **b**: significant vs. hypothyroidism group, **c**: significant vs. Hyperthyroidism group, *p < 0.05, **p < 0.01, **p < 0.001			

Table3: Clinical Characteristics of Lipid Profile Information

Parameters	Control (n=30)	Hypo - TR (n= 30)	Hyper - TR (n=30)
Cholesterol (mg/dl)	188.9± 27.28	235.3 ± 14.02 $^{a*, c*}$	189.91 ± 42.15^{b*}
TG (mg/dl)	148.84± 61.18	196.93 ± 17.62 ^{a*}	189.10± 92.35
VLDL (mg/dl)	29.76 ± 12.23	38.74 ± 17.9	37.82 ± 18.47
HDL-C (mg/dl)	42.31± 7.39	42.30 ± 8.59	40.706± 8.95
LDL-C (mg/dl)	111.52± 36.80	155.86 ± 13.21 ^{a*}	111.382± 32.66
a: Significant vs. control group, **b**: Significant vs. hypothyroidism group, **c**: Significant vs. hyperthyroidism group, *p < 0.05*			

Table4: Clinical Characteristics of Liver Function Test

Parameters	Control (n=30)	Hypo - TR (n= 30)	Hyper - TR (n=30)
ALT (IU/L)	22.19± 12.6	23.34± 11.04	26.99± 23.20
AST (IU/L)	22.59± 15.91	22.71± 11.08	31.19± 30.84
ALT: Alanine aminotransferase – a liver enzyme used to assess liver cell function and injury. **AST**: Aspartate aminotransferase – a liver-related enzyme also found in other tissues such as the heart and muscles; used in conjunction with ALT to evaluate liver health. Values are presented as mean ± standard deviation. No statistically significant differences were observed among the groups.			

With no statistically significant differences noted in the Table1, the mean age distribution within the three different groups i.e., control, hypothyroid (Hypo-TR), and hyperthyroid (Hyper-TR) was similar (control: 48.89 ± 10.03 years, hypothyroid (Hypo-TR: 50.12 ± 11.53 years, hyperthyroid (Hyper-TR: 52.70 ± 11.57 years). This suggests that, among the study groups, age was very equal; this is one indication of normal distribution of data variables. On the contrary, body mass index (BMI) varied significantly across groups. With a BMI of 29.76 ± 3.38 kg/m², the hypothyroid (Hypo-TR) group was far higher than both the control ($p < 0.001$) and hyperthyroid ($p < 0.05$). Though the difference was less clear ($P < 0.05$), the Hyper-TR group also displayed a greater BMI (27.87 ± 4.31 kg/m²) than the control group (26.21 ± 4.26

kg/m²). These findings reflect the identified metabolic consequences of thyroid malfunction, especially the link between hypothyroidism and weight increase, as well as the somewhat low BMI values usually connected with hyperthyroidism. Table2 explains the clinical characteristics of thyroid function tests for women patients and the study biomarker galanin between three groups: control apparently healthy, hypothyroid group (Hypo-TR), and hyperthyroid group (Hyper-TR). The levels of TSH were markedly elevated in the Hypo-TR group (6.95 ± 1.83 μIU/ml) in comparison to both the control group (2.08 ± 0.40 μIU/ml, $p < 0.001$) and the Hyper-TR group (0.29 ± 0.20 μIU/ml, $p < 0.001$). Free T3 levels were markedly reduced in the hypothyroid group (2.12 ± 0.55 pg/dL) versus control group (3.00 ± 0.58 pg/dL, $P < 0.001$), while levels were significantly elevated in the hyperthyroid group (6.54 ± 1.05 pg/dL) when compared with both the control and hypothyroid groups ($P < 0.001$ for both group comparisons). Relative to both the control and hypothyroid groups, free T4 levels showed the same pattern: they were significantly raised in the hyperthyroidism group (3.43 ± 0.81 ng/dL) and greatly reduced in the hypothyroid group (0.57 ± 0.28 ng/dL). It is noteworthy that compared to the control group as seen in Table2, both groups with thyroid malfunction had increased serum galanin levels. The hypothyroid group (412.41 ± 261.41pg/mL) had the highest mean galanin level, followed by the hyperthyroid group (280.15 ± 183.03 pg/mL); the control group recorded the lowest mean galanin level (176.85 ± 89.52 pg/mL). Although every group showed significant variation, the data suggest to a trend towards higher galanin concentrations associated with thyroid malfunction, particularly hypothyroidism as seen in Fig.1. The results of the investigation revealed variations in lipid parameters in comparison with the control group as seen in the Table 3. With statistically significant differences ($P < 0.05$), total cholesterol levels increased somewhat in the hypo-TR group (235.3 ± 14.02 mg/dL) and in the hyper-TR group (189.91 ± 42.15 mg/dL) relative to the control group (188.9 ± 27.28 mg/dL). With statistically significant differences in the hypo-TR group ($P < 0.05$), triglyceride (TG) levels similarly raised considerably in the hyper-TR group (189.10 ± 92.35 mg/dL) and the hypo-TR group (196.93 ± 17.62 mg /dL) ($P < 0.05$), compared to the control group (148.84 ± 61.18 mg/dL). Analogously, very low-density lipoprotein (VLDL) values were greater in both groups with thyroid dysfunction, especially in the hypothyroid group (38.74 ± 17.9 mg/dL) relative to the control group (29.76 ± 12.23 mg/dL). With a little, non-statistically significant increase in the hypo-TR group (42.92 ± 8.59 mg/dL) and a modest decline in the hyper-TR group (40.71 ± 8.95 mg/dL), compared to the control group (42.31 ± 7.39 mg/dL), high-density lipoprotein (HDL-C) levels were very stable across all groups. While LDL-C in the hyper-TR group remained similar (111.38 ± 32.66 mg/dL), LDL-C levels revealed a marked slightly higher in the hypo-TR group (155.86 ± 13.21mg/dL) compared to the control group (111.52± 36.80 mg/dL).

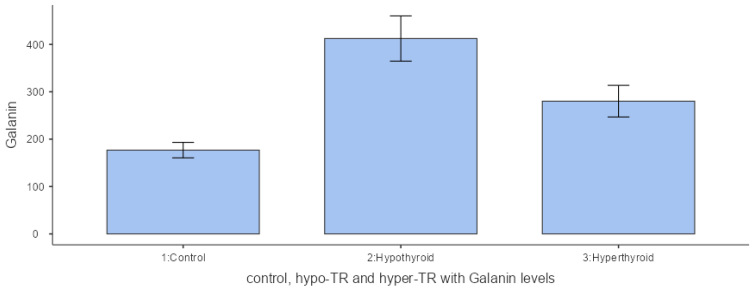

Figure1: Plasma Galanin Levels in Control, Hypothyroid, and Hyperthyroid Groups
The bar chart illustrates the mean plasma galanin concentrations (pg/ml) among the three study groups: **Control** group: lowest galanin level, **Hypothyroid** group: significantly elevated galanin levels, **Hyperthyroid** group: intermediate levels between control and hypothyroid. Error bars represent the standard deviation. These findings suggest that **galanin levels are notably increased in hypothyroid patients**, indicating a potential role in thyroid hormone regulation or compensatory neuroendocrine mechanisms.

3.2. The Correlation Analysis Between Galanin Hormone and Biochemical Parameters

In liver function tests as shown in Table 4, alanine aminotransferase (ALT) levels were slightly elevated in the Hyper-TR group (26.99 ± 23.20 IU/L) relative to the Hypo-TR group (23.34 ± 11.04 IU/L) and the control group (22.19 ± 12.6 IU/L), though these variations did not reach statistical significance. AST levels were elevated in the Hyper-TR group (31.19 ± 30.84 IU/L) relative to the Hypo-TR group (22.71 ± 11.08 IU/L) and the control group (22.59 ± 15.91 IU/L); however, these differences were not statistically significant. Fig.2 and detailed in Tables5 and Table6 show the correlation analysis between serum Galanin levels and diverse clinical and biochemical indicators in the hyperthyroid (hyper-TR) and hypothyroid (hypo-TR) patient groups, respectively. The magnitude and direction of relationships were evaluated using Pearson's correlation coefficient (r), and statistical significance was determined by the Pearson correlation test. This investigation aimed to investigate potential correlations between Galanin and variables including lipid profile, thyroid hormones, liver enzymes, and anthropometric measurements as shown in Fig.3. The correlation patterns between galanin hormone levels and the examined biomarkers seem different in the hypothyroid group. However, they did not show a significant association. This indicates that the association between galanin and various metabolic indicators may be affected by the degree of hypothyroidism development as seen in the Table6.

Table5: Correlation Between Galanin and Parameters Between Hyper-TR Patients Group

Parameters	Galanin (pg/ml)	
	P value	Pearson's correlation coefficient (r)
Age (years)	0.804	0.047
BMI (Kg/m²)	0.208	-0.237
TSH(µIU/ml)	0.664	0.083
Free T3(Pg/dL)	0.932	0.016
Free T4(ng/dL)	0.273	0.207
Cholesterol(mg/dl)	0.506	-0.126
HDL (mg/dl)	0.221	0.140
LDL (mg/dl)	0.037	-0.383
VLDL (mg/dl)	0.0857	0.021
TG (mg/dl)	0.657	-0.085
ALT (mg/dl)	0.432	0.254
AST (mg/dl)	0.543	0.351

Values shown are for the **hyperthyroid group**. Significant correlation ($p < 0.05$) was observed only between **galanin and LDL levels**, with a moderate negative correlation (**r = -0.383**).

Table6: Correlation Between Galanin and Parameters Between Hypo-TR Patients Group

Parameters	Galanin (pg/ml)	
	P value	Pearson's correlation coefficient (r)
Age (years)	0.893	0.26
BMI (Kg/m²)	0.446	-0.144
TSH(µIU/ml)	0.766	0.057
Free T3(Pg/dL)	0.632	-0.091
Free T4(ng/dL)	0.631	0.082
Cholesterol(mg/dl)	0.557	- 0.112
HDL (mg/dl)	0.411	0.156
LDL (mg/dl)	0.607	- 0.098
VLDL (mg/dl)	0.769	0.34
TG (mg/dl)	0.623	-0.093
ALT (mg/dl)	0.313	0.118
AST (mg/dl)	0.221	0.140

Galanin (pg/ml): A neuropeptide involved in metabolic and endocrine regulation. **BMI:** Body Mass Index (kg/m²). **TSH:** Thyroid Stimulating Hormone (µIU/ml). **Free T3 / Free T4:** Unbound triiodothyronine and thyroxine hormones (pg/dL and ng/dL, respectively). **Cholesterol, HDL, LDL, VLDL, TG:** Lipid profile parameters measured in mg/dL. **ALT / AST:** Liver enzymes (Alanine and Aspartate aminotransferase, respectively) measured in mg/dL. **r:** Pearson's correlation coefficient, indicating the strength and direction of association. **p-value:** Statistical significance ($p < 0.05$ is considered significant).

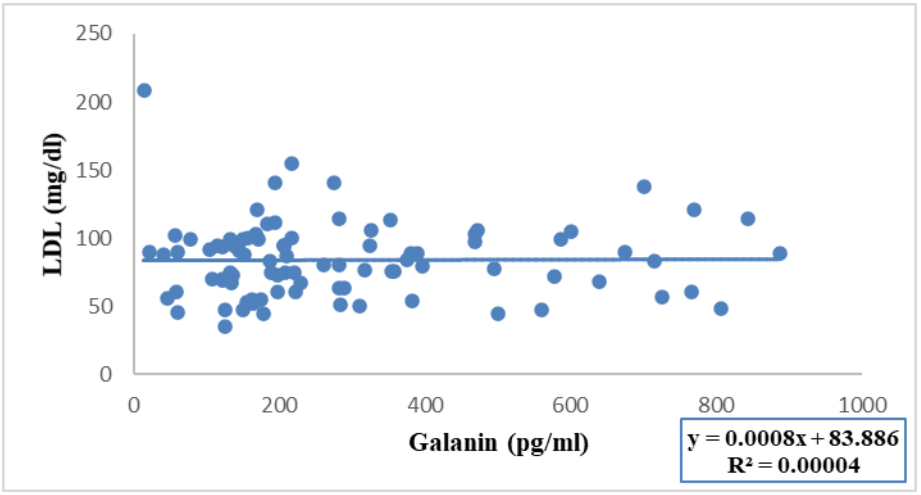

Figure2: Correlation Between Plasma Galanin Levels and LDL Concentration
The scatter plot shows the relationship between plasma galanin levels (pg/ml) and LDL cholesterol levels (mg/dl). The linear regression line indicates a **very weak positive correlation** (*$R^2 = 0.00004$*), with the regression equation: **$y = 0.0008x + 83.886$**. This suggests **no meaningful association** between galanin and LDL levels in the study population.

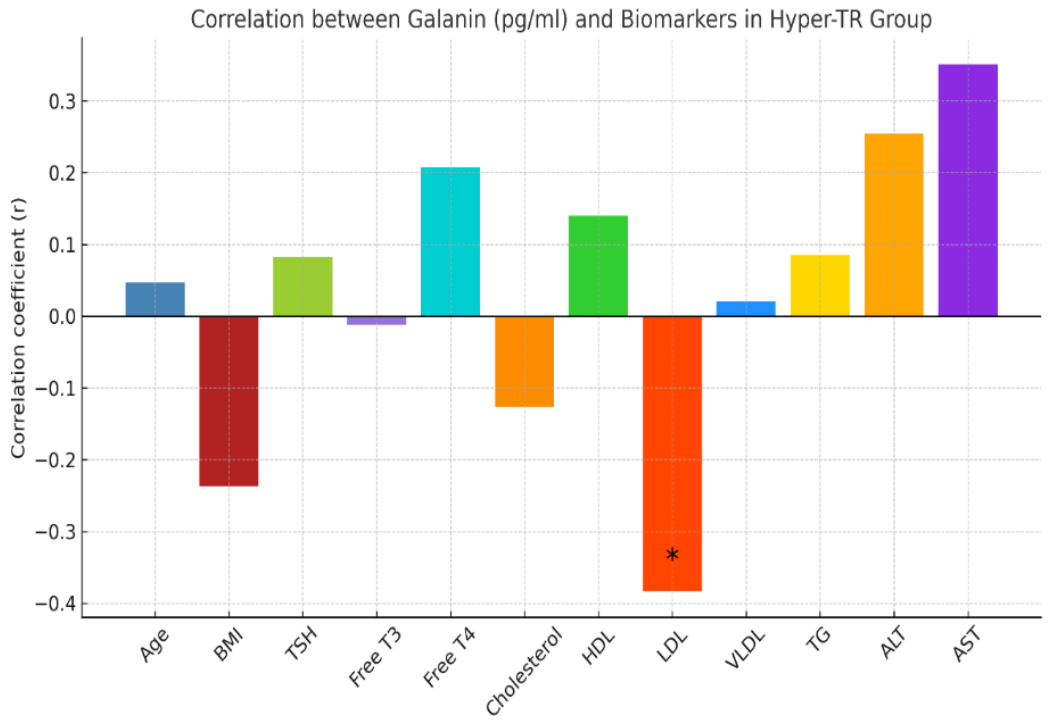

Figure3: Correlation Between Serum Galanin Levels and Various Biomarkers in the Hyperthyroid Group

The bar chart displays the correlation coefficients (r) between galanin (pg/ml) and several clinical biomarkers in patients with hyperthyroidism.

Notably, LDL shows a significant negative correlation with galanin ($p < 0.05$), indicated by the asterisk (*). Other variables such as AST, ALT, and HDL exhibit positive correlations, while BMI and LDL show inverse relationships. This suggests a possible regulatory link between galanin and lipid/liver parameters in hyperthyroid individuals.

Fig.4 and Fig.5 show the correlation coefficients (r) between serum levels of the galanin hormone and various biomarkers in the hypothyroid and hyperthyroid groups. Comparing the correlation patterns between these groups, several differences were revealed: In the hyperthyroid patient group, Galanin levels exhibited a statistically significant negative correlation with LDL cholesterol levels ($r = -0.383$, $p = 0.037$), indicating an inverse relationship between Galanin and LDL in this group as seen in the Figure 2. No further indicators exhibited significant relationships with Galanin in the hyperthyroid cohort. BMI had a negative connection ($r = -0.237$), although the association lacked statistical significance ($p = 0.208$). Conversely, among hypothyroid patients, none of the assessed measures exhibited a statistically significant connection with Galanin. The relationship between Galanin and LDL cholesterol was weak and non-significant ($r = -0.098$, $p = 0.667$), contrasting with the strong link found in the hyperthyroid cohort. BMI had a weak and non-significant association with Galanin in the hypothyroid cohort ($r = -0.144$, $p = 0.446$). The data indicate that the association between Galanin and lipid metabolism, specifically LDL cholesterol, may be modified in hyperthyroidism but remains unaffected in

hypothyriodism

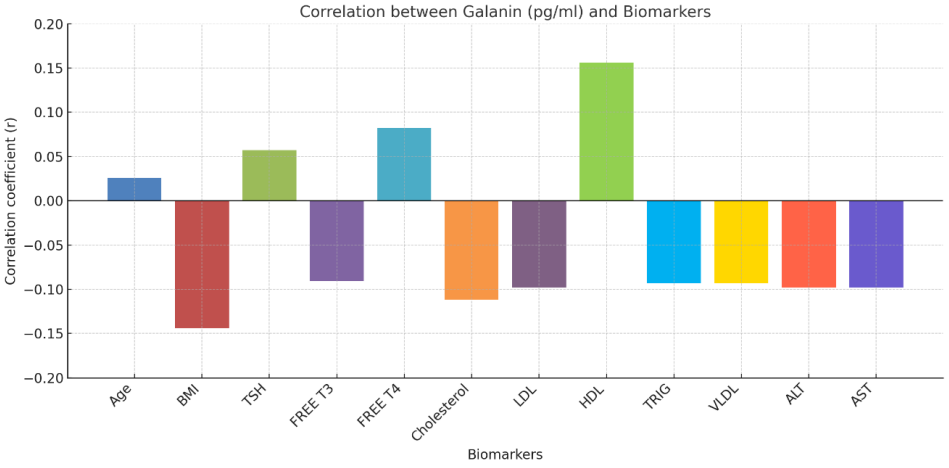

Figure4: Correlation Between Serum Galanin Levels and Various Biomarkers in the Overall Study Population

This bar chart displays the **Pearson correlation coefficients (r)** between **serum galanin levels (pg/ml)** and a range of clinical biomarkers across the full study population. **Positive correlations** were observed with HDL, Free T4, TSH, and Age. **Negative correlations** were found with BMI, Free T3, Cholesterol, LDL, VLDL, Triglycerides (TRIG), ALT, and AST. The strongest positive association was with **HDL**, while the most notable negative correlation appeared with **BMI**. No statistically significant correlations were indicated in this chart. Error bars are not shown.

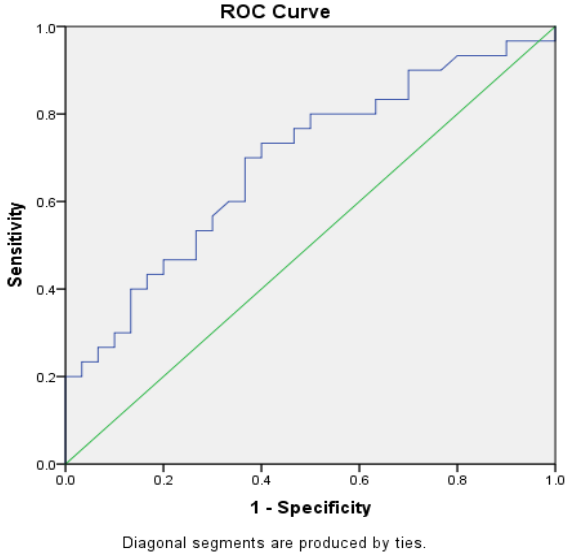

Figure5: ROC Curve Analysis of Serum Galanin Levels for Differentiating Hyperthyroid Patients from Controls

This Receiver Operating Characteristic (ROC) curve evaluates the diagnostic performance of **serum galanin levels** in distinguishing **hyperthyroid patients** from **healthy controls**. The curve plots **sensitivity** versus **1 − specificity** at various cutoff values. The **area under the curve (AUC)**, although not displayed here, reflects the test's overall accuracy. The curve deviates above the diagonal reference line, indicating **diagnostic value** above random chance.

3.3. Receiver Operating Characteristics (ROC) Curve Diagnostic Performance of Serum Levels of Galanin

Table7 illustrates the findings of the ROC (Receiver Operating Characteristic) curve analysis assessing the diagnostic efficacy of serum galanin levels in women diagnosed with hyperthyroidism. The area under the curve (AUC) was 0.703, signifying a moderate discriminating capacity. The ideal cut-off value, found via Youden's index, was 0.333 pg/ml, resulting in a sensitivity of 73.3% and a specificity of 60.0%. The 95% confidence interval for the AUC range from 0.556 to 0.824, indicating a moderate diagnostic accuracy of Galanin in differentiating hyperthyroid state in female patients

Table7: AUC, Optimal Threshold, Sensitivity, and Specificity of Serum Galanin Among Hyperthyroid and Healthy Control

Test Result Variable(s)	Area under the Curve (AUC)	Cut off value Youden's index	Specificity	Sensitivity	95% Confidence Interval	
					Lower Bound	Upper Bound
Galanin (pg/ml) In hyperthyroid patient's women	0.703	0.333	0.600	0.733	0.556	0.824
AUC (Area Under the Curve): Reflects the diagnostic accuracy of galanin; AUC = 0.703 indicates fair diagnostic performance. Cut-off Value: The optimal threshold of galanin for distinguishing hyperthyroid patients from controls. Youden's Index: Summarizes the performance of the test (Youden's Index = Sensitivity + Specificity – 1) Specificity: The proportion of true negatives correctly identified. Sensitivity: The proportion of true positives correctly identified. 95% Confidence Interval: Range in which the true AUC value lies with 95% certainty.						

Table8 illustrates the findings of the ROC (Receiver Operating Characteristic) curve analysis assessing the diagnostic efficacy of serum galanin levels in women diagnosed with hypothyroidism. The area under the curve (AUC) was 0.763, signifying a moderate discriminating capacity. The ideal cut-off value, found via Youden's index, was 123.15pg/ml, resulting in a sensitivity of 83.3% and a specificity of 23.3 %. The 95% confidence interval for the AUC range from 0.636 to 0.889, indicating a moderate diagnostic accuracy of Galanin in differentiating hypothyroid state in female patients

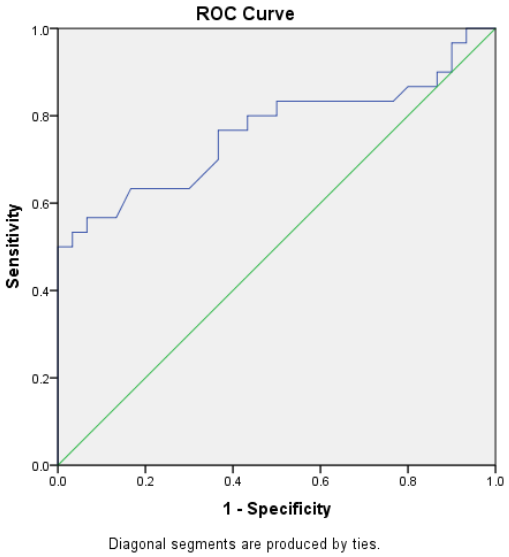

Figure6: ROC Curve of Serum Galanin Levels in Hypothyroid Female Patients Compared to Control Group

This ROC curve evaluates the diagnostic performance of **serum galanin levels** in distinguishing **hypothyroid patients** from **healthy controls**. The curve plots **sensitivity** versus **1 − specificity** at various cut-off values. The position of the curve above the diagonal reference line suggests **good diagnostic discrimination**.

This analysis helps determine the **optimal threshold** for galanin to **predict**

Table8: AUC, Optimal Threshold, Sensitivity, And Specificity of Serum Galanin Among Hypothyroid and Healthy Control

Test Result Variable(s)	Area under the Curve (AUC)	Cut off value Youden's index	Specificity	Sensitivity	95% Confidence Interval	
					Lower Bound	Upper Bound
Galanin (pg/ml) In hypothyroid patient's women	0.763	123.15	0.233	0.833	0.636	0.889
AUC (Area Under the Curve): AUC = 0.763 indicates **good diagnostic ability** of galanin for identifying hypothyroidism. **Cut-off Value:** 123.15 pg/ml is the optimal galanin level for diagnosis. **Youden's Index:** Measures overall test performance (Sensitivity + Specificity − 1). **Specificity:** Proportion of true negatives (healthy controls) correctly identified. **Sensitivity:** Proportion of true positives (hypothyroid patients) correctly identified. **95% Confidence Interval:** Range of uncertainty around the AUC estimate.						

4. Discussion

Since age showed no appreciable variation across the three study groups (control, hypothyroid, and hyperthyroid), the present study implies that age was not a major factor determining the reported metabolic or hormonal parameters. This age-matching raises group comparison dependability.

Body mass index (BMI), especially in the hypothyroid group whose levels above those of the control group and the hyperthyroid patients, varied significantly, nevertheless. This result is in line with the well-documented link between hypothyroidism and higher body weight, largely resulting from lower basal metabolic rate, fluid retention, and reduced thermogenesis connected with low thyroid hormone levels(Qiu et al., 2023). Interestingly, in this study those with hyperthyroidism had a somewhat higher BMI than the control group; although, given their greater metabolism, one would usually expect their BMI to be lower. This could represent personal variation, disease length, or other compensating elements (like a higher hunger). Previous research indicates that not all hyperthyroidism sufferers are underweight, particularly in the early or subclinical phases when the catabolic effects could not be clearly evident(Lee and Pearce, 2023). These results highlight the effect of thyroid dysfunction on body weight control, therefore generally supporting the importance of considering BMI as a clinical indicator and a possible outcome of changed thyroid hormone activity. This study investigated alterations in thyroid hormones, their impacts, and their association with the neuropeptide hormone galanin. We compared individuals with hypothyroidism (Hypo-TR), hyperthyroidism (Hyper-TR), and healthy controls. The results showed clear and significant differences in all thyroid measurements between the groups, which matched the known characteristics of thyroid problems. The data clearly reveal that TSH hormone levels were strongly raised in hypothyroid patients and conversely in the hyperthyroid group. These findings determine negative feedback systems in response to thyroid hormone levels. While they were raised in hyperthyroid patients compared to the control group, free T3 and T4 hormones levels were noticeably lowered in hypothyroid patients, therefore verifying the usual biochemical composition of each thyroid disease. Significantly, the study showed a gradual increase in galanin levels across all thyroid disorders; hypothyroid individuals had the greatest values. Such an increase could be either a compensatory neuroendocrine reaction linked with hypothyroidism or a possible function of galanin in thyroid hormone control. Though they were less evident than in the hyperthyroid group, the raised galanin levels in hypothyroid patients suggest their role in thyroid-related neuroendocrine signaling(Can et al., 2024b). Previous research has detected the function of galanin in regulating the hypothalamic-pituitary-thyroid axis, namely in controlling the secretion of TRH and TSH. The findings indicated that circulating galanin levels fluctuate markedly with thyroid functional state (Abed, n.d.). This indicates that the heightened galanin levels in hyperthyroid people may signify higher sympathetic and metabolic activity, whereas the elevated levels in hypothyroidism may reflect modified neural feedback due to hormone shortage. These results taken together indicate galanin as a prospective biomarker associated with thyroid dysfunction. Current studies keep clarifying its particular function and relation to thyroid dysfunction. Future research could look into galanin's therapeutic target or diagnostic marker possibilities for thyroid disorders. This study investigates the lipid profile of patients with thyroid dysfunction as a significant indicator associated with thyroid hormones and body mass index. Participants with thyroid hormone insufficiency showed elevated levels of total cholesterol and triglycerides(Arce-Sánchez et al., 2021; Mutalazimah et al., 2022). This corresponds to previous studies connecting hypothyroidism to dyslipidemia. These alterations are likely attributable to diminished activation of low-density lipoprotein

(LDL) receptors and reduced lipid clearance, both of which are significant characteristics of hypothyroidism. Indeed, the elevation of VLDL in the hypothyroidism group significantly supports the existence of elevated LDL lipid profiles in these individuals. Triglycerides and VLDL were higher in the hyperthyroid group than in the control group, although these differences were not statistically significant. Total cholesterol increased slightly but significantly. Thyroid hormones' complicated metabolic activities alter lipid production and blood filtration, causing this rise. Additionally, in this study, the combination of low thyroid hormones causes a decrease in fat metabolism, which affects total cholesterol, causing it to rise, as well as LDL and triglycerides, and a slight decrease in HDL levels. The levels of lipid profile are affected by the severity of hypothyroidism(Arce-Sánchez et al., 2021), meaning that higher TSH levels are associated with a greater increase in fat. The liver enzyme levels in the hyperthyroid group were elevated compared to the hypothyroid group and the healthy control group; however, these differences did not achieve statistical significance. This is due to increased thyroid hormones inducing toxic effects on the liver, resulting in oxidative stress and subsequently impacting liver enzyme activity(Piantanida et al., 2020). These results confirm that thyroid dysfunction affects the metabolic system. Additional research using larger sample sizes and longitudinal methods is necessary to validate these findings and clarify the underlying mechanisms.

5. Conclusion

The results indicate that elevated galanin levels in women with thyroid disorders are a potential biomarker associated with impaired thyroid function. Current studies continue to elucidate its specific function and its relationship to thyroid dysfunction. Future research may investigate galanin's therapeutic target or its potential as a diagnostic marker for thyroid disorders.

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Anxiety Disorders among Patients in Primary Care Centers

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Abstract

Background: Anxiety disorders are some of the most common mental health issues globally, with significant consequences for individuals' quality of life and overall well-being. In primary care settings, anxiety disorders frequently co-occur with somatic

Aim of study: This study aimed to investigate the prevalence of anxiety disorders among patients presenting with somatic complaints in a primary care setting.

Patients and Methods: A cross-sectional study was conducted on 106 patients visiting a primary care unit. Participants completed self-report questionnaires assessing somatic symptoms and anxiety disorders. Diagnostic interviews were also conducted to confirm anxiety disorder.

Results: The study included a total of 106 participants. The majority of participants were young adults aged 26-35 years (32.1%), followed by the 36-45 age group (26.4%). A smaller proportion of participants were in the older age group of 56-65 years (7.5%). In terms of gender, the sample was predominantly female (66%), with a smaller proportion of male participants (34%). results were shown that the prevalence of anxiety among a sample of 106 participants reached about 83%, which reported experiencing some level of anxiety, ranging from simple to severe. Income level emerged as a significant predictor of anxiety. Individuals with lower income levels were more likely to experience higher levels of anxiety. On the other hand, Age, sex, marital status, and number of children were not found to be significant predictors of anxiety in this study. The high prevalence of anxiety highlights the need for increased mental health awareness and access to mental health services. The significant association between income level and anxiety suggests that socioeconomic factors may play a crucial role in mental health.

Conclusion: Anxiety disorders are highly prevalent among primary care patients with somatic complaints. These findings highlight the importance of screening for and managing anxiety in this patient population to improve clinical outcomes.

اضطرابات القلق بين المرضى في مراكز الرعاية الأولية

زينب محمد حسن، سلام فيصل لفتة، علا بدري رسول

الخلاصة

المقدمة

تُعد اضطرابات القلق من أكثر مشكلات الصحة النفسية شيوعًا على مستوى العالم، ولها آثار كبيرة على جودة حياة الأفراد ورفاههم العام. وفي بيئات الرعاية الأولية، غالبًا ما تتزامن اضطرابات القلق مع الشكاوى الجسدية.

هدف الدراسة

هدفت هذه الدراسة إلى التحقيق في مدى انتشار اضطرابات القلق بين المرضى الذين يعانون من شكاوى جسدية في بيئة الرعاية الأولية.

المرضى والطرق

أُجريت دراسة مقطعية على 106 مرضى زاروا وحدة رعاية أولية. أكمل المشاركون استبيانات ذاتية لتقييم الأعراض الجسدية واضطرابات القلق، كما أُجريت مقابلات تشخيصية لتأكيد الإصابة باضطرابات القلق.

النتائج

شملت الدراسة 106 مشاركين. كانت النسبة الأكبر من المشاركين من فئة الشباب الذين تتراوح أعمارهم بين 26–35 سنة (32.1%)، تليها الفئة العمرية 36–45 سنة (26.4%)، في حين كانت النسبة الأقل من الفئة العمرية 56–65 سنة (7.5%). من حيث الجنس، كانت الغالبية من الإناث (66%)، مقارنة بنسبة أقل من الذكور (34%). أظهرت النتائج أن انتشار القلق بين العينة بلغ حوالي 83%، حيث أبلغ المشاركون عن مستويات مختلفة من القلق، تتراوح بين البسيطة إلى الشديدة.

وقد تبين أن مستوى الدخل يُعد مؤشرًا هامًا للتنبؤ بالقلق، حيث أن الأفراد ذوي الدخل المنخفض كانوا أكثر عرضة للإصابة بمستويات أعلى من القلق. من ناحية أخرى، لم تُظهر المتغيرات مثل العمر، الجنس، الحالة الاجتماعية، وعدد الأطفال دلالة إحصائية كمؤشرات للتنبؤ بالقلق. وتشير النسبة المرتفعة لانتشار القلق إلى الحاجة إلى زيادة الوعي بالصحة النفسية وتحسين إمكانية الوصول إلى خدمات الصحة النفسية. كما أن العلاقة المهمة بين الدخل والقلق توحي بأن العوامل الاجتماعية والاقتصادية قد تلعب دورًا حاسمًا في الصحة النفسية.

الاستنتاج

تُعد اضطرابات القلق شائعة بشكل كبير بين مرضى الرعاية الأولية الذين يعانون من شكاوى جسدية. وتسلّط هذه النتائج الضوء على أهمية الفحص المبكر ومعالجة القلق ضمن هذه الفئة من المرضى لتحسين النتائج السريرية.

1. Introduction

Somatic complaints, such as pain, fatigue, and gastrointestinal issues, are common reasons for seeking care in primary care settings. While these symptoms can have a variety of underlying medical causes, research has shown that psychological factors, particularly anxiety disorders, play a significant role in the experience and presentation of somatic symptoms (Simon *et al.*, 1999; Richardson and Brahmbhatt, 2021; Swainston *et al.*, 2023). Anxiety disorders, such as generalized anxiety disorder, panic disorder, and social anxiety disorder, are characterized by persistent feelings of worry, fear, and physiological arousal. These psychological states can manifest as physical symptoms, leading patients to seek medical care for their somatic complaints. However, the prevalence of anxiety disorders among primary care patients with somatic complaints is not well-established (Pihkala, 2020).

The current study aimed to investigate the prevalence of anxiety disorders in a sample of primary care patients presenting with somatic complaints. Understanding the relationship between anxiety and somatic symptoms can inform clinical practice and improve the management of these patients in primary care settings.

2. Patients and Methods

2.1. Participants and Procedure

The study was conducted at a primary care clinic in a metropolitan area. Over 6 months, 106 patients who presented with somatic complaints were recruited to participate. Eligible participants were adults (18 years or older) who reported experiencing physical symptoms, such as pain, fatigue, or gastrointestinal issues, as their primary reason for seeking care. Participants completed self-report questionnaires assessing their somatic symptoms and anxiety levels. They also underwent a diagnostic interview conducted by a trained clinician to determine the presence of any anxiety disorders based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria.

2.2. Measurement of Anxiety Disorder

Anxiety Disorders: The Structured Clinical Interview for DSM-5 Disorders (SCID-5) was used to diagnose anxiety disorders, including generalized anxiety disorder(Alhadi AN).

2.3. Data Analysis

Descriptive statistics were used to calculate the prevalence of anxiety disorders among the study participants. Chi-square tests and independent samples t-tests were conducted to examine the differences in somatic symptom severity between participants with and without an anxiety disorder diagnosis.

3. Results and Discussion

3.1. Demographic and Clinical Characteristics

The present study examined the demographic characteristics of 106 participants. The majority of participants were 26-35 years (N = 34, 32.1%,) followed by 36-45 (N = 28, 26.4%]. A smaller proportion of participants were in the 56-65 group, N = 8, 7.5%. Regarding marital status, most participants were married (N = 79, 74.5%). About (N = 23, 21.7%) were single, while a small percentage were divorced (N = 3, 2.8%) or widowed (N =1, 0.9%). In terms of gender, the sample was predominantly female (N = 70, 66%), with a less proportion of males (N = 36, 34%). Finally, the participants were distributed relatively evenly across the three income categories: 7.5% reported having low income, 67% had middle income, and 25.5% had high income as shown in Table1.

Table1: Frequency of the Participants According to Demographic Information

Variables	Groups	N	%
Age Group (Years)	16-25 Years	18	17.0
	26-35 Years	34	32.1
	36-45 Years	28	26.4
	46-55 Years	18	17.0
	56-65 Years	8	7.5
Status	Married	79	74.5
	Single	23	21.7
	Divorce	3	2.8
	Widow	1	0.9
Sex	Male	36	34.0
	Female	70	66.0
Income state	Low	8	7.5
	Middle	71	67.0
	Good	27	25.5

3.2. Scoring of Anxiety Symptoms

The results show that 42.5% of participants reported experiencing simple anxiety, followed by moderate anxiety (33.0%) and severe anxiety (7.5%). A relatively smaller proportion of participants (17.0%) reported no anxiety, overall, the Prevalence of anxiety was 83%, as presented in Fig.1.

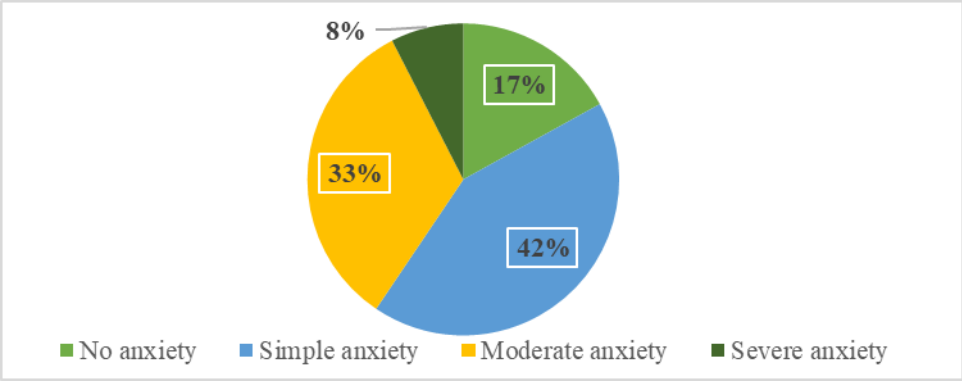

Figure1: Distribution of Anxiety Levels Among Study Participants
The pie chart illustrates the percentage of participants experiencing different levels of anxiety.**42%** had simple anxiety, **33%** had moderate anxiety, **17%** had no anxiety, **8%** experienced severe anxiety

The results of this study indicate a high prevalence of anxiety among the participants, with 83% reporting experiencing some level of anxiety. This is a concerning finding, as anxiety can have a significant negative impact on individuals' mental health and well-being. The most common level of anxiety was simple anxiety, reported by 42.5% of participants. This indicates that a significant portion of the population is experiencing mild anxiety symptoms. A substantial number of participants reported moderate (33.0%) and severe (7.5%) anxiety, highlighting the presence of more severe forms of anxiety within the population. The high prevalence of anxiety suggests a significant mental

health burden within the study population. Addressing anxiety is crucial for improving the overall well-being of individuals. There was no significant association between sex, Age of the participants and marital status with anxiety level ($p > 0.05$), as presented in Table2 Interestingly, there was a significant association between income level and anxiety level ($p < 0.05$). Individuals with lower income levels were more likely to report higher levels of anxiety compared to those with middle- or good-income levels. Furthermore, there was a significant association between the severity of anxiety and the reported difficulty it caused in daily life ($p < 0.05$). Participants with higher levels of anxiety (moderate or severe) were more likely to report significant difficulties in their lives compared to those with no anxiety or simple anxiety. Results were shown that financial difficulties may contribute to higher levels of anxiety. Also, it was highlighting the significant negative impact that anxiety can have on individuals' daily lives, particularly for those with moderate or severe anxiety.

Table2: The Association Between Different Variables with Anxiety Score

Variable	Groups	Anxiety				P-value
		No Anxiety	Simple Anxiety	Middle Anxiety	Sever Anxiety	
Sex	**Male**	5	16	12	3	0.543
	Female	13	29	23	5	
Age Group (Years)	**16-25**	5	9	4	0	0.065
	26-35	4	14	13	3	
	36-45	5	11	10	2	
	46-55	2	6	8	2	
	56-65	2	5	0	1	
Status	**Married**	11	33	29	6	0.134
	Single	7	10	5	1	
	Divorce	0	2	0	1	
	Widow	0	0	1	0	
Money	**Bad**	0	1	5	2	**0.001**
	Middle	9	33	24	5	
	Good	9	11	6	1	
Effect of anxiety on their lives	**Difficult**	13	12	3	0	**0.002**
	Extremely difficult	5	30	24	3	
	Very difficult	0	3	7	3	
	No difficulty	0	0	1	2	
Chi-Square Results are presented as N (%), $p<0.05$ considered significantly different						

The incidence pattern of anxiety disorders changes with age. Anxiety disorders are among the most common mental disorders in young people, especially for age group (26-45) which was similar as reported in previous study engagement with technology can be a risk factor for anxiety. Individuals have higher social media and technology use rates (Chan, Hsieh and Usak, 2021; Liao, Widowati and Hsieh, 2021; Rapee *et al.*, 2023; Zisopoulou and Varvogli, 2023). An increasing body of evidence has shown that technology and engagement with social media are positively correlated with anxiety disorders in young people. This study also found that a significantly large burden of anxiety disorders, as measured by the total number of cases, was present in middle & high-income. this finding is consistent with previous research demonstrating a link between affluence and anxiety. The reason that anxiety is more prevalent in higher-income individual could be due to a range of factors, like the typical lifestyle in affluent compared to low

income(22:495–499) (Hawes *et al.*, 2022; Prasad *et al.*, 2023; Zisopoulou and Varvogli, 2023). In more prosperous societies, jobs are typically more sedentary in nature. People obtain less physical activity; individuals are more likely to consume a diet heavy in processed foods, sodium, and sugar, which can catalyze psychophysiological reactions that impact mood and affect. High sodium levels can result in elevated blood pressure, resulting in psychological symptoms like stress and anxiety. Simple sugars can cause sharp spikes in blood glucose levels, resulting in anxiety symptoms. These factors can negatively impact mental health and increase the risk of developing an anxiety disorder over time. Anxiety impacts everyone differently. There are both physical and emotional symptoms, varying in severity. Not only does anxiety impact the person with the symptoms, but it can also often impact family and friends. Being diagnosed with anxiety can affect a person's career, hobbies, and self-esteem (Baxter *et al.*, 2013, 2014; Villasante Fricke and Miteva, 2015). A mental health professional may diagnose an anxiety disorder if the level of anxiety is to such a degree that symptoms are overpowering and interfere with daily life. The healthcare professional may ask about physical symptoms and thoughts and how these impacts regular life. Table3 and Fig.2 presented the distribution of anxiety levels across different income groups. Results were shown that only 7 out of 8 Individuals in the low-income group reported the mild to moderate levels of anxiety. While the middle-income group had a higher prevalence of anxiety compared to the high-income group, it was notably higher than the low-income group. In high-income group, 9 out of 27 were not reported any level of anxiety, 11, 6 were reported a simple to moderate anxiety and only 1 were reported severe level These findings highlight the importance of addressing socioeconomic disparities to improve mental health outcomes (Baxter *et al.*, 2013, 2014; Bandelow and Michaelis, 2015; Bandelow, Michaelis and Wedekind, 2017; Commodari and La Rosa, 2021). Policies and interventions that aim to reduce poverty, increase access to healthcare, and promote social inclusion can be effective in mitigating the impact of income on anxiety and other mental health conditions.

Table3: Anxiety Level According to the Income of the Participants

Anxiety	Low Income	Middle income	High Income
No anxiety	0	9	9
Simple anxiety	1	33	11
Moderate anxiety	5	24	6
Severe anxiety	2	5	1

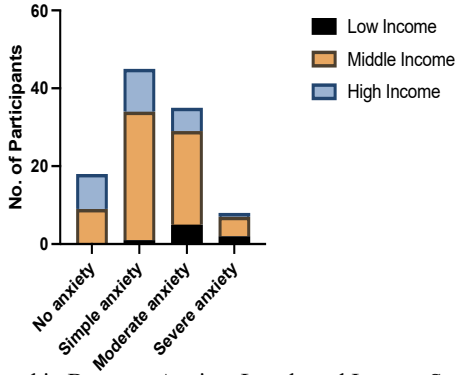

Figure2: Relationship Between Anxiety Levels and Income Status of Participants
This stacked bar chart illustrates the distribution of anxiety levels (simple, moderate, severe) among participants categorized by their income status: **Low income**, **Middle income**, **Good income**. Each bar represents the total number of participants within an income group, with segments showing the proportion experiencing different anxiety levels. The data suggests that anxiety, particularly moderate and simple anxiety, is more prevalent among participants with middle income, while severe anxiety is less reported across all income levels.

Income inequality pertains to disparities in income between the rich and the poor and is recognized as one of the world's most serious social problems. It has been found to be associated with less sustainable economic growth, lower civic engagement, more health problems, and lower psychological well-being Studies have found that the effects of inequality remain robust after controlling for income levels, suggesting that its effects are distinct from absolute income. In the educational context, inequality has most often been explored in relation to achievement outcomes, and past studies have shown a negative association between the two (Maddocks, 2010; Bandelow, Michaelis and Wedekind, 2017). However, less attention has been paid to affective outcomes such as test anxiety. Although we are not aware of any previous empirical study that has linked income inequality to test anxiety, income inequality "may serve as a contextual stressor" and make test anxiety more prevalent. Indeed, studies have shown that income inequality is perceived as threatening and stressful. studies have shown that income inequality is perceived as threatening and stressful Indirect empirical evidence for the potential linkage between income inequality and test anxiety can also be found in two interrelated yet distinct strands of literature (Pickett and Wilkinson, 2015; Carey, 2022). The first strand is from epidemiological research. Epidemiological studies have shown that people living in areas with higher levels of income inequality have worse mental health outcomes, including higher levels of depression and anxiety. This pattern has been found both within and across countries, and applies to both those of lower and higher socioeconomic status (SES). Research has also shown that students who have high test anxiety are more likely to suffer from mental health and socio-emotional problems. The second strand of work comes from sociological literature which focuses on income inequality and status anxiety (Patel *et al.*, 2018; Shimonovich *et al.*, 2022). In highly unequal societies, individuals can gain more material and social resources by doing better than their peers. Inequality also makes one's position in the social hierarchy more important and salient. Thus, people become stressed and anxious about their relative social position and fearful of being left behind by their peers. Although test anxiety is distinct from status anxiety, these different forms of anxiety nevertheless share a common conceptual dimension. The data in Table4 shown a clear link between the severity of anxiety and the reported quality of life. Individuals with no or simple anxiety were more likely to report no difficulty in their daily lives (Patel *et al.*, 2018). Conversely, those with moderate or severe anxiety were more likely to report experiencing difficulties in their daily lives. These findings suggest that anxiety can significantly impair a person's ability to function in daily life. It highlights the importance of addressing anxiety to improve overall well-being (Beidel and Turner, 2007; Hofmann, 2007; 'Shy children, phobic adults: nature and treatment of social anxiety disorder', 2007).

Table4: Frequancy of the Anxiety Level Based on the Quality of Life

Anxiety	No Difficulty	Difficult	Very Difficult	Extremely Difficult
No anxiety	13	5	0	0
Simple anxiety	12	30	3	0
Moderate anxiety	3	24	7	1
Severe anxiety	0	3	3	2

As shown in Table5, Multiple linear regression analysis was conducted to examine the association between anxiety level and demographic characteristics. Results were shown that the strongest predictor of anxiety was the number of children, with a significant negative association ($p = 0.002$). Interestingly, this suggests that individuals who have

more children are more likely to experience of decreasing level of anxiety While none of Age, Sex, status, and Money were found to be significant predictors of depression in this analysis (Beidel and Turner, 2007; Hofmann, 2007; 'Shy children, phobic adults: nature and treatment of social anxiety disorder', 2007; Patel *et al.*, 2018; Carey, 2022; Shimonovich *et al.*, 2022).

Table5: Multiple Linear Regression for the Association of Anxiety and Demographic Characteristics

Groups	Anxiety			
	B	**S.E**	**t**	**P value**
Age	0.035	0.037	0.945	0.347
Sex	0.458	0.839	0.546	0.586
Status	0.399	0.717	0.557	0.579
Child	-2.207	0.705	-3.132	**0.002**
Money	0.035	0.037	0.945	0.347
Results are presented as N(%), p<0.05 considered significantly different				

Anxiety disorder showed neither age nor gender has a difference in the prevalence, these results were consistence with the previous finding by those who reported that there were no differences between men and women about the age of onset and the estimated chronicity of anxiety disorders. Significant gender effects were observed in the patterns of comorbidity and in the dysfunction associated with having an anxiety disorder, which together underscores the importance of gender to the epidemiology of anxiety (Yang *et al.*, 2021; Farhane-Medina *et al.*, 2022). The multiple linear regression analysis reveals a significant negative association between the number of children and anxiety levels, suggesting that individuals with more children are less likely to experience anxiety. This finding is somewhat counterintuitive, as having children is often associated with increased stress and responsibilities. The possible explanations might be that having children can foster strong social bonds and provide a sense of purpose and fulfillment, which may contribute to reduced anxiety. Or it might be that parenting can necessitate the development of coping mechanisms to manage stress and challenges, which may also help in reducing anxiety. Other parents often have a strong support network of family and friends, which can provide emotional support and reduce feelings of anxiety. Finally, having children may lead individuals to prioritize their needs and focus on the well-being of their family, which can help to mitigate anxiety-inducing thoughts and behaviors (Bekker and van Mens-Verhulst, 2007; Hofmann, 2007; McLean *et al.*, 2011; Asher and Aderka, 2018).

4. Conclusion

Patients in primary care who present with somatic complaints, such as physical symptoms without a clear medical explanation, often have underlying depression. These individuals may not recognize or report their emotional distress, leading to underdiagnosis and undertreatment of depression. Proper screening for depression in such patients can improve overall care and address both their psychological and physical health needs. Early identification and treatment, including therapy or medication, can lead to better health outcomes for these patients.

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Evaluation the Risk of Atherosclerosis Among Some Iraqi Hyperlipidemic Patients Taking Atorvastatin

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Abstract

Background: Hyperlipidemia is a family of disorders that are characterized by abnormally high levels of lipids in the blood. While fats play a vital role in the body's metabolic processes, high blood levels increase the risk of atherosclerosis and cardiovascular diseases, especially coronary heart disease (CHD). This research aims to provide an investigation of hyperlipidemia and will focus on the atherogenic index of plasma (AIP) and The Castelli's risk indexes (CRI-I & CRI-II) which are strong markers to predict the risk of atherosclerosis and coronary heart disease.

Methodology: In this cross-sectional study, one hundred forty-nine Iraqi male and female patients with primary hyperlipidemia of the age of 28 to 85 years, who were treated with atorvastatin 40mg for at least 6 months were recruited. Lipid profile and liver functions were assessed, and the AIP and CRI-I & CRI-II were calculated to predict the risk of atherosclerosis and coronary heart disease.

Results: The study's finding shows there are 42 patients (28.2%) with good response to the statin therapy (atorvastatin 40 mg), about 35% of studied patients with a moderate response and about 39% of patients had poor or non-response after at least 6 months of the treatment. Additionally, there are 17 patients (11.4%) with a low risk of IHD, 3.4% with a moderate risk, and 85% of studied patients had a high risk of IHD according to the results of AIP. According to CRI-I and CRI-II there are 68 and 118 patients at low risk and 81, 31 patients at high risk to IHD respectively. Significant differences were observed in the levels of TC, BMI, and AIP between the age groups of the studied patients. Moreover, there are significant differences in the levels of TC, and AST regarding to the duration of treatment groups of the studied patients.

Conclusion: The study highlights varying responses to atorvastatin, with 39% showing poor or no response. AIP results indicate that 85% of patients are still at high IHD risk, supported by CRI-I and CRI-II assessments.

تقييم خطر تصلب الشرايين بين بعض المرضى العراقيين المصابين بفرط شحميات الدم الذين يتناولون الأتورفاستاتين

خالد قاسم محمد، مازن حامد عودة، سوزان جبير

الخلاصة

المقدمة

فرط شحميات الدم هو مجموعة من الاضطرابات التي تتميز بارتفاع غير طبيعي في مستويات الدهون في الدم. وعلى الرغم من أن الدهون تلعب دورًا حيويًا في العمليات الأيضية داخل الجسم، إلا أن ارتفاع مستوياتها في الدم يزيد من خطر الإصابة بتصلب الشرايين وأمراض القلب والأوعية الدموية، وخاصة مرض الشريان التاجي .(CHD) يهدف هذا البحث إلى دراسة فرط شحميات الدم مع التركيز على مؤشر تصلب الشرايين في البلازما (AIP) ومؤشري كاستيلي للمخاطر(CRI-I وCRI-II)، اللذين يُعتبران مؤشرات قوية للتنبؤ بخطر الإصابة بتصلب الشرايين ومرض القلب التاجي.

العينات وطرق العمل

في هذه الدراسة المقطعية، تم تجنيد 149 مريضًا ومريضة عراقيين يعانون من فرط شحميات الدم الأولي، تتراوح أعمارهم بين 28 و85 عامًا، وخضعوا للعلاج بعقار أتورفاستاتين بجرعة 40 ملغ لمدة لا تقل عن 6 أشهر. تم تقييم ملف الدهون ووظائف الكبد، كما تم حساب مؤشرات AIP و CRI-Iو CRI-IIللتنبؤ بخطر تصلب الشرايين ومرض القلب التاجي.

النتائج

أظهرت نتائج الدراسة أن 42 مريضًا (28.2%) استجابوا بشكل جيد لعلاج الستاتين (أتورفاستاتين 40 ملغ)، في حين كان لدى حوالي 35% استجابة معتدلة، و39% لم يكن لديهم استجابة كافية أو لم يستجيبوا للعلاج بعد ستة أشهر على الأقل. بالإضافة إلى ذلك، تبين أن 17 مريضًا (11.4%) لديهم خطر منخفض للإصابة بمرض القلب الإقفاري(IHD) ، و3.4% لديهم خطر معتدل، في حين أن 85% من المرضى المشمولين بالدراسة كانوا معرضين لخطر مرتفع وفقًا لنتائج مؤشر .AIP ووفقًا لمؤشري-CRI IوCRI-II، كان هناك 68 و118 مريضًا على التوالي في فئة الخطر المنخفض، و81 و31 مريضًا على التوالي في فئة الخطر المرتفع للإصابة بمرض القلب الإقفاري. كما لوحظت فروق ذات دلالة إحصائية في مستويات الكوليسترول الكلي(TC) ، ومؤشر كتلة الجسم(BMI) ، ومؤشر AIP بين الفئات العمرية للمرضى. علاوة على ذلك، وُجدت فروق ذات دلالة إحصائية في مستويات الكوليسترول الكلي (TC) وإنزيم AST بين مجموعات المرضى وفقًا لمدة العلاج.

الاستنتاج

تسلط الدراسة الضوء على التباين في استجابة المرضى لعقار أتورفاستاتين، حيث أظهر 39% منهم استجابة ضعيفة أو معدومة. كما تشير نتائج مؤشر AIP إلى أن 85% من المرضى لا يزالون معرضين لخطر مرتفع للإصابة بمرض القلب الإقفاري، وهو ما تؤكده أيضًا تقييمات مؤشري CRI-I و.CRI-II

1. Background

Hyperlipidemia is recognized as a significant risk factor for the development of cardiovascular diseases (CVDs) (Alloubani et al., 2021). It involves an elevated level of one or more plasma lipids, such as triglycerides, cholesterol, cholesterol esters, and phospholipids, or plasma lipoproteins, including very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), along with a decrease in high-density lipoprotein (HDL) levels (Sheeba and Gandhimathi, 2021). Elevated cholesterol (hypercholesterolemia) and triglyceride levels (hypertriglyceridemia) are primary contributors to atherosclerosis, a condition closely associated with ischemic heart disease (IHD) (Shao et al., 2020). Atherosclerosis is characterized by the hardening of arteries caused by cholesterol buildup within the arterial walls, leading to their narrowing. Hyperlipidemia contributes to the progression of atherosclerosis and increases the risk of related conditions, such as coronary, cerebrovascular, and peripheral vascular diseases (AL-Ezzy and Hameed, 2021). Hyperlipidemia is associated with heightened oxidative stress, resulting in the excessive production of oxygen free radicals. These radicals can cause oxidative modifications in low-density lipoproteins (LDL), which play a crucial role in the onset and progression of atherosclerosis and related CVD (Pappan et al., 2024). Statins are a group of medications that inhibit the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, thereby reducing the production of cholesterol in the body. They are the most commonly prescribed drugs for lowering lipid levels and are widely used for both the prevention and management of CVDs (Zhou and Liao, 2009, Mills et al., 2011) which remain the leading cause of death globally (Mc Namara et al., 2019). The development of CVDs is influenced by several established risk factors, including diabetes, elevated blood glucose levels, lack of physical activity, smoking, low levels of HDL cholesterol, and increased levels of LDL cholesterol (Sun et al., 2015). Therefore, effectively managing hypertension and dyslipidemia is essential for reducing the risks associated with CVD. Today, satins are the most effective oral medications for preventing and treating cardiovascular events caused by hypercholesterolemia (Ramkumar et al., 2016). Clinical trials have demonstrated that statins not only reduce LDL cholesterol and triglyceride (TG) levels (Ramkumar et al., 2016), but also decrease the morbidity and mortality linked to coronary heart disease, cerebrovascular disease, and peripheral arterial disease (Mills et al., 2011).The atherogenic index of plasma (AIP) serves as a reliable indicator for assessing the risk of atherosclerosis and coronary heart disease (Wu et al., 2021). It represents the balance between protective and atherogenic lipoproteins and correlates with the size of lipoprotein particles involved in atherogenesis (Niroumand et al.). AIP is calculated according to the formula, log(TG/HDL-C) (Askin and Tanriverdi, 2023). Castelli's risk indexes (CRI-I and CRI-II), also known as cardiac risk indexes, are two lipid ratios associated with CVD risk. CRI-I represents the ratio of total cholesterol (TC) to HDL-C, while CRI-II is the ratio of LDL-C to HDL-C (Olamoyegun et al., 2016). These indexes were introduced by William Castelli in the late 20th century (Abid et al., 2021), and subsequent studies have confirmed their strong positive correlation with CVD risk (Gómez-Álvarez et al., 2020, Igharo et al., 2020). CRI-I has been specifically shown to reflect the formation of coronary plaques and the thickness of the carotid artery intima-media in young adults(Nair et al., 2009, Frontini et al., 2007). This research aims to evaluate the risk of atherosclerosis and CVD among some Iraqi hyperlipidemic patients on atorvastatin treatment.

2. Materials and Methods

2.1. Study subjects

This research was conducted as a cross-sectional observational study at Imam Al-Hussain Medical City and the Kerbala Center for Cardiac Diseases and Surgery in Karbala, spanning from November 2023 to April 2024. The study received approval from the Scientific and Ethical Committee of the College of Pharmacy, University of Kerbela. Informed consent was obtained from all participants after explaining the purpose and details of the study. The study included 149 participants (80 males and 69 females), aged between 28 to 85 years, who had been receiving a daily oral dose of 40 mg atorvastatin as monotherapy for hyperlipidemia for a minimum of six months. During blood sample collection, participants were asked about any additional medications that could potentially influence atorvastatin metabolism.

Data were gathered from both medical records and direct interviews with participants. The collected information included age, weight, height, smoking habits, treatment duration, adverse drug reactions, other medical conditions, and concurrent medications.

Five milliliters of venous blood were drawn from the participants, and was kept in gel tube, centrifuged to get the serum, which was then used for the biochemical analysis. These parameters included lipid profile (HDL, LDL, TC, and TG levels) which performed by The cobas c 311 analyzer, developed by Roche Diagnostics, HDL cholesterol is measured using a homogeneous enzymatic colorimetric assay, Roche's HDL-C Gen.4 reagent is utilized for this analysis. The determination of LDL cholesterol is performed using a direct homogeneous assay, Roche's LDL-C Gen.3 reagent is utilized for this analysis, an enzymatic colorimetric method for total cholesterol (TC) determination Roche's Cholesterol Gen.2 reagent is specifically designed for this purpose and triglyceride levels (TG) measure. liver function tests which involve ALT level which is determined using the International Federation of Clinical Chemistry (IFCC) recommended method without pyridoxal phosphate. This kinetic assay measures the rate of NADH oxidation, which is directly proportional to ALT activity. Roche's ALTL reagent is employed for this test. Similar to ALT, AST activity is measured via the IFCC method without pyridoxal phosphate. The assay monitors the decrease in NADH absorbance, reflecting AST activity in the sample. The ASTL reagent from Roche is used for this purpose. Total bilirubin levels are measured using a colorimetric diazo method. In acidic conditions, bilirubin reacts with a diazo reagent to form azobilirubin, a colored compound. The intensity of the color is proportional to the total bilirubin concentration. Roche provides the BIL-T Gen.3 reagents for total bilirubin measurements, and random blood sugar RBS.

2.2. Statistical Evaluation

Statistical analysis was performed using IBM SPSS software, version 26. The data were entered into a computerized database to identify and address any errors or inconsistencies, as well as to organize, process, and analyze the information. Scale variables were presented as mean values with standard error of the mean (SEM), while categorical variables were reported as frequencies and percentages. A parametric test was used due to the normal distribution of the scale variables. One-way analysis of variance (ANOVA) was applied to compare means across more than two groups, with statistical significance $P \leq 0.05$.

3. Results

A total of 149 Iraqi participants, both male and female, with primary hyperlipidemia were included in this study. Their demographic details are outlined in Table1. Among the participants, 42 patients (28.2%) showed a good response to statin therapy (40 mg atorvastatin). Approximately 35% had a moderate response, while about 39% exhibited poor or no response. Table 1 also shows that 17 patients (11.4%) were classified as having a low risk of ischemic heart disease (IHD), 3.4% had a moderate risk, and 85% had a high risk of IHD based on AIP results. Regarding CRI-1, about 46% of patients were classified as low risk, while 54% were considered at high risk for CVD and atherosclerosis. For CRI-2, 80% of the participants were at low risk, and approximately 20% were at high risk for CVD and atherosclerosis.

Table1: The Demographic and Atherogenic Characteristics of the Hyperlipidemia Patients.

Variable		N	Percent
Gender	Male	80	53.7
	Female	69	46.3
Age	25-45	25	16.8
	46-65	95	63.8
	66-85	29	19.5
BMI	Under weight	1	0.7
	Normal	32	21.5
	Over weight	88	59.1
	Obese	27	18.1
Duration of treatment (months)	6-12	112	75.2
	13-24	25	16.8
	25-36	6	4.0
	37-48	6	4.0
Smoking	No smoke	100	67.1
	Smoker	49	32.9
Response (LDL level)	<50 Good	42	28.2
	51-100 Moderate	52	34.9
	>100 None	55	36.9
AIP	<0.11	17	11.4
	0.11-0.21	5	3.4
	>0.21	127	85.2
CRI-I	<3.5	68	45.6
	>3.5	81	54.4
CRI-II	<3	118	79.2
	>3	31	20.8
BMI: body mass index, AIP: Atherogenic Index of Plasma, CRI-1: Castelli's risk indexe-1, CRI-2**:** Castelli's risk indexe-2			

Table2 presents the mean levels of laboratory biomarkers in the study participants, categorized by age. Significant differences were observed in body mass index (BMI) between groups B and A, with a p-value of 0.014. Additionally, TC levels showed a significant difference between groups C and B, with a p-value of 0.036. A significant variation in AIP results was found between groups A vs. C and B vs. C, with p-values of 0.048 and 0.05, respectively. Furthermore, total bilirubin levels showed significant differences between groups B vs. A and C vs. A, with p-values of 0.034 and 0.042, respectively. Lastly, CRI-1 results revealed significant differences between groups A vs. C and B vs. C, with p-values of 0.034 and 0.032, respectively.

Table2: The Laboratory Biomarkers in the Hyperlipidemia Patients Categorized by Age

Parameter	A:25-45 years (n=25)	B:46-65 years (n=95)	C:66-85 years(n=29)	MC	P value
BMI	26.25±0.67	28.32±0.41	26.86±0.47	B vs A	0.014
SBP	129.60±4.93	134.01±1.86	135.45±3.51	NS	0.512
DBP	81.36±2.25	83.72±1.08	82.90±2.50	NS	0.646
RBS	149.32±23.50	139.74±5.66	154.48±15.22	NS	0.603
LDL	91.40±8.49	89.78±4.92	74.34±8.79	NS	0.268
HDL	43.20±3.46	46.15±1.72	50.10±5.51	NS	0.441
VLDL	29.52±3.45	28.70±1.47	23.20±2.06	NS	0.160
TC	160.44±10.78	163.14±5.12	140.28±9.60	C vs B	0.036
TG	147.60±17.28	143.55±7.38	116.03±10.32	NS	0.160
ALT	22.28±2.97	20.60±0.98	19.77±1.57	NS	0.669
AST	51.00±24.81	32.38±4.79	33.66±6.61	NS	0.434
TBL	0.820±0.14	0.544±0.05	0.500±0.08	B vs A C vs A	0.034 0.042
AIP	0.49±0.049	0.46±0.026	0.35±0.044	A vs C B vs C	0.048 0.050
CRI-1	4.20±0.324	3.83±0.151	3.15±0.250	A vs C B vs C	0.034 0.032
CRI-2	2.27±0.261	2.11±0.126	1.61±0.231	NS	0.105

BMI stands for body mass index, SBP refers to systolic blood pressure, DBP is diastolic blood pressure, RBS indicates random blood sugar, LDL represents low-density lipoprotein, HDL denotes high-density lipoprotein, VLDL refers to very low-density lipoprotein, TC stands for total cholesterol, TG indicates triglycerides, ALT represents alanine transaminase, AST refers to aspartate transaminase, TBL denotes total bilirubin, AIP for The Atherogenic Index of Plasma, and CRI-1 and CRI-2 for The Castelli's risk indexes (I & II)

Table3 presents the mean levels of laboratory biomarkers in the study participants, categorized by treatment duration. Significant differences were observed in total cholesterol (TC) levels between groups C and B, as well as in AST levels between groups C and A, and C and B.

Table3: Description of the Laboratory Biomarkers of the Studied Patients According to Duration of Treatment

Parameter	A-6-12 months (n=112)	B- 13-24 months (n=25)	C-25-36 months (n=6)	D-37-48 months (n=6)	MC	P value
BMI	27.61±0.35	28.36±0.922	25.87±1.09	28.26±0.61	NS	0.505
SBP	132.01±1.85	140.16±3.72	135.00±6.70	133.33±8.79	NS	0.310
DBP	82.76±0.97	84.56±2.50	85.83±4.90	82.17±8.83	NS	0.826
RBS	143.53±7.09	143.80±14.35	161.83±43.14	141.17±11.95	NS	0.949
LDL	84.21±3.97	89.13±12.59	120.86±22.71	97.60±21.08	NS	0.280
HDL	46.07±2.02	48.32±3.29	45.33±4.90	46.16±4.83	NS	0.965
VLDL	28.90±1.43	24.69±2.66	25.96±3.35	21.40±1.83	NS	0.383
TC	159.71±4.42	145.80±12.87	192.33±26.19	148.50±22.70	C vs B	0.047
TG	144.50±7.16	123.48±13.32	129.83±16.76	107.00±9.18	NS	0.383
ALT	20.52±0.86	19.58±1.71	35.83±10.33	14.06±1.46	NS	0.669
AST	29.57±2.21	44.04±17.20	130.33±102.94	22.06±2.87	C vs A C vs B C vs D	0.001 0.003 0.003
TBL	0.596±0.05	0.504±0.09	0.700±0.11	0.533±0.55	NS	0.851
AIP	0.47±0.025	0.37±0.04	0.45±0.77	0.37±0.06	NS	0.300
CRI-1	3.83±0.133	3.28±0.388	4.26±0.476	3.29±0.402	NS	0.258
CRI-2	2.09±0.106	1.75±0.361	2.64±0.440	1.79±0.350	NS	0.385

BMI stands for body mass index, SBP refers to systolic blood pressure, DBP is diastolic blood pressure, RBS indicates random blood sugar, LDL represents low-density lipoprotein, HDL denotes high-density lipoprotein, VLDL refers to very low-density lipoprotein, TC stands for total cholesterol, TG indicates triglycerides, ALT represents alanine transaminase, AST refers to aspartate transaminase, TBL denotes total bilirubin, AIP for The Atherogenic Index of Plasma, and CRI-1 and CRI-2 for The Castelli's risk indexes (I & II)

Table4 shows the correlation matrix for the relation of AIP on some related parameters under the study. The relation between TG and AIP is 0.745 with a P-value of 0.001.

Table4: Correlation Matrix for the Relation of AIP with Some Related Parameters Under the Study

Parameter	Status	AIP	SBP	LDL	TG
AIP	Correlation	1	0.041	0.618	0.745**
	p Value		0.618	0.153	0.001
SBP	Correlation	0.041	1	-0.106	0.057
	P value	0.618		0.200	0.492
LDL	Correlation	0.153	-0.106	1	0.134
	P value	0.062	0.200		0.103
TG	Correlation	0.745**	0.001	0.134	1
	P value	0.001	0.057	0.103	

4. Discussion

This study aimed to evaluate hyperlipidemia as a risk factor for atherosclerosis among the Iraqi hyperlipidemic patients taking atorvastatin. A total of 149 Iraqi participants, both male and female, with primary hyperlipidemia were included in this study. According to the guidelines for the management of dyslipidemia (European Society of Cardiology/European Atherosclerosis Society, ESC/EAS) (Mach et al., 2020) 28% of the patients showed a good response to statin therapy with LDL levels below 50 mg/dL. Approximately 35% had a moderate response, with LDL levels ranging from 51 to 100 mg/dL, while about 39% exhibited poor or no response, with LDL levels exceeding 100 mg/dL after at least six months of treatment. On evaluation of lipid ratios in the current study, AIP was significantly high in about 85% of studied patients which indicate high risk to IHD and atherosclerosis. AIP is a ratio calculated as

(log TG/HDL-C). Studies have shown an inverse relationship between TG and HDL-C and that the ratio of TG to HDL-C is a strong predictor of infarction (Sami Khaza, 2013). AIP is being used by some practitioners as a significant predictor of atherosclerosis. It has been suggested that AIP values of less than 0.1 are associated with low, 0.1 to 0.21 with medium and above 0.21 with high cardiovascular risk (Sadeghi et al., 2021). CRI is based on three important lipid profile parameters i.e. TC, LDL-C and HDL-C. CRI-I calculated as the ratio of (TC/HDL-C) and CRI-II as (LDL-C/HDL-C) (Yıldız et al., 2016). In about 54% of our patients, CRI-I was greater than 3.5, which represents the upper normal limit (Subia Jamil and Afshan Siddiq, 2012). This level of CRI-I associated with coronary plaques formation and increase risk to IHD. In our study, CRI-II was also found to be above the upper limit for normal range (>3) (Subia Jamil and Afshan Siddiq, 2012) in 21% of the studied patients. In PROCAM study, it was observed that subjects with LDL-C/HDL-C greater than 5 had six times higher rate of coronary events (Bhardwaj et al., 2013).Dyslipidemias is characterized by lipid circulatory disorders, including high levels of LDL-C, elevated triglycerides, high levels of TC, and low levels of HDL-C (Kopin and Lowenstein, 2017). LDL carries about 60–70% serum cholesterol (DiPiro et al., 2014) and transports the liver's cholesterol to the peripheral tissues. A high level of LDL-C is harmful because it can build up to initiate the formation of atherosclerotic plaques on the arterial walls (Elshourbagy et al., 2014). Atherosclerosis develops when the endothelial layer of blood vessels is damaged by factors such as hypertension, smoking, diabetes, and high LDL-C levels (Frąk et al., 2022). This damage compromises the integrity of the arterial wall, making it more permeable and allowing LDL particles to accumulate in the subendothelial space (Khatana et al., 2020). Once trapped by proteoglycans in the extracellular matrix, these LDL particles undergo oxidative changes due to reactive oxygen species (ROS) and enzymes like lipoxygenase, Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and myeloperoxidase (Gianazza et al., 2021). The oxidation of LDL makes it highly reactive and toxic to cells, initiating a strong immune response. When oxidized LDL (oxLDL) accumulates in the arterial wall, the immune system perceives it as a harmful substance. In response, endothelial cells produce adhesion molecules such as vascular cell adhesion molecule 1 and intercellular adhesion molecule 1 , which facilitate the recruitment of white blood cells, particularly monocytes, to the affected area(Razeghian-Jahromi et al., 2022). Once monocytes infiltrate the arterial wall, they transform into macrophages that engulf oxLDL through specialized scavenger receptors like SR-A and CD36 (Lee and Choi, 2020). Unlike normal LDL uptake, this process lacks regulation, leading to continuous lipid accumulation within macrophages. As a result, these lipid-filled macrophages become foam cells, a key feature of early atherosclerotic plaques. Foam cells contribute to inflammation by releasing cytokines such as tumor necrosis factor-alpha , interleukin-1 beta , and monocyte chemoattractant protein-1 (Chistiakov et al., 2017). This inflammatory response further attracts immune cells and promotes additional LDL retention, accelerating plaque development. As the plaque grows, smooth muscle cells, influenced by growth factors like platelet-derived growth factor, migrate to the site and produce extracellular matrix proteins such as collagen, forming a fibrous cap over the lipid core to provide stability. However, persistent inflammation and mechanical stress can weaken this fibrous cap, making it prone to rupture(Jansen et al., 2024). If the cap breaks, the exposed thrombogenic material interacts with circulating blood, triggering platelet activation and the formation of a blood clot (thrombus). This can obstruct blood flow, leading to severe cardiovascular events such as a heart attack or stroke (Alkarithi et al., 2021). Large randomized trials have shown that lowering LDL-C with 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors

("statins") reduces coronary mortality and morbidity in some high-risk patients (Feingold, 2024).In this study, group C (ages 66–85 years) exhibited significantly lower TC levels and a reduced risk of IHD and atherosclerosis, as indicated by AIP and CRI-I results, compared to the younger groups (Table 2 and Table 3). This can be attributed to several physiological, pharmacological, and behavioral factors such as variations in drug metabolism, particularly in the liver, play a significant role in the effectiveness of atorvastatin. Aging can slow liver metabolism, which is responsible for processing the drug. As a result, higher circulating levels of atorvastatin may enhance its cholesterol-lowering effects(Hirota et al., 2020). Lifestyle factors also influence cholesterol management. Older adults tend to adhere more consistently to prescribed medications and lifestyle modifications, such as maintaining a healthier diet, which contributes to more effective cholesterol control. Additionally, they are more likely to attend regular follow-up appointments with healthcare providers, ensuring continuous monitoring and better management of their cholesterol levels. In contrast, younger individuals with high cholesterol may be less consistent in adopting lifestyle changes, such as improving their diet or increasing physical activity, as they often perceive that they have more time to address their health concerns. Regarding the duration of treatment, the results in (Table 3) indicates that group C, with a treatment duration of 25–36 months, shows significantly higher total cholesterol (TC) levels compared to group B, whose treatment duration ranges from 13–24 months. This difference may be attributed to factors such as reduced adherence to treatment, lifestyle influences, disease progression, or inadequate dosing. Group C also exhibits significantly higher levels of AST compared to the other groups. This may be explained by the mechanism of atorvastatin, which inhibits HMG-CoA reductase in the liver to reduce cholesterol production (Jiang et al., 2018). Over time, this process could slightly strain liver cells, potentially causing mild and chronic elevations in liver enzymes for some patients. Additionally, prolonged exposure to the drug might result in minor liver cell injury or increased cell turnover, when hepatocytes are injured, the breakdown of both the plasma membrane and mitochondrial membranes causes the release of intracellular enzymes like AST into the blood (Contreras-Zentella and Hernández-Muñoz, 2016). This happens due to structural damage, oxidative stress, inflammation, and impaired energy production, all of which compromise the cell's ability to maintain its integrity and lead to the leakage of AST (Herrick et al., 2016, Ziolkowska et al., 2021). Prolonged use of atorvastatin may lead to mild muscle injury in certain individuals, a condition known as statin-induced myopathy. Because AST is present in muscle tissue as well as the liver, muscle damage could be a contributing factor to increased AST levels (Taha et al., 2014). According to the correlation between AIP and the other parameters, as demonstrated in (Table 4), there is a strong direct correlation between AIP and TG; as TG levels increase AIP levels also rise. This elevation in AIP is associated with a higher risk of IHD (Kim et al., 2021).

5. Conclusion

The study reveals variability in the patient's response to atorvastatin (40 mg) with only one third of patients showing a good response, while a significant portion exhibited moderate or poor/non-response. The AIP results indicate that the majority of patients are at high risk of IHD, with only a small fraction at low or moderate risk. Risk assessment using CRI-I and CRI-II further supports these findings, showing a considerable number of the patients at high risk. Additionally, significant differences in TC, BMI, and AIP levels were observed across different age groups, suggesting that age may influence lipid profile and cardiovascular risk.

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Vit D and Interleukin-17 Levels in Patients with Acne Vulgaris Severity in Anbar Governorate.

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Abstract

Acne vulgaris is a common inflammatory skin disorder influenced by immune responses, particularly involving interleukin-17 (IL-17) and vitamin D (Vit.D). This study aimed to evaluate the relationship between serum IL-17 and Vit.D levels with acne severity. Blood samples were collected from 96 patients with acne vulgaris and 84 healthy controls in Haditha District, Anbar Governorate. Participants were matched by age and gender. Serum IL-17 and Vit.D levels were measured using Sandwich-ELISA. Results showed significantly higher IL-17 levels and lower Vit.D levels in acne patients compared to controls ($P < 0.0001$). Acne severity was positively correlated with IL-17 and inversely with Vit.D levels ($P < 0.001$). Female patients had higher IL-17 levels and more pronounced Vit.D deficiency than males. Although no direct correlation was found between IL-17 and Vit.D levels, both markers were significantly associated with disease severity and gender. ROC analysis demonstrated their diagnostic potential. In conclusion, elevated IL-17 and Vit.D deficiency are strongly linked to acne pathogenesis and may serve as biomarkers or therapeutic targets in acne management.

مستويات فيتامين د و الانترلوكين 17 لدى المرضى حب الشباب الشديد في محافظة الانبار

عبد الله زيد خلف، وجيه يونس العاني , عبد الله صالح حسن

خلاصة

يعد الجهاز المناعي أحد العوامل التي يمكن أن تؤثر على شدة حب الشباب الشائع، وهو حالة جلدية التهابية شائعة. يعد Interleukin-17 (IL-17) وفيتامين د (Vit.D) مكونين أساسيين يساهمان في تطور الالتهاب في حب الشباب. إن فهم العلاقة بين IL-17 ومستويات فيتامين د وشدة حب الشباب قد يوفر رؤى مهمة حول الأهداف المحتملة للعلاج وطرق علاج حب الشباب لدى الأفراد. تضمنت الدراسة 96 مريضا مصابا بحب الشباب و 84 مجموعة ضابطة في عينات الدم المستخدمة في هذه الدراسة. قسم المرضى والضوابط بالتساوي على أساس العمر والجنس وشدة الحالة. جمعت العينات في قضاء حديثة بمحافظة الأنبار. في هذه الدراسة استخدمت تقنية ELISA. لوحظ نقص فيتامين د. مصحوبًا بزيادة في IL-17 في المرضى الذين يعانون من حب الشباب الشائع مقارنةً بالأشخاص الأصحاء (قيمة P <0.0001). وارتبطت مستويات فيتامين د عكسيا مع شدة حب الشباب (P <0.001). وارتبطت مستويات IL-17 طرديا مع شدة المرض (P <0.001)، وشوهدت مستويات أعلى من IL-17 في الإناث مقارنة بالذكور (P <0.001). وكان الانخفاض في فيتامين د أكثر وضوحا بين الإناث منه بين الذكور. علاوة على ذلك، لم يلاحظ أي ارتباط بين مستويات مصل IL-17 وفيتامين د. ومع ذلك، كان هناك ارتباط سلبي قوي بين مستويات مصل IL-17 وفيتامين. د مقارنة بالجنس وأيضا على مستوى شدة المرض. وقد لوحظ وجود ارتباط سلبي ضعيف بين IL-17 وفيتامين د- مستويات المصل مقارنة بخطورة المرض. كما لوحظ وجود علاقة سلبية قوية بين مستويات IL-17 وفيتامين د في مصل الدم مقارنة بخطورة المرض والجنس. استخدم منحنى ROC لتقييم أداء IL-17 وفيتامين د. خاتمة. أظهرت هذه الدراسة أن IL-17، وهو السيتوكين الالتهابي، يؤدي إلى تفاقم حب الشباب. ومن ناحية أخرى، فقد تبين أيضًا أن نقص فيتامين د يزيد من شدة حب الشباب. هناك علاقة سلبية بين انخفاض فيتامين د ومستويات عالية من IL-17. يكون نقص أكثر انتشارًا في مرضى حب الشباب مقارنة بالأشخاص الأصحاء.

1. Introduction

The common dermatological condition known as acne vulgaris is characterized by inflammation and blockage of sebaceous glands and hair follicles (Alajeel & Hasan, 2021). is a chronic inflammatory disorder of pilosebaceous units with polymorphic nodules, including closed and open comedones, pimples, pustules, and nodules. It is detected in 85% of teenagers worldwide (Kutlu et al., 2023).The pathogenesis extrinsic to P. acnes colonization and infection is well known, and includes hormones, hyper keratinization and plugging of sebaceous ducts, sebum production, and the ensuing colonization insecurity and irritation of certain facts. In contrast, the formations and their interactions are not well understood in sequence (Alajeel & Hasan, 2021; Kang et al., 2005; Kutlu et al., 2023). The severity of AV is influenced by the immune system, particularly by T helper (Th) 1, Th2, and Th17 cells. Interleukin-17 (IL-17), which is produced by Th17 cells, has a part in acne, according to a study conducted by Kim's group. Another study highlighted how crucial Th1 and Th17 cytokines are to acne vulgaris. Both protein levels and the mRNA of IL-17A, a crucial Th17 signaling pathway effector cytokine, were significantly elevated in acne lesions. Moreover, they discovered an increase in Th17-related cytokines at the mRNA level, including IL-6, TGF-β, IL-1β, and IL-23 (Agak et al., 2014; Kelhälä et al., 2014). Vitamin D (Vit D) plays a crucial role in immune regulation, keratinocyte and sebocyte proliferation, and differentiation. Moreover, it functions as an antioxidant by inducing critical enzymes such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), thereby helping to prevent comedone formation. Inflammatory papulopustular acne patients often exhibit reduced levels of these antioxidant enzymes. Deficiency of these antioxidants is frequently observed in papulopustular acne. Additionally, Vit D enhances the production of antimicrobial peptides like cathelicidin, highlighting its importance in acne pathogenesis (Alhetheli et al., 2020; Chang & Lee, 2019; Iqbal et al., 2023; Spiro & Buttriss, 2014). Importantly, the active form, 1,25-dihydroxyvitamin D, directly suppresses IL-17 gene transcription and impairs IL-17 production by Th17 cells in response to *P. acnes*, highlighting a potential therapeutic mechanism in acne. Although Vit D and IL-17 each have documented roles in acne vulgaris pathophysiology, few studies have simultaneously evaluated their relationship (A. Singh et al., 2021). Our study addresses this gap by comparing serum IL-17 and Vit.D levels between acne patients a know if you'd like this formatted in Harvard style or need full reference details.

2. Patients and Methods

This study involved a total of 96 patients diagnosed with acne vulgaris, comprising 48 males and 48 females. Each group was further categorized by a specialist physician into three subgroups based on disease severity: mild, moderate, and severe. Participants were matched for age, ranging from 13 to 20 years. An additional 84 healthy individuals served as the control group, equally divided into 42 males and 42 females, and matched by age with the patient cohort. Participants were recruited from intermediate and preparatory schools in Haditha City, as well as from the College of Basic Education/Haditha. The study procedures were conducted in the laboratories of the College of Basic Education/Haditha and Haditha General Hospital. Venous blood samples (5 mL) were collected using sterile, disposable gel tubes. Samples were allowed to clot at 37°C for 10–15 minutes, followed by centrifugation at 3000 rpm for 10–15 minutes to separate the serum, which was then stored at −20°C. Serum levels of interleukin-17 (IL-17) and vitamin D were measured using a sandwich ELISA kit (SunLong Biotech Co., Ltd., China) on a Human Reader Hs ELISA system (Germany).

2.1. Statistical Analysis

The statistical analysis was performed using IBM SPSS Statistics version 26. One-way and two-way ANOVA with Tukey's post hoc test, along with the Student's t-test, were used to assess group differences. Pearson's correlation coefficient (r) was applied to determine associations between variables. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values and assess the predictive performance (AUC) of serum biomarkers. The Shapiro–Wilk test confirmed the normal distribution of quantitative data, which were expressed as mean ± standard deviation (SD).

3. Results

Patient and control groups were similar in age and gender Table1. However, the patients' mean BMI ± SD was higher than the AV patients. For the IL-17 relationship between control groups and acne patients, the mean ± SD for the control group was (404.07±71.125) (pg/ml) with a percentage of (46.67%). For acne patients, it was (708.28±165.114) (pg/ml) and by a rate of (53.33%), which gave a p-value higher than (0.0001), which indicates that there are statistically significant differences in IL-17 between the control group and the patient group. The relationship between gender and IL-17, as the mean ± SD for the control group for males, was (344.95±49.419) (pg/ml) (N=42), while its value for females was (463.18± 25.284) (pg/ml) (N=42). Its value for acne vulgaris patients was (835.73± 95.976) (pg/ml) (N=48) for males and (580.83±112.756) (pg/ml) (N=48) for females, where the P value for males was higher than (0.0001) While the P value for females was higher than (0.0001), Table1. This suggests a significance between the IL-17 variant and acne vulgaris in males and females for both the patient and the control groups.

Table1: Clinical and Demographic Characteristics of Acne Patients and Control Subjects

Parameter	Mean Parameter ± SD		*p*-Value
	Controls	patients	
Age (year)	16.64±2.342	16.50±2.303	0.681
BMI (kg/m2)	18.994±2.378	24.086±4.576	< 0.0001
IL-17 (pg/mL)	404.07±71.125	708.28±165.114	<0.0001
Male	344.95±49.419	835.73±95.976	< 0.0001
Female	463.18±25.284	580.83±112.756	< 0.0001
Vit. D (ng/ml)	8.13±1.746	20.08±0.537	<0.0001
Male	19.60±0.218	9.21±1.544	< 0.0001
Female	20.55±0.256	7.05±1.181	< 0.0001
SD= Std. Deviation.			

The results showed the IL-17 for AV severity Table2 as the mean ±SD of mild, moderate, and severe stimulation (607.38±134.428, 695.24±166.814, and 822.22±116.456), respectively. The P value was higher than 0.001 between mild and severe, the P value = 0.0014 between moderate and severe, and the P value = 0.0 377between mild and moderate, all significant. The results of the study showed two-way ANOVA of variance (Tukey's test for comparative analysis) that the P values were statistically significant for the average acne severity of males as shown in Table2 where it is mean ± SD was mild (728.81±58.852), moderate (855.23±35.356) and severe (923.16±58.270) for males P values mild vs. moderate was higher than (0.001), mild vs. severe was higher than (0.001) and moderate vs. severe (0.007) were significant for all comparisons. The relationship between acne

severity and gender with IL-17 Results for females were mean ± SD (485.94±49.244), (535.26 ±40.658), (721.28±53.830) mild, moderate, and severe, respectively, P values mild vs. moderate (0.0179) mild vs. severe was higher than (0.001) moderate vs. severe was higher than (0.001) were significant for all comparisons.

Table2: Relationship Between Acne Severity with IL-17 And Vit.D and Genders

Parameter	Mean Parameter ± SD			Tukey's multiple comparisons test	P Value
	Mild	Moderate	Severe		
IL-17 (pg/ml)	607.38±134.428	695.24±166.814	822.22±116.456	mild vs. moderate	0.0377
				Mild vs. Severe	<0.001
				Moderate vs. Severe	0.0014
Male	728.81±58.852	855.23±35.356	923.16±58.270	Mild vs. Moderate	<0.001
				Mild vs. Severe	<0.001
				Moderate vs. Severe	0.007
Female	485.94±49.244	535.26 ±40.658	721.28±53.830	Mild vs. Moderate	0.0179
				Mild vs. Severe	<0.001
				Moderate vs. Severe	<0.001
Vit. D (ng/ml)	9.21±1.714	8.12±1.429	7.07±1.411	Mild vs. Moderate	0.0141
				Mild vs. Severe	<0.001
				Moderate vs. Severe	0.0188
Male	10.42±1.385	9.16 ±1.166	8.07±1.115	Mild vs. Moderate	0.0036
				Mild vs. Severe	<0.001
				Moderate vs. Severe	0.0137
Female	8.01±1.031	7.08 ±0.742	6.07±0.862	Mild vs. Moderate	0.0418
				Mild vs. Severe	<0.001
				Moderate vs. Severe	0.0243

The association between control groups and acne patients regarding Vit. D. Table1 for the control group, the mean ± SD was (8.13±1.746) (ng/ml) with a percentage of 46.67%, and for the acne patients, it was (20.08±0.537) (ng/ml) with a rate of 53.33%. This resulted in a p-value greater than (0.0001), indicating statistically significant differences in Vit. D between the patient group and the control group. The relationship between gender and Vit.D, as the mean ± SD for the control group for males, was (19.60±0.218) (ng/ml) (N=42), while its value for females was (20.55±0.256) (ng/ml) (N=42). Its value for acne vulgaris patients was (9.21±1.544) (ng/ml) (N=48) for males and (7.05±1.181) (ng/ml) (N=48) for females, where the P value for males was higher than (0.0001) While the P value for females was higher than (0.0001). This indicates a significance between the control group and acne vulgaris for males and females. The results showed the Vit.D for acne severity [Table 2] according to the mean ±SD of mild, moderate, and severe stimulation (9.21±1.714, 8.12±1.429 and 7.07±1.411) respectively. The P value was <0.001 between mild and severe, the P value 0.0188 between moderate and severe, and the P value 0.0141 between mild and moderate, all significant. The study's results showed that a two-way ANOVA of variance (Tukey's test for comparative analysis) showed that the P values were statistically significant for the average acne severity of males, where its mean ± SD was mild (10.42±1.385), moderate (9.16 ±1.166), and severe (8.07±1.115) for males. The p-value for Mild vs. Severe was greater than (0.001), Mild vs. Moderate (0.0036), and Moderate vs. Severe (0.0137). The relationship between acne severity and gender with VitD results for females were mean ± SD (8.01±1.031), (7.08±0.742), and (6.07±0.862) for mild, moderate, and severe, respectively. P values for Mild vs. Moderate (0.0418) and Mild vs. Severe greater than (0.001) and Moderate vs. Severe (0.0243) were significant for all comparisons. The results of linear regression analysis showed a non-significant correlation between IL-17 and (Vit. D) blood serum AV patient $p = 0.512$, $p = (0.068)$. Still, when compared with gender, it gave a strong negative correlation $p < 0.01$, $r = (-0.894)$ of IL-17 with male blood serum Vit.D concentration and a strong negative correlation $p < 0.01$, $r = (-0.843)$ of IL-17 with female blood serum Vit.D concentration. Also, when

studying the linear correlation between IL-17 and Vit. D in the AV patients' severity showed a weak positive correlation $p < 0.05$, $r = (0.415)$ of IL-17 with acne patients' mild blood serum (Vit. D) concentration, a medium positive correlation $p < 0.01$, $r = (0.587)$ of IL-17 with acne patients moderate blood serum (Vit. D) concentration and a weak positive correlation $p < 0.05$, $r = (0.377)$ of IL-17 with acne patients sever blood serum (Vit. D) concentration. So was the linear correlation between IL-17 and (Vit. D) AV patient's severity and gender. A strong negative correlation $p < 0.01$, $r = (-0.895)$ of IL-17 with AV patients' mild male blood serum (Vit. D) concentration, strong negative correlation $p < 0.01$, $r = (-0.922)$ of IL-17 with AV patients moderate male blood serum (Vit. D) concentration and There was a strong negative correlation $p < 0.01$, $r = (-0.782)$ of IL-17 with AV patients sever blood serum (Vit. D) concentration. There was a strong negative correlation $p < 0.01$, $r = (-0.813)$ of IL-17 with AV patients' mild female blood serum (Vit. D) concentration, a strong negative correlation $p < 0.01$, $r = (-0.932)$ of IL-17 with Av patients moderate female blood serum (Vit. D) concentration and a strong negative correlation $p < 0.01$, $r = (-0.785)$ of IL-17 with acne patients sever blood serum (Vit. D) concentration. We used a receiver operating characteristic curve (ROC) analysis to get the diagnostic values for IL-17 and Vit. D about patient and control differentiation. With an area under the curve (AUC) of 0.960 and a 95% confidence interval ranging from 0.921 to 0.984, $p = < 0.0001$, IL-17 demonstrated an excellent ability to differentiate between the control and acne vulgaris patient groups. At cutoff values of >512.94, the test's sensitivity and specificity were 83.33% and 100.00%, respectively Fig.1. With an area under the curve (AUC) of 0.970 and a 95% confidence interval ranging from 0.933 to 0.990, $p = < 0.0001$, vitamin D demonstrated an excellent ability to distinguish between the control and acne vulgaris patient groups. The test's sensitivity and specificity were 100.00% and 94.05%, respectively, at cutoff values of ≤11.975 Fig.2. The ROC curve showed good diagnostic accuracy for the area under the curve (AUC) for IL-17 and Vit D in the control and AV patient groups, which may help diagnose some conditions. It can also be used to distinguish people with acne from healthy people.

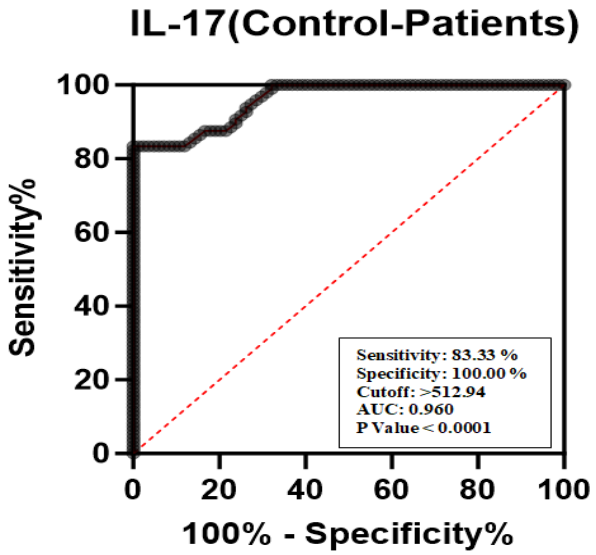

Figure1: ROC Curve Analysis of Serum IL-17 Levels Distinguishing AV Patients from Healthy Controls The ROC curve shows an area under the curve (AUC) of 0.960, indicating excellent diagnostic accuracy. At a cutoff value of >512.94 pg/ml, IL-17 demonstrates a sensitivity of 83.33% and a specificity of 100%. The analysis was statistically significant ($P < 0.0001$).

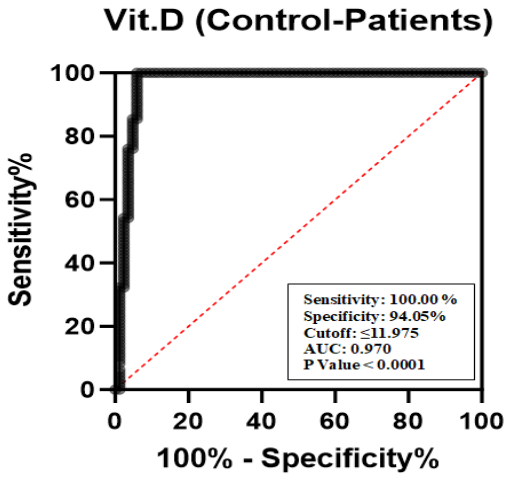

Figure2: Receiver Operating Characteristic (ROC) Curve Analysis of Serum Vitamin D Levels Differentiating AV Patients from Healthy Controls

The area under the curve (AUC) was 0.970, with a cutoff value of ≤11.975 ng/mL, yielding a sensitivity of 100.00% and a specificity of 94.05% ($P < 0.0001$).

4. Discussion

The pathogenesis of acne has lately been connected to several cytokines, including IL-17. Vitamin A (ATRA) and Vit.D inhibit P. acnes-driven Th17 differentiation and downregulate IL-17 synthesis at the mRNA and protein levels because they share a signaling pathway with the retinoid X receptor. Interestingly, greater suppression of retinoid orphan receptors α (RORα) and RORγ expression was found to indicate the synergistic activity of ATRA and Vit.D on retinoid receptors. The relationship between IL-17 and the development of acne in patients has been the subject of much research in the last several years. Proinflammatory cytokine IL-17 is involved in the etiology of numerous inflammatory disorders, including acne (A. Singh et al., 2021). A substantial relationship between IL-17 and acne was identified in their cross-sectional observational study on the link between interleukins and acne. In a survey by Egyptians to evaluate the level of interleukin 17 (IL-17) in the blood of patients suffering from acne vulgaris, they concluded that IL-17 is significantly higher in acne patients compared to the control group. Moreover, it increases considerably with Increased disease severity and in patients with scarring lesions. In a study that did not agree with our results they conducted on 80 acne patients, there was no statistically significant relationship between the severity of AV lesions and the level of IL-17. He conducted a study that agreed with our results on a group of acne patients (Akdeniz et al., 2018; El Husseiny et al., 2012; Maalmi et al., 2012). The results showed significant differences in levels between cases and controls for interleukin 17 levels. In another study that agreed with our results on a group of acne patients, the results were that the average levels of IL-17 in serum were significantly higher ($P < 0.001$) in acne sufferers in contrast to the group under control. There was a noteworthy relationship ($P < 0.001$) between the severity of acne and the levels of IL-17. His findings corroborated the association between IL-17 and the severity of acne in another study (Agak et al., 2014; Ebrahim et al., 2019; S. Singh et al., 2023). According to their findings, the severity of acne vulgaris was correlated with higher levels of IL-17. Furthermore, contrary to our findings, studies revealed that girls with acne vulgaris had higher levels of IL-17 than males with the same condition. This suggests that IL-17 may have a greater effect on women's acne severity. Our results showed a lower mean Vit.D for patients compared to control groups, a lower mean for females compared to males, and a correlation of Vit.D level with disease severity, as it was an inverse relationship with disease severity. In addition to providing the body with

Vit.D, the skin can respond to 1,25(OH) 2D, which is the active metabolite of Vit. D. Sebocytes exhibit robust expression of the critical components of the Vit.D system, including the 25-hydroxylase, 1α-hydroxylase, 24-hydroxylase, and Vit.D receptor. The interaction between 1,25(OH) 2D and calcium regulates skin differentiation. Vit.D is critical for the immune system and skin health. Vitamin D, in its active form, calcitriol, has anti-inflammatory (Akdeniz et al., 2018; Ebrahim et al., 2019; El Husseiny et al., 2012; S. Singh et al., 2023). Properties and may help regulate the skin's immune response. Moreover, Vit.D has been linked to the regulation of keratinocyte proliferation and differentiation, which is essential for maintaining skin health. Depending on the concentration, Vit.D can either promote or inhibit the growth of keratinocytes (≥10-8 M). According to Krämer et al., 1,25(OH) D has a biphasic effect on sebocytes, boosting Z95 sebocytes that proliferate slowly while repressing those that do so rapidly. Moreover, it has been demonstrated that in situations of low vitamin D, cultured sebocytes release higher levels of inflammatory cytokines (IL6, IL8, and MMP9) (Krämer et al., 2009; Lee et al., 2013; S. Singh et al., 2023). It has been demonstrated that Vit.D increases the skin's innate immune response and modulates the responses of T and B lymphocytes, dendritic cells, Toll-like receptor 2 (TLR2), and its co-receptor CD. Furthermore, Vit.D deacetylates and dephosphorylates the protein known as Forkhead box O (FoxO), activating it and further preventing the liver's synthesis of IGF-1. Furthermore, Vit.D triggers the FoxO signaling pathway, which successfully inhibits the mTORC1 (mammalian target of rapamycin complex 1) and prevents IGF-1 signaling, an important route in the development of acne. The emergence or worsening of acne symptoms may be linked to a deficiency of Vit.D. Maranda et al. conducted a thorough study and meta-analysis (Ahmed Mohamed et al., 2021; Lee et al., 2013; Lim et al., 2016; Rasti et al., 2022). In cases of Vit.D deficiency accompanied by acne vulgaris, the research showed that Vit.D supplements were linked to a decrease in the severity of acne, indicating a possible therapeutic role. Vit.D in acne therapy. The effect of vit was investigated by Chun et al. in a different investigation. Vit.D levels in relation to acne vulgaris severity. The researchers enrolled individuals with mild to moderate acne vulgaris and divided them into two groups. “One group received a placebo, while the other was administered Vitamin D tablets. After 12 weeks, the group receiving Vitamin D medication and the group receiving a placebo exhibited notably distinct levels of acne vulgaris”.

Conclusion

The current study recorded that there is no correlation between serum IL-17 levels and Vit.D levels. However, it showed that there are correlations between IL-17 Vit.D levels in terms of gender and in terms of disease severity, in addition to the existence of a direct relationship between IL-17 levels and AV severity. This study also revealed that There is an inverse relationship between the severity of AV and Vit.D. Therefore, it is worth studying the modification of Vit.D and IL-17 levels and their effect on the severity of AV, especially in patients with severe degrees of acne. Our results, along with previous studies, suggest that acne severity can be predicted by measuring IL-17 and Vit.D levels.

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Viral Hepatitis: Types, Symptoms, Treatment and Prevention: A Review

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Abstract: Viral hepatitis is an infectious liver disease caused by several viruses, primarily Hepatitis A, B, C, D, and E. These viruses lead to liver inflammation, with symptoms ranging from mild jaundice to severe liver damage. Hepatitis A is often transmitted via contaminated food or water, particularly affecting children, while Hepatitis B, C, and D spread through blood and bodily fluids, leading to chronic liver conditions. Hepatitis E, common in regions with poor sanitation, spreads through water contamination. Although there is no definitive cure for most types, vaccines are available for Hepatitis A and B, and preventive measures like safe hygiene and avoiding contaminated sources can reduce infection risks. The hepatitis B vaccine has been recommended for infants since 1991 and is typically administered in three doses. It generates immunity in 95% of vaccinated children, with protection declining slightly in older adults. Immunity remains long-lasting even if antibody levels fall below the protective threshold. For infants born to hepatitis B-infected mothers, vaccination combined with immune globulin is highly effective in preventing transmission. The vaccine is crucial for those at risk, such as healthcare workers and individuals in contact with infected persons.

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التهاب الكبد الفيروسي: الأنواع، الأعراض، العلاج والوقاية : مراجعة

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الخلاصة

التهاب الكبد الفيروسي هو مرض معدٍ يصيب الكبد، تسببه عدة فيروسات، أهمها فيروسات التهاب الكبد A و B و C و D و E. تؤدي هذه الفيروسات إلى التهاب في الكبد، وتتنوع الأعراض بين اليرقان الخفيف وتلف الكبد الشديد. ينتقل فيروس التهاب الكبد A عادة عبر الطعام أو الماء الملوث، ويصيب الأطفال بشكل خاص، في حين أن فيروسات التهاب الكبد B و C و D تنتقل عبر الدم وسوائل الجسم، مما يؤدي إلى حالات مزمنة تصيب الكبد. أما فيروس التهاب الكبد E ، الشائع في المناطق التي تفتقر إلى الصرف الصحي الجيد، فينتقل عبر تلوث المياه. ورغم عدم وجود علاج نهائي لمعظم أنواع التهاب الكبد، تتوفر لقاحات لفيروس التهاب الكبد A و B، كما أن التدابير الوقائية مثل الحفاظ على النظافة وتجنب مصادر التلوث يمكن أن تقلل من خطر العدوى. وقد تم التوصية بتطعيم الأطفال ضد التهاب الكبد B منذ عام 1991، وعادة ما يُعطى على ثلاث جرعات. يولد اللقاح مناعة لدى 95% من الأطفال المطعّمين، مع انخفاض طفيف في مستوى الحماية لدى البالغين الأكبر سناً. وتظل المناعة طويلة الأمد حتى لو انخفضت مستويات الأجسام المضادة إلى ما دون العتبة الوقائية. أما بالنسبة للرضع المولودين لأمهات مصابات بالتهاب الكبد B، فإن الجمع بين التطعيم والغلوبيولين المناعي يعد فعالاً للغاية في منع انتقال العدوى. ويعتبر اللقاح ضرورياً لأولئك المعرضين للخطر، مثل العاملين في مجال الرعاية الصحية والأشخاص الذين يتعاملون مع المصابين.

1. Introduction

Viral hepatitis, or epidemic hepatitis, is an infectious ailment induced by viruses that impair liver cells. This damage may be either transient or irreversible. The illness is marked by the infiltration of inflammatory cells in liver tissues, resulting in diverse clinical manifestations. One of the hallmark symptoms of viral hepatitis is jaundice, particularly common among children, where the skin and eyes take on a yellowish. There are five primary types of viral hepatitis: A, B, C, D, and E. In addition to these, there are other, less well-defined types, such as Hepatitis G, which are not as clearly linked to the disease. Some forms of hepatitis can be transmitted through sexual contact. Acute liver failure, a serious complication often leading to coma and death, is a common cause of mortality in patients with viral hepatitis (Murray, Rosenthal and Pfaller, 2019). Although children tend to experience less severe symptoms, the infection can later result in liver cirrhosis, tissue damage, or even liver failure. Currently, there is no specific treatment for viral hepatitis. When the liver is affected by viral hepatitis, the death of liver cells may result in various complications, such as repeated bleeding due to the liver's decreased production of clotting factors. Hepatitis A is primarily transmitted through contact with an infected person's feces, urine, or saliva. In contrast, hepatitis B, C, and D are mainly spread through blood transfusions and other blood-related procedures (Shepard, 2006).

1.1. Types of Viral Hepatitis

1.1.1. Hepatitis A

It is highly contagious but rarely fatal. It is common among children, in densely populated areas, and during travel to regions where the virus is prevalent. The virus is found in the feces of infected individuals, and transmission usually occurs through contaminated food and water. It can also spread through raw or undercooked foods, such as shellfish, vegetables, and fruits, especially if washed with contaminated water (Murray, Rosenthal and Pfaller, 2019).

Symptoms

Common symptoms include body aches, dark urine (similar to tea), diarrhea, fever, weakness, nausea, vomiting, jaundice (yellowing of the skin and eyes), loss of appetite, and light-colored stools. The virus is often contracted through contaminated water, ice, raw seafood, and unwashed fruits and vegetables. Rarely, saliva, semen, vaginal fluids, or urine transmit the disease. Severe dehydration, confusion, extreme drowsiness, or loss of consciousness, along with swelling in the face, hands, and feet, water retention in the body, and bleeding from the nose, mouth, or under the skin, are key indicators of hepatitis A (Shepard, 2006).

Treatment

Physicians recommend a high-protein diet and advise patients to stay hydrated, especially during vomiting episodes. Drinking water, soups, and fruit juices is encouraged, while alcohol and liver-damaging medications should be avoided. Preventive measures, such as handwashing with hot water and soap after contact with the patient or their feces, are crucial. To avert infection, immune globulin (IG) may be injected within two weeks of potential exposure to the virus. However, the hepatitis A vaccine does not work once the infection is present. Symptoms typically subside within two weeks, during which time the patient's feces remain contagious. Hepatitis A is considered less dangerous than other types (Murray, Rosenthal and Pfaller, 2019).

1.1.2. Hepatitis B

Acute and chronic hepatic infections are caused by the highly contagious Hepatitis B virus (HBV). When they first get an illness, many people don't feel sick. However, for some, the symptoms may come on quickly and last for weeks. These may include nausea, vomiting, jaundice, lethargy, black urine, and stomach discomfort. From thirty to one hundred and eighty days is the incubation period. About 90% of people infected at birth develop chronic illness, but less than 10% of those infected after 5 years old do. Liver cirrhosis and cancer are long-term consequences that can occur in 15–25% of people with chronic liver disease, even though most people with this condition experience no symptoms at all (Seeger and Mason, 2000). Transmission of the virus occurs primarily through direct contact with infected blood or body fluids. In highly endemic areas, infection is commonly acquired during childbirth or early childhood through close contact with infected individuals. In low-endemic regions, The two most common ways that this virus can spread are through intravenous drug use and having sex without protection. Infections can also spread through healthcare settings, blood transfusions, hemodialysis, close contact with an infected individual, and visiting regions with a high infection rate (Liaw and Chu, 2009). In the 1980s, tattoos and acupuncture were significant transmission sources, though this has become less common due to improved sterilization techniques. Hepatitis B is not transmitted through casual contact such as handshakes, shared eating utensils, or breastfeeding. Since 1982, vaccination has played a crucial role in preventing hepatitis B infection. The World Health Organization recommends vaccination starting at birth, followed by two or three doses to ensure complete immunity. The vaccine is effective in approximately 95% of cases, and as of 2006, around 180 countries had implemented vaccination programs (*Weekly epidemiological record Relevé épidémiologique hebdomadaire*, 2009). Additional preventive measures include blood screening prior to transfusions and the use of condoms during sexual activity. Antiviral medications, such as Tenofovir or Interferon, are often prescribed for chronic infections but can be expensive. Liver transplantation remains the final treatment option for cirrhosis patients (Lampertico *et al.*, 2017). Globally, approximately one-third of the world's population has been infected with HBV at some point, with 240-350 million people suffering from chronic infections. In 2013, there were 129 million new infections, and annually, over 750,000 people die due to hepatitis B-related complications, such as liver cancer. The disease is highly endemic in East Asia and Sub-Saharan Africa, where 5-10% of the population is chronically infected. In contrast, infection rates are less than 1% in Europe and North America. Despite its global impact, efforts continue to improve treatments and develop oral vaccines to combat this viral threat. In summary, hepatitis B remains a significant global health issue, particularly in developing regions. Through vaccination and preventive healthcare measures, progress is being made to reduce its spread and the severe complications associated with chronic infection (Schweitzer *et al.*, 2015).

Prevention

Since 1991, the United States has recommended that infants receive the hepatitis B vaccine. Most of these vaccines are given in three doses over a period of months. The presence of anti-HBs antibodies in the blood serum of a vaccinated person, at a concentration of 10 mIU/ml or higher, indicates a positive response to the vaccine, meaning the recipient has developed immunity against hepatitis B virus. The hepatitis B vaccine is highly effective in generating immunity in children, with 95% of vaccinated children developing sufficient antibodies to protect them from the virus. However, the level of protection decreases with age, with 90% efficacy in individuals around 40 years old and 75%

efficacy in those 60 years or older. The protection provided by the vaccine is long-lasting, and immunity remains even if anti-HBs antibody levels drop below 10 mIU/ml. Vaccination at birth is particularly important for infants if the mother is infected with hepatitis B. A combination of hepatitis B immune globulin and emergency doses of the hepatitis B vaccine can prevent mother-to-child transmission during birth, with a success rate of 86% to 99%. It is essential for individuals who may need blood or fluid transfusions to be vaccinated against hepatitis B if they have not already done so. Certain tests can determine whether a person has developed effective immunity against the hepatitis B virus, and additional doses of the vaccine may be administered if the person does not have adequate protection (Weekly epidemiological record Relevé épidémiologique hebdomadaire, 2009). In cases involving assisted reproductive technologies, there is no need for sperm washing if the male partner is infected with hepatitis B, as long as the female partner has been properly vaccinated. The risk of viral transmission from an infected mother to a child conceived through assisted reproductive technologies is the same as the risk of transmission during natural pregnancy. There are specific tests for individuals at high risk of contracting hepatitis B, and effective treatment is available for those infected. These tests are particularly necessary for individuals who have not been vaccinated (Liaw and Chu, 2009).

Vaccine Duration

studies conducted over a period of 10 to 22 years, no cases of hepatitis B infection were reported among individuals with a normal immune system who had received the full course of vaccination. However, rare cases of chronic infection have been documented (Weekly epidemiological record Relevé épidémiologique hebdomadaire, 2009).

Treatment

Acute hepatitis B generally does not require treatment, as the infection often resolves spontaneously in adults. Early antiviral treatment is only needed in rare cases—less than 1% of those who contract a severe infection (fulminant hepatitis) or those with compromised immune systems. On the other hand, treating chronic infections is crucial to reduce the risk of cirrhosis and liver cancer. Patients with chronic hepatitis B who show persistent elevation of alanine aminotransferase (ALT) levels, indicating liver damage, and high HBV-DNA levels are candidates for treatment. Treatment duration ranges from six months to a year, depending on the therapy and the patient's genetic makeup (Lampertico *et al.*, 2017). Although no available treatments can completely eradicate the infection, they can stop viral replication, reducing liver damage. As of 2008, seven antiviral drugs have been approved in the United States to treat chronic hepatitis B. Remivudine, entecavir, telbivudine, tenofovir, and adefovir are antiviral medications that fall under this category. Two more immune system modulators are pegylated interferon-alpha 2A and interferon-alpha 2A itself. As a first line of defence against cirrhosis, the World Health Organisation suggests tenofovir and entecavir (Lampertico *et al.*, 2017). Interferon treatment requires daily or thrice-weekly injections but has largely been replaced by pegylated interferon, which is typically administered once weekly. Response to treatment varies between individuals, possibly due to genetic factors, the viral genotype, or the patient's own genetic makeup. Treatment helps reduce viral replication in the liver, lowering the viral load in the blood. The response to treatment also varies by genetic factors, with the production of anti-HBe antibodies occurring in 37% of those with genotype A, compared to 6% for genotype B. Genotype B has a similar response to genotype A regarding anti-HBe production, while genotype C produces anti-HBe at a rate of 15%. Sustained loss of HBeAg production following treatment is 45% for genotype A and B but only 25% to 30% for genotypes C and D (Schweitzer *et al.*, 2015)(Ghafil *et al.*, 2023).

1.1.3. Hepatitis C

Primarily spreads through blood or blood products contaminated with the virus. Approximately 80% of those infected develop chronic hepatitis, with 20% of them developing cirrhosis. Within 10 years, 5% may develop liver cancer (Murray, Rosenthal and Pfaller, 2019). Chronic hepatitis C is the leading cause of liver transplants in many countries, particularly the United States, costing an estimated $600 million annually in medical expenses and lost work hours according to the World Health Organization (WHO).

Symptoms

Early infection often presents as acute hepatitis with general fatigue, loss of appetite, nausea, vomiting, mild fever, dark urine, and skin rashes, which are common signs of viral liver diseases. Symptoms last several weeks, followed by gradual recovery in most cases. However, liver damage may result in liver failure and death in some cases. The virus infects over 170 million people worldwide, many of whom suffer from chronic liver disease. Chronic hepatitis C can progress to cirrhosis and, in some cases, liver cancer Patients with chronic hepatitis should avoid alcohol, as it accelerates liver damage (Shepard, 2006). Acute symptoms of hepatitis C occur in only about 15% of cases. These symptoms are usually mild and vague, including loss of appetite, fatigue, nausea, muscle or joint pain, and weight loss. Jaundice (yellowing of the skin and eyes) is rare in severe cases. Without treatment, the infection resolves on its own in 10–50% of cases, with young females more likely to clear the virus (Seeff, 2002).

Chronic Infection

Approximately 80% of those exposed to HCV get chronic infection. Most individuals endure minor or no symptoms for decades, however chronic hepatitis C may be linked to fatigue (Younossi et al., 2007). Hepatitis C is the primary aetiology of cirrhosis and hepatocellular carcinoma in chronic patients. Between 10–30% of those infected for more than 30 years will eventually develop cirrhosis (Di Bisceglie, 1997). Cirrhosis is more prevalent among those co-infected with hepatitis B or HIV, thos e with a history of alcohol abuse, and males (Fattovich et al., 1997)(Fattovich *et al.*, 2002). Patients with cirrhosis face a twentyfold increased risk of liver cancer, with an annual incidence of 1–3% (El-Serag, 2012). In alcoholics, the risk increases 100 times (Donato, 2002). Hepatitis C accounts for 27% of cirrhosis cases and 25% of liver cancer cases globally (Perz *et al.*, 2006). Cirrhosis can lead to complications such as high blood pressure in the veins leading to the liver, fluid accumulation in the chest, easy bruising or bleeding, enlarged veins in the stomach and esophagus, jaundice, and brain damage (Grebely and Dore, 2011).

Extrahepatic Manifestations

Hepatitis C is also linked, though rarely, to Sjögren's syndrome (an autoimmune disorder), low platelet counts, chronic skin conditions, diabetes, and non-Hodgkin’s lymphoma .Other effects may include oral manifestations like lichen planus, inflammation of the salivary glands, dry mouth, smooth tongue, teeth grinding, and perioral rashes (AL-HASHIMI, 2001).

Causes

Hepatitis C is a diminutive, enveloped, positive-sense single-stranded RNA virus classified within the genus Hepaciviral of the family Flaviviridae. There exist seven primary genotypes of the hepatitis C virus. In the United States, genotype 1 constitutes 70% of instances, whereas genotype 2 comprises 20%. All other genotypes comprise

approximately 1% of cases. Genotype 1 is the predominant variant in South America and Europe (Simmonds *et al.*, 2005).

Transmission

In developed countries, HCV is primarily spread through intravenous drug use. In developing countries, the primary routes of transmission are blood transfusions and unsafe medical procedures (Hajarizadeh, Grebely and Dore, 2013).

Prevention

Since 2011, there has been no vaccine for hepatitis C. Vaccines are still under development. Preventive measures, including needle exchange programs and substance addiction treatment, diminish the risk of hepatitis C transmission among injection drug users by 75%. National blood donor screening is essential, along with compliance with international safety protocols in healthcare settings. In nations with inadequate sterile syringe availability, the administration of oral medications is advised. In 20% of instances, the transmission source remains unidentified; nevertheless, a significant portion is probably associated with intravenous drug use (Nelson *et al.*, 2011).

Treatment

Hepatitis C virus (HCV) causes chronic infection in approximately 50–80% of infected individuals. Of these, around 40–80% can be cured with treatment. In rare cases, the infection resolves without treatment. Individuals with chronic hepatitis C should avoid alcohol, liver-toxic medications, and should be vaccinated against hepatitis A and hepatitis B. The main treatment for HCV involves two medications: interferon and ribavirin. Approximately 50–80% of patients administered these medications achieve a cure. Patients who develop cirrhosis or liver cancer may require a liver transplant, although the virus often recurs after transplantation. Notably, there is no vaccine for hepatitis C (Strader *et al.*, 2004).

1.1.4. Hepatitis D

Also known as the delta virus, cannot replicate on its own and needs the occurrence of hepatitis B. It coexists with hepatitis B and is found in approximately 8% of hepatitis B patients and less than 2% of hepatitis B carriers.

Transmission

Hepatitis D spreads through blood transfusions and sexual contact. Risk factors are similar to those of hepatitis B, with intravenous drug users being particularly vulnerable.

Prevention

Hepatitis D infection can be prevented through the hepatitis B vaccine (Murray, Rosenthal and Pfaller, 2019).

1.1.5. Hepatitis E

It is primarily an epidemic disease linked to water contamination. It is transmitted through the consumption of contaminated food and drink. Since the virus is excreted in feces, contaminated drinking water is often the source of infection. The incubation period is 2 to 9 weeks. Individuals aged 15-40 are most susceptible, with pregnant women being at the highest risk, with mortality rates as high as 20%, compared to less than 1% for others (Shepard, 2006). Clinically, hepatitis E is indistinguishable from hepatitis A and typically resolves on its own (Murray, Rosenthal and Pfaller, 2019).

1.1.6. Hepatitis G

It was discovered in 1996, and research on this virus is still ongoing. Initially thought to cause viral hepatitis, later studies did not conclusively link it to the disease. As more research emerges, understanding of this virus may evolve. Hepatitis G belongs to the Flaviviridae family and shares structural similarities with the hepatitis C virus. It spreads through blood transfusions and sexual contact. Although it has been found in cases of chronic hepatitis, its direct association with the disease is uncertain. Studies on human immune deficiency virus (HIV) patients have shown that those with co-infections of HIV and hepatitis G tend to survive longer than those infected with HIV alone. Approximately 2% of healthy blood donors in the U.S. carry the hepatitis G virus, though they do not exhibit symptoms. Ninety to one hundred percent of carriers develop chronic infections, though these rarely cause significant harm compared to other viral hepatitis families (Shepard, 2006).

2. Prognosis After Treatment

An infection with hepatitis B can be either short-lived (acute) or long-lasting (chronic). Acute, self-limiting infections typically resolve in a matter of weeks or months for those affected. Children have a poorer healing rate compared to adults. If an adult or older child gets the virus, almost 95% of them will get well and even develop immunity. The percentage of children that recover falls to 30% when they are younger, and if they are infected at birth, just 5% of babies will be able to eliminate the virus. Cirrhosis and cancer of the liver threaten the lives of 40% of this population. In youngsters between the ages of 1 and 6, 70% of cases are resolved (Lampertico *et al.*, 2017).

Hepatitis D can only occur in individuals co-infected with hepatitis B, as hepatitis D uses the surface antigen of hepatitis B to form its viral envelope. Co-infection with hepatitis D increases the risk of cirrhosis and liver cancer (Liaw and Chu, 2009). Polyarteritis nodosa is more common in people infected with hepatitis B.

Hepatitis C is a viral infection that predominantly impacts the liver. The hepatitis C virus (HCV) is the etiological agent of this disease. Hepatitis C often manifests asymptomatically; however, chronic infection can cause liver fibrosis, potentially progressing to cirrhosis over time (Lauer and Walker, 2001). Patients with cirrhosis may develop liver failure, liver cancer, or markedly distended veins in the esophagus and stomach, potentially resulting in substantial hemorrhaging that could be fatal. HCV is mainly transmitted through blood-to-blood contact, often due to intravenous drug use, unsterilized medical equipment, and blood transfusions. It is estimated that between 130 to 170 million people worldwide are infected with hepatitis C (Lavanchy, 2009). Research on the hepatitis C virus commenced in the 1970s, with its existence being verified in 1989. It remains unclear whether the virus affects any other animals (Choo *et al.*, 1989).

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Assessment of The Level of Protein S100B as a Potential Clinical Biomarker for Epilepsy and Correlation with HBA1C Level

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Abstract

Background

Epilepsy is a chronic brain disorder marked by a tendency for recurrent seizures and associated neurobiological, psychological, and social effects. Seizures are sudden, stereotyped episodes reflecting abnormal brain activity. Epilepsy can be primary (genetic causes affecting neurotransmission and ion channels) or secondary (due to brain injuries like trauma, stroke, infections, or tumors). The protein S100B, mainly produced by astrocytes, is elevated in the serum or CSF of epilepsy patients. HbA1c, a key marker for glucose control, is also relevant, as blood sugar fluctuations can trigger seizures. This study aimed to assess serum S100B and HbA1c levels in epilepsy patients and explore their correlation.

Methods

The study enrolled 90 subjects grouped as primary epileptic patients which have the disease due to genetic reasons, secondary epileptic patients which have the disease due to acquired reasons such as fall in blood sugar level and an age-gender matched control group (n=30). The serum samples were collected at the six-month interval and were analyzed for protein S100B using ELISA.

Results

The results showed a significantly decreased levels of HBA1C in patients with secondary epilepsy in comparison with control and primary epilepsy. Suggest that blood sugar levels should be taken into consideration when developing a treatment plan for a patient with epilepsy. Also, there were statistically significant differences in the level of protein S100B biomarker between the control group and patients with primary and secondary epilepsy as well as the comparation between patient groups primary and secondary are statistically significant with higher protein S100B level in the secondary group. S100B also shows a negative correlation with HBA1C levels which means that as S100B increases, the HBA1C levels decrease.

Conclusion

Serum protein S100B levels are a useful tool to assess and diagnose patients with epilepsy as well as blood HBA1C levels are useful in diagnosing and making treatment plan for epileptic patients

تقييم مستوى البروتين S100B كمؤشر حيوي سريري محتمل للصرع وارتباطه بمستوى الهيموغلوبين السكري HBA1C

عذراء الجعفر، غصون غانم، وحيدر شافي

الخلاصة

المقدمة

الصرع مرض دماغي، يتميز بميل مستمر للإصابة بنوبات، وتُعدّ التبعات العصبية الحيوية والمعرفية والنفسية والاجتماعية لتكرار النوبات من السمات المميزة للصرع. تُعرّف النوبات الانتيابية المتكررة، أو نوبات الصرع، باضطرابات سلوكية نمطية تُشير إلى الآليات الدماغية الكامنة وراء المرض. هناك العديد من الاضطرابات السريرية التي تُدرج في التشخيص التفريقي للصرع، وتتميز بتغيرات وجيزة في الوعي و/أو السلوك. يُصنف الصرع حسب سبب المرض إلى صرع أولي قد يحدث نتيجة لتغيرات جينية. يمكن أن تؤثر هذه الطفرات على إطلاق النواقل العصبية، والقنوات الأيونية، والمرونة المشبكية، وآليات بيولوجية أخرى. أما الصرع المكتسب (الصرع الثانوي) فيشير إلى اضطرابات النوبات التي تتطور نتيجة لإصابات أو أمراض دماغية محددة، مثل إصابات الدماغ الرضحية، والسكتة الدماغية، والالتهابات، والأورام.

Protein S100Bهو بروتين رابط للكالسيوم، يُعبَّر عنه بشكل أساسي في الخلايا النجمية. يمتلك مرضى الصرع مستويات أعلى من التعبير عن S100B في مصل الدم أو سائل السائل الدماغي الشوكي مقارنةً بالأشخاص الأصحاء. يُعد الهيموغلوبين السكري (HbA1c) أحد المؤشرات الحيوية المهمة لتقييم التحكم طويل الأمد في مستوى الجلوكوز لدى مرضى السكري. يساعد رصد HbA1c في تشخيص المرضى الذين يعانون من نوبات صرع، حيث يكون الارتفاع أو الانخفاض غير الطبيعي في سكر الدم أحد أسبابها. هدفت الدراسة إلى تقدير تركيز بروتين S100B في مصل دم مرضى الصرع، وتحديد مستويات HBA1C وارتباطها بمستويات.S100B

العينات وطرق العمل

شملت الدراسة 90 مريضًا، مُصنَّفين إلى مجموعتين: مرضى صرع أولي (لأسباب وراثية)، ومرضى صرع ثانوي (لأسباب مكتسبة مثل انخفاض مستوى سكر الدم)، ومجموعة ضابطة متطابقة من حيث العمر والجنس (عددهم 30). جُمعت عينات المصل بفاصل ستة أشهر، وحُللت للكشف عن بروتين S100B باستخدام تقنية.ELISA

النتائج

أظهرت النتائج انخفاضًا ملحوظًا في مستويات HBA1C لدى مرضى الصرع الثانوي مقارنةً بالمجموعة الضابطة والمجموعتين الأوليتين. تشير هذه النتائج إلى ضرورة أخذ مستويات سكر الدم في الاعتبار عند وضع خطة علاجية لمريض الصرع. كما وُجدت فروق ذات دلالة إحصائية في مستوى المؤشر الحيوي لبروتين S100B بين المجموعة الضابطة ومرضى الصرع الأولي والثانوي. كما أن المقارنة بين مجموعتي المرضى الأوليين والثانويين كانت ذات دلالة إحصائية مع ارتفاع مستوى بروتين S100B في المجموعة الثانوية. كما أظهرت الدراسة ارتباطًا سلبيًا مع مستوياتHBA1C ، مما يعني أنه مع زيادةS100B ، تنخفض مستويات.HBA1C

1. Introduction

The epilepsies are chronic neurological disorders in which clusters of nerve cells, or neurons, in the brain sometimes signal abnormally and cause seizures. Many neurons fire (signal) simultaneously during a seizure, up to 500 times per second, which is far quicker than usual. *(National Institute of Neurological Disorders and Stroke. (2022).* In addition to causing involuntary actions, sensations, emotions, and behaviors, this simultaneous spike in excessive electrical activity may also result in a temporary loss of consciousness (Shorvon et al., 2011). Epilepsy can have many different causes, including acquired (secondary epilepsy) and genetic factors (primary epilepsy). Genetic mutations are a significant contributor. For example, in severe childhood epilepsy, mutations in the CACNA1E gene cause calcium channels in neurons to be disrupted, which results in excessive electrical activity and seizures (Macdonald et al., 2010; Staněk et al., 2018). Some adult epilepsies, including temporal lobe epilepsy, are also influenced by somatic mutations that develop after conception. Certain genetic pathways, such as the RAS/MAPK pathway, which is also linked to cancer, may be impacted by these mutations (Beltrán-Corbellini et al., 2022; Montanaro et al., 2023). Additionally, research has identified the TMEM184B gene as a possible factor in epilepsy, as its absence or alteration can cause neurons to fire excessively, affecting normal neural communication (Beltrán-Corbellini et al., 2022; Gesche & Beier, 2022; McCormack et al., 2020). Numerous conditions that also impact the structure and function of the brain can result in epilepsy. Because they can cause aberrant electrical activity in the brain, neurological disorders like stroke, traumatic brain traumas, and brain tumors are important causes of epilepsy. Seizures can also be caused by illnesses that inflame the brain or surrounding tissues, such as encephalitis or meningitis. Another component may be autoimmune disorders, in which the brain is mistakenly attacked by the immune system, resulting in epileptic episodes. Additionally, epilepsy has been connected to vascular abnormalities and degenerative diseases like Alzheimer's disease especially in older people. These secondary causes frequently draw attention to epilepsy as a sign of more serious underlying problems (Kenney & Mann, 2013; O'Neill et al., 2020). A calcium-binding protein called protein S100B is mostly expressed by astrocytes in the central nervous system (CNS). Its participation in intracellular and extracellular regulatory functions makes it an important biomarker for a number of neurological disorders. Increased S100B serum levels have been used in clinical settings to gauge the degree of traumatic brain injury (TBI) and are suggestive of astrocytic damage. S100B has been used as a screening tool in the treatment of TBI patients, which has been noteworthy since it helps with patient outcome prediction and monitoring (Mondello et al., 2021; Zimmer et al., 2023). S100B has been studied in relation to epileptic seizures in addition to TBI. According to research, S100B may contribute to the pathophysiology of epilepsy by playing a part in the neuroinflammatory processes linked to the condition. S100B may be a biomarker for epileptic activity because elevated levels of the protein have been seen in seizure patients (Reddy & Volkmer, 2017; van Vliet et al., 2017; Zimmer et al., 2023). One indicator of long-term blood glucose levels, hemoglobin A1c (HbA1c), has been linked to epilepsy treatment and prognosis. Increased seizure intensity and recurrence have been linked to elevated HbA1c levels, which are a sign of inadequate glycemic control. In research, individuals with hyperglycemia diabetes who had their first seizure had significantly higher HbA1c levels if they had another seizure (11.8% vs. 8.6%, $p<0.05$) than those who did not. Additionally, patients who had HbA1c levels higher than 9% were more likely to experience seizure clustering and recurrence (Bellon et al., 2017; He et al., 2023; Phoswa & Mokgalaboni, 2023). Seizures can also occur in individuals with low hemoglobin A1c (HbA1c)

levels, primarily due to hypoglycemia. Hypoglycemia, defined as blood glucose levels falling below normal, can lead to neurological symptoms, including seizures. While seizures are relatively uncommon, they are more likely to happend when glucose levels fall significantly. A study found that generalized tonic-clonic seizures occurred when serum glucose levels fell below 2.0 mM, and focal seizures were noted at glucose levels as high as 3.3 mM. (He et al., 2023; Phoswa & Mokgalaboni, 2023; Reddy & Volkmer, 2017; Zimmer et al., 2023). Additionally, lower HbA1c levels have been linked to a higher risk of severe hypoglycemia and hypoglycemic coma, which includes seizures, in young patients with type 1 diabetes. But with time, patients with lower HbA1c levels had a lower relative risk of severe hypoglycemia, maybe due to improved management strategies. Furthermore, HbA1c has been investigated as a possible biomarker for tracking systemic ketosis and diet adherence in individuals with drug-resistant epilepsy on ketogenic diets. Higher blood ketone levels were linked to lower HbA1c levels, indicating its potential use in the management of such dietary treatments (Gulati & Panda, 2019; Teng et al., 2022). This study aims to Comparison between primary and secondary types of epilepsy by measure the level of PROTEIN S100 B biomarker and its role in patients with epilepsy. Measurement level of HBA1C and its effect in patients with epilepsy. Measure progression after the condition is established and reduce the cost of clinical trials of potential ant epileptogenic interventions by enriching the trial population with patients at high risk for developing epilepsy.

2. Patients & Methods

The study enrolled 90 subjects grouped as primary epileptic patients which have the disease due to genetic reasons, secondary epileptic patients which have the disease due to acquired reasons such as fall in HBA1C level and an age-gender matched control group (n=30). The serum samples were collected at the six-month interval and were analyzed for protein S100B using ELISA.

2.1. Detection of HBA1C

2.1.1. Principles

When the sample is added to the sample port on the test card, HbA1c and Hb in the sample combines with mouse anti-human HbA1c and Hb monoclonal antibodies which are coupled to fluorescent particles to form fluorescent particles - antibody - antigen complexes. This immune complex reaches the test area (T) along the nitrocellulose membrane and binds with the pre-coated mouse anti-human HbA1c monoclonal antibody, The amount of HbA1c in the sample is directly correlated with its fluorescence intensity. A quality control line is created when the remaining fluorescent antibody particle reaches the quality control area (C) and combines with the pre-coated goat anti-human Hb monoclonal antibody. The ratio of HbA1c to Hb was calculated by the fluorescence signal intensity. The test area (T) will not appear fluorescence, if the sample does not contain HbA1c.

2.1.2. Procedure

Bring all reagents to room temperature (18-25℃) before use.

1. Startup: Click “STD Mode” in the main menu to enter the measurement interface, click “Item” to select the desired test item and click “Type” to select the sample type.
2. Click “Lot No.” to enter the card swiping interface, put mag card of the corresponding item into the magnetic induction zone and when hearing a “di” sound this mean that the mag card is swiped successfully. Make sure the mag card and the test card are from the same batch.

3. Sampling: Add 10μL of whole blood into a centrifuge tube with 1000μL of the sample diluent, mix for 1 minute. Take 100μL diluted sample, drop vertically to the sample port directly on the test card and start timing.
4. Insert it into the analyzer's test slot (the sample port ends toward the inside). Click "Measure", the instrument will detect and print out the results automatically after 15 minutes (If using "Fast Mode", keep it for 15 minutes and quickly insert into the analyzer's test slot)

2.2. Determination of Protein S100-B

2.2.1. Principle

The kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with Human S100B antibody. S100B present in the sample is added and binds to antibodies coated on the wells. And then biotinylated Human S100B Antibody is added and binds to S100B in the sample. Then Streptavidin HRP is added and binds to the Biotinylated S100B antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Then added substrate solution, and color develops in proportion to the amount of Human S100B. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

2.2.2. Calculation of Results

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.

3. Results and Discussion

3.1. Demographic Characteristics of Study Groups

The current study was conducted on (90) people suffering from epilepsy and healthy people. This study takes 30 samples from patients suffering from primary epilepsy (group1), 30 samples from secondary epilepsy (group2), and 30 samples as a control, Table1.

Table1: Demographic Characteristics of Study Groups

Category	Groups	N
Patients	Group-1: primary epilepsy	30
	Group-2: secondary epilepsy	30
Healthy	Control	30

3.2. Protein S100B in Epileptic Patients and Control

There were statistically significant differences in the level of protein S100B biomarker between the control group and other groups (group 1 and 2 of patients); (43.161 ±9.267 VS 50.537 ±2.711, 64.206 ±9.534), P-value was 0.00032, Table2.

Table2: Comparison of the Research Parameter of All Patients Compared with Control Group

Parameters	Control		Patient primary		Patient secondary		P value
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	
Protein S100B	43.161	9.267	50.537	2.711	64.206	9.534	0.00032

In Table3 and Fig.1 the comparation between patient groups primary and secondary are statistically significant (50.537 ±2.711VS 64.206 ±9.534), the P-value was 0.00005, indicating a highly significant difference between the groups with higher protein S100B level in the secondary group.

Table3: Comparison of the Research Parameters Between Patients in Primary Compared with Secondary Group

Parameters	Patient primary		Patient secondary		P value
	Mean	Std. Deviation	Mean	Std. Deviation	
Protein S100B	50.537	2.711	64.206	9.534	0.00005

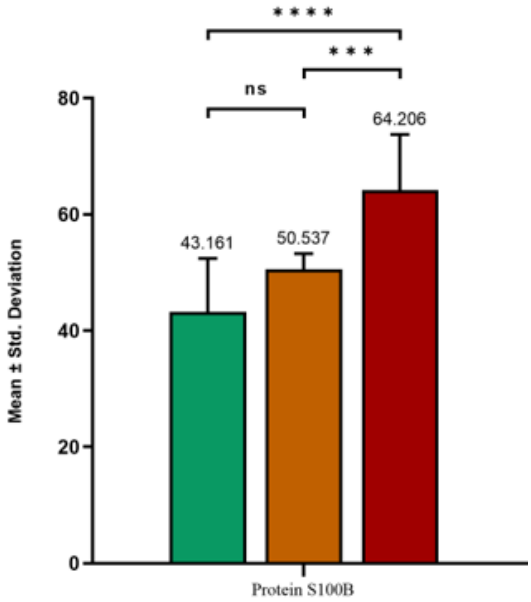

Figure1: Comparison of Serum Protein S100B Levels (Mean ± Standard Deviation) Among Control Subjects, Patients with Primary Epilepsy, And Patients with Secondary Epilepsy
Statistical analysis shows a significant increase in Protein S100B levels in secondary epilepsy patients compared to both control and primary epilepsy groups (***p < 0.001, ****p < 0.0001), while the difference between control and primary groups was not statistically significant (ns).

Beyond just acting as a marker, S100B plays an active involvement in the pathophysiology of epilepsy. S100B promotes neuronal survival and function at physiological concentrations, but when levels are high, it can cause neurotoxicity by triggering stress-induced enzymes and pro-inflammatory cytokines, which can lead to neuronal apoptosis and worsen epileptic disorders. Additionally, the development of neuroinflammatory responses has been linked to S100B's interaction with the receptor for advanced glycation end products (RAGE), underscoring its dual function of neuroprotection and neurodegeneration contingent on its concentration (Liang et al., 2019; Seçen et al., 2023). The table's results are consistent with findings from other research studies. Patients with epilepsy exhibited significantly higher serum S100B levels than healthy controls, according to a systematic review and meta-analysis of

18 studies with 1,057 individuals. The pooled effect size was Hedges g = 1.568 (95% CI = 1.431–1.706, P < 0.001). This lends credence to the idea that elevated S100B levels are linked to epilepsy. Additionally, case-control research on mesial temporal lobe epilepsy (MTLE) revealed that patients had significantly higher plasma S100B levels than healthy controls (P = 0.018), which may indicate that raised S100B levels are a biomarker for MTLE." Furthermore, serum S100B levels were significantly higher in patients with epileptic seizures (SMD = 0.80; 95% CI 0.18 to 1.42), according to a meta-analysis looking at blood-based brain biomarkers in these patients. This suggests that S100B levels are higher in epileptic patients than in healthy controls (Chen et al., 2015; Keshavarz & Amiri, 2019; Lipatova et al., 2018; Seçen et al., 2023; Xu et al., 2012).

3.3. HBA1C In Primary and Secondary Epilepsy:

The result showed the difference of the HBA1C between the patients of primary and secondary epilepsy and control group (6.877 ±0.564 mg/dl, 5.320 ± 1.851 mg/dl VS 6.613 ±0.580 mg/dl) was significant. The P-value of 0.00603 shows a significant difference in HBA1C levels, especially between the primary and secondary groups, Table4.

Table4: Comparison of the HBA1C Level of All Patients Compared with Control Group

Parameters	Control		Patient primary		Patient secondary		P. value
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	
HBA1C	6.613	0.580	6.877	0.564	5.320	1.851	0.00603

The comparison between primary and secondary patient groups (table 4-5) showed that the primary group had (6.877 ±0.564 mg/dl) while the secondary group had a lower mean of (5.320 ± 1.851 mg/dl), this difference was statistically significant, with a P-value of 0.00300.

Table5: Comparison of the HBA1C Level Between Patients in Primary Compared with Secondary Group

Parameters	Patient primary		Patient secondary		P value
	Mean	**Std. Deviation**	**Mean**	**Std. Deviation**	
HBA1C	6.877	0.564	5.320	1.851	0.00300

These findings agreed with a study that was published in the Journal of Epilepsy in 2017. The study found that patients who had poor glycemic control (HbA1c > 9%) were much more likely to experience generalized seizures (46.7% vs. 7.7%, p < 0.05). The importance of your findings is supported by the suggestion that raised HbA1c levels are linked to an increased risk of seizures (Mondello et al., 2021). Furthermore, an investigation that was published in the Seizure: European Journal of Epilepsy (2010) discovered a correlation between a significant increase in HbA1c levels and occipital lobe seizures. This lends more credence to the link between high HbA1c levels and seizures (Zimmer et al., 2023).

3.4. Correlation Coefficient Among Parameters According to Research Parameters

The correlation analysis presented in Table6 explains the relationships between various biological and clinical parameters in epilepsy patients. A notable finding is the negative correlation between S100B and HBA1C levels (r = -0.302, p = 0.019), suggesting a potential link between S100B and altered metabolic or mineral states in epilepsy patients.

Table6: Correlation Coefficient Among Parameters According to Research Parameters

Parameters	Value	Protein S100B	HBA1C
Protein S100B	R. value	1.000	-.302*
	P. value		.019
HBA1C	R. value		1.000
	P. value		

A study published in Diabetes Research and Clinical Practice investigated the relationship between glycemic control and S100B levels in diabetic patients. Higher S100B levels were linked to better glycemic control, according to the study, which also identified a negative correlation between S100B levels and HbA1c levels. This study indicates a possible association between S100B and metabolic parameters that may be pertinent to individuals with epilepsy, even though it was carried out on diabetic patients (Celikbilek et al., 2014; Katsanou et al., 2018; Ruchkin et al., 2022).

4. Conclusion

significantly decreased levels of HBA1C in patients with secondary epilepsy in comparison with control and primary epilepsy. Suggest that blood sugar levels should be taken into consideration when developing a treatment plan for a patient with epilepsy. Also, there were statistically significant differences in the level of protein S100B biomarker between the control group and patients with primary and secondary epilepsy as well as the comparation between patient groups primary and secondary are statistically significant with higher protein S100B level in the secondary group. S100B also shows a negative correlation with HBA1C levels which means that as S100B increases, the HBA1C levels decrease.

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Correlation Of IL-5 And IL-17 With Specific Allergens in Pediatric Asthmatic Patients

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Abstract

Background: This study aimed to investigate the correlation between interleukin-5 (IL-5) and interleukin-17 (IL-17) with specific aeroallergens in pediatric asthmatic patients.

Patients and Methods: A cross-sectional study included (100) pediatric asthmatic patients, focusing on demographics, Biomarkers, and aeroallergens.

Results: The results showed that eosinophilic asthma patients had considerably higher levels of IL-5 (p=0.0015), especially those who were sensitized to allergens like sorrel, white ash, and sweet vernal mix. IL-17 levels were associated with sensitivity to firebush and CCD indicators and were higher in neutrophilic asthma (p=0.0072). Overlapping biomarker elevations in mixed granulocytic asthma suggested intricate inflammatory processes.

Conclusion: The significance of IL-5 and IL-17 as biomarkers for asthma phenotype in juvenile patients is highlighted by this study. IL-5 plays a crucial role in allergen-induced asthma, as seen by the strong association it has with particular allergens. Asthma treatment could be completely transformed by personalized therapy based on biomarker analysis.

العلاقة بين IL-5 وIL-17 ومسببات الحساسية المحددة لدى مرضى الربو الأطفال

خالد جبار سالم، الاء سعد العتابي، ضمياء مكي حمزة، حيدر عبد الامير نجم عبود

الخلاصة

المقدمة

هدفت هذه الدراسة إلى دراسة العلاقة بين الإنترلوكين-5 (IL-5) والإنترلوكين-17 (IL-17) مع مسببات الحساسية الجوية المحددة لدى مرضى الربو الأطفال.

المرضى وطرق العمل

شملت هذه الدراسة المقطعية (100) مريضًا بالربو من الأطفال، مع التركيز على التركيبة السكانية والعلامات الحيوية ومسببات الحساسية الجوية.

النتائج

أظهرت النتائج أن مرضى الربو الحمضي لديهم مستويات أعلى بكثير من IL-5 (p=0.0015)، وخاصة أولئك الذين كانوا حساسين لمسببات الحساسية مثل الحميض والرماد الأبيض ومزيج الربيع الحلو. ارتبطت مستويات IL-17 بالحساسية لمؤشرات شجيرة النار وCCD وكانت أعلى في الربو العدلي (p=0.0072). تشير ارتفاعات المؤشرات الحيوية المتداخلة في الربو الحبيبي المختلط إلى عمليات التهابية معقدة.

الاستنتاج

وُجِد في هذه الدراسة أهمية IL-5 وIL-17 كعلامات حيوية لنمط الربو لدى المرضى الأحداث. يلعب IL-5 دورًا حاسمًا في الربو الناجم عن المواد المسببة للحساسية، كما يتضح من الارتباط القوي الذي له بمسببات الحساسية المحددة. يمكن تحويل علاج الربو تمامًا من خلال العلاج الشخصي القائم على تحليل العلامات الحيوية.

1. Introduction

Asthma, a chronic respiratory disorder that affecting over 300 million individuals worldwide, is still particularly prevalent among children with significant morbidity and economic burden (Levy *et al.*, 2023). This disorder condition is characterized by hyper responsiveness of airway, obstruction, and persistent inflammation driven by diverse immunological pathways. In pediatric; asthma often manifested in distinct phenotypes, including eosinophilic and neutrophilic asthma each of them associated with the inflammatory profiles (Wenzel, 2012). Interleukin-5 (IL-5) a cytokine pivotal to eosinophilic inflammation that promoting eosinophil differentiation with activation and survival (Takatsu, 2011). In the context, interleukin-17 (IL-17) Produced by Th17 cells, IL-17 has been implicated in neutrophilic asthma and associated with the severe and steroid-resistant asthma in patients (Rahmawati *et al.*, 2021). Association of biomarkers with environmental allergens, such as pollen and animal dander were recognized as primary triggers for asthma exacerbations especially in atopic individuals. Interleukin-5 is closely associated with to allergen driven eosinophilic inflammation, while interleukin-17 had role extends to non-atopic triggers such as pollution and infections (Hammad and Lambrecht, 2021). This study aims to investigate the association between IL-5, IL-17, and specific allergens to enhance asthma diagnosis and allocate appropriate treatments.

2. Material and Methods

2.1. Study Design

A cross-sectional study included 100 pediatric asthmatic patients aged from 6 to 15 years. Participants were divided into four group asthma phenotypes: (eosinophilic, neutrophilic, mixed granulocytic, and allergic asthma).

2.2. Inclusion Criteria

1. Diagnosed asthmatic children aged (6-15) years.
2. Children is receiving inhaled corticosteroids.

2.3. Exclusion Criteria

1. Patients on systemic corticosteroids or biologic therapy.
2. All patients' upper and lower respiratory infection diseases.
3. Patients with autoimmune disease and these with cystic fibrosis also should be excluded.

2.4. Data Collection and Analysis

1. Demographic and Clinical data will be collected using a specific detailed questionnaire
2. Approximately 5 ml of venous blood were drawn from each subject which were obtained by disinfecting antecubital fossa with 70% ethanol and then make vein puncture by disposal syringes after applying a tourniquet. One ml of blood was dispensed into (1) EDTA tube for the haematological tests. Four ml of blood was dispensed into gel tube and allowed to clot then serum was separated by centrifugation at

3000 round per minutes (RPM) for 5 minutes. Then the serum was transferred to new eppendorf tube (2 ml) and stored in deep freeze (-20°C) to be used for immunological assays.

3. Immunological measurement of IL-5, IL-17, and specific IgE (S.IgE) by ELISA Kit
4. The study was used Enzyme-linked immunosorbent assay (ELISA): for biomarkers IL5, IL17 ,total IgE (T.IgE) and specific immunoglobulin E (S.IgE) inhalation in peripheral blood for all patients
5. Correlation between biomarkers and allergen sensitivity was analyzed.

2.5. Ethical Considerations

Ethical approval was obtained from the Karbala College of Medicine and patient consent was secured.

2.6. Statistical Analysis

Statistical analysis of data in the present study was performed by SPSS. version 25.0 on the basis of one way analysis of variance (ANOVA) using significant levels ($P<0.05$).

3. Results

3.1. Demographic Data for Asthma Patients

The demographic data of asthmatic patients were included in current study were illustrated in Table (3-1). These data were involved: age, sex, residency, passive smoking, family history, Food allergy, Allergic Rhinitis, and Pets at home. patients were divided according to their ages into three groups: 5-8 y, 9-12 y, and 13-16 y, the results of statistical analysis using Chi-square test revealed a highly significant ($p=0.0001$) differences between groups, where the highest percent (58.0%) of patients within the age group 9-12 y. The majority (72.5%) of asthma patients were male, while only (27.5%) were female, the results of statistical analysis showed a highly significant ($p=0.0001$) difference. In the context of residency, a significantly ($p=0.0009$) highest percent (66.4%) of patients were resided in urban, while only (33.6%) resided in rural. As for passive smoking, patients were divided into two groups: non-passive smoking and passive smoking, but statistical analysis did not show any significant differences between the two groups; where $p=0.3173$. Regarding family history; significantly ($p=0.0001$), most patients (70.2%) had a family history of asthma, while only 29.8% had no family history of the disease. As for food allergy, patients were divided into two groups according to present or absent food allergy. The results of the statistical analysis showed that the majority (82.4%) of patients in the current study did not have food allergy, while the smaller percentage (17.6%) had a food allergy; $p=0.0001$. with respect to Allergic Rhinitis and presence a pet at home, the results of statistical analysis found non-significant ($p>0.05$) differences between groups of asthma patients divided according to these mentioned characteristics as, as shown in Table1.

Table1: Demographic Data for Asthma Patients

Age group (year)					
	5-8 y	**9-12 y**	**13-16 y**	**Total**	***P value***
Count (%)	29(29%)	59(59%)	12(12%)		0.0001*
Mean ± SD	9.71 ± 2.328				
Gender					
	Male	**Female**		**Total**	***P value***
Count (%)	75 (75%)	25(25%)		100	0.0001*
Residency					
	Urban	**Rural**		**Total**	***P value***
Count (%)	65 (65%)	35(35%)		100	0.0027*
Passive Smoking					
	Non-2nd smoking	**2nd smoking**		**Total**	***P value***
Count (%)	52(52%)	48 (48%)		100	0.6892
Family hX					
	Non- Family hX	**Family hX**		**Total**	***P value***
Count (%)	30 (30%)	70 (70%)		100	0.0001*
Food allergy					
	Non- food allergy	**Food allergy**		**Total**	***P value***
Count (%)	82(82%)	18 (18%)		100	0.0001*
Allergic Rhinitis					
	Non-allergic Rhinitis	**Allergic Rhinitis**		**Total**	***P value***
Count (%)	43(43%)	57 (57%)		100	0.1615
Pets at home					
	No	**Yes**		**Total**	***P value***
Count (%)	48(48%)	52(52%)		100	0.6892
*Mean significant difference P< 0.05 level by chi-square test					

3.2. Biomarkers Concentrations in Asthma Patients

3.2.1. Biomarkers Concentrations in Asthma Patients According to Eosinophilic Group

Table2 displays the concentrations of studied markers (IL-17, IL-5) according to Eosinophilic group (Eosinophil scores) in asthmatic patients, which divided into three groups: high, low and normal. Statistical analysis using one way-ANOVA test showed non-significant ($p>0.05$) differences in the distributions of IL-17, while highly significant ($P=0.0015$ and $P=0.0054$ respectively) for IL-5.

Table2: Biomarkers Concentrations in Asthma Patients According to Eosinophil Scores

Biomarkers/units	Eosinophil level	No.	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17 (unit)	High	65	221.54969	112.862866	0.212
	Low	14	184.11750	108.190582	
	Normal	21	255.21229	133.607551	
	Total	100	223.37833	117.529254	
IL-5 (unit)	High	65	262.74848	194.840479	**0.0015***
	Low	14	142.91371	45.396255	
	Normal	21	196.93794	141.376216	
	Total	100	203.19476	148.838171	
*Mean significant difference $p \leq 0.05$ by One way – ANOVA					

3.2.2.Biomarkers Concentrations in Asthma Patients According to Neutrophil Scores

Table3 shows the concentrations of studied markers (IL-17, IL-5) according to Neutrophil scores in asthma patients, which divided into three groups: high, low and normal. Statistical analysis using one way-ANOVA test showed highly-significant (P=0.0072 respectively) for IL-17 while for these markers IL-5 there was no significant

Table3: Biomarker Concentration in Patients According to Neutrophil Levels

Biomarkers/units	Neutrophil level	No.	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17 (unit)	High	32	200.51786	288.88537	**0.0072***
	Low	34	153.85226	218.18139	
	Normal	34	195.29070	285.54717	
	Total	100	200.05798	246.69868	
IL-5 (unit)	High	32	167.40991	412.99727	0.416
	Low	34	124.63665	536.83347	
	Normal	34	89.64867	302.64733	
	Total	100	187.51196	354.87756	
*Mean significant difference $p \leq 0.05$ by One way – ANOVA					

3.2.3.Biomarkers Concentrations in Asthma Patients According to Mix Granulocytic Score

Table4 shows the concentrations of studied markers (IL-17, IL-5) according to mix granulocytic score in asthmatic patients, which divided into two groups: Mix and non-mix. Statistical analysis using one way-ANOVA test showed highly-significant (P=0.0074, P=0.0021) for IL-17, IL-5.

Table4: Biomarker Concentration in Patients According to Mix Granulocytic Score

Biomarkers/units	mix granulocytic score	No.	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17	Mix	22	274.02077	134.435195	0.0074*
	Non	78	209.09456	109.047451	
	Total	100	223.37833	117.529254	
IL-5	Mix	22	412.86282	600.165762	0.0021*
	Non	78	231.23710	350.852946	
	Total	100	271.19476	421.742178	
*Mean significant difference p ≤ 0.05 by One way – ANOVA					

3.4. Frequencies of All Aeroallergens (Aag) in Asthma Patients

The frequencies of all (Aag) in asthma patients were illustrated in Table5. The results of the statistical analysis using chi-square test, indicated that the percentages of patients without antigens were significantly (p=0.0001) higher than those with (Aag), and this applies to all (Aag) included in the current study. The highest frequency (Aag) among asthma patients in the current study were Cat (18%), cultivated oat (17%) Meadow foxtail (19%), Goosefoot (15%), Russian thistle (22%), Rough pigweed (16%), Cockroach Germany (9%) and Altrenaria Altrenaria(12%)

Table5: Frequency of all Aeroallergens in Asthma Patients

Aeroallergens		Count	%	Total	P value
Cat	Positive	18	18%	100	0.0001
	Negative	82	8 %		
Cultivated oat	Positive	17	17%	100	0.0001
	Negative	83	83%		
Meadow foxtail	Positive	19	19%	100	0.0001
	Negative	81	81%		
Goosefoot	Positive	15	15%	100	0.0001
	Negative	85	85%		
Russian thistle	Positive	22	22%	100	0.0001
	Negative	78	78%		
Rough pigweed	Positive	16	16%	100	0.0001
	Negative	84	84%		
Cockroach Germany	Positive	9	9%	100	0.0001
	Negative	91	91%		
Alternaria	Positive	12	12%	100	0.0001
	Negative	88	88%		
Significant difference P< 0.05 level by chi-square test					

3.3. Biomarkers Concentration in Patients According to Aeroallergens

3.3.1. Biomarkers Concentration in Patients According to Aeroallergens (Sweet Vernal Mix) .

Table6 shows the effects of Aeroallergen (Sweet vernal mix) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that highly significant in IL-5 (P=0.002), were is it increased in patients that have inhaled (positive) Aeroallergen (Sweet vernal mix). While other remaining biomarkers did not show any significant IL-17 (P=0.421).

Table6: Biomarkers Concentration in Patients According to Aeroallergens (Sweet Vernal Mix)

Biomarkers/units	Aeroallergen (Sweet vernal mix)	N	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17	Positive	13	219.70298	115.652329	0.421
	Negative	78	247.97492	131.709794	
	Total	100	223.37833	117.529254	
IL-5	Positive	13	597.63077	861.481380	**0.002***
	Negative	78	222.41697	287.545651	
	Total	100	271.19476	421.742178	
* Significant difference p ≤ 0.05 by One way – ANOVA					

3.3.2. Biomarkers Concentration in Patients According to Aeroallergens (Firebush).

Table7 shows the effects of Aeroallergen (Sweet vernal mix) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that significantly in biomarkers IL-17 (P=0.003 respectively), were is it increased in patients that have inhaled (positive) Aeroallergen (Firebush). While the other remaining biomarkers did not show any significant IL-5 (P=0.210)

Table7: Biomarkers Concentration in Patients According to Aeroallergens (Firebush)

Biomarkers/units	Aeroallergen/(Firebush)	N	Biomarker concentration In patients		P value
			Mean	Std. Deviation	
IL-17	Positive	10	231.52541	118.741221	**0.003***
	Negative	90	150.05460	76.672808	
	Total	100	221.38722	113.128273	
IL-5	Positive	10	111.88650	49.079889	0.210
	Negative	90	288.89568	440.952878	
	Total	100	261.17474	430.651267	
*** Significant difference under p ≤ 0.05 by One way – ANOVA**					

3.3.3. Biomarkers Concentration in Patients According to Aeroallergens (Sorrel)

Table8 shows the effects of Aeroallergen (Sorrel) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that significantly only in IL-5 (P=0.004), were is it increased in patients that have inhaled (positive) Aeroallergen (Sorrel). While the other remaining biomarkers did not show any significant SAA1 and IL-17 (P=0.317 and P=0.244)

Table8: Biomarkers Concentration in Patients According to Aeroallergens (Sorrel)

Biomarkers/units	Aeroallergen (Sorrel)	N	Biomarker concentration In patients		P value
			Mean	Std. Deviation	
IL-17	Positive	4	220.57337	116.632690	0.244
	Negative	96	290.69725	137.008823	
	Total	100	203.24836	107.332244	
IL-5	Positive	4	684.58900	1026.119471	**0.004***
	Negative	96	253.97000	380.276282	
	Total	100	273.17446	441.766168	
* Significant difference p ≤ 0.05 by One way – ANOVA					

3.3.4. Biomarkers Concentration in Patients According to Aeroallergens (White Ash)

Table9 shows the effects of Aeroallergen (White ash) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that highly significant in IL-5 (P=0.006), were is it increased in patients that have inhaled (positive) Aeroallergen (White ash). While the other remaining biomarkers did not show any significant IL-17 (P=0.306)

Table9: Biomarkers Concentration in Patients According to Aeroallergens (White Ash)

Biomarkers/units	Aeroallergen (White ash)	No.	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17	Positive	7	220.05989	115.792100	0.306
	Negative	93	267.46614	141.038177	
	Total	100	221.656315	113.459334	
IL-5	Positive	7	688.49429	936.238586	**0.006***
	Negative	93	239.78512	346.391350	
	Total	100	241.29467	454.743848	
* Significant difference p ≤ 0.05 by One way – ANOVA					

3.3.5. Biomarkers Concentration in Patients According to Aeroallergens (Tree Mix 4)

Table10 shows the effects of Aeroallergen (Tree Mix 4) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that significantly in IL-5 (P=0.005), were is it increased in patients that have inhaled (positive) Aeroallergen (Tree Mix 4). While the other remaining biomarkers did not show any significant IL-17 (P=0.640).

Table10: Biomarkers Concentration in Patients According to Aeroallergens (Tree Mix 4)

Biomarkers/units	Aeroallergen (Tree Mix 4)	N	Biomarker concentration In patients		P value
			Mean	Std. Deviation	
IL-17	Positive	4	222.24922	117.558341	0.640
	Negative	96	250.47700	131.076512	
	Total	100	213.67599	117.765844	
IL-5	Positive	4	673.42075	1168.957312	**0.005***
	Negative	96	254.43534	367.570818	
	Total	100	272.19825	411.747654	
* Significant difference $p \leq 0.05$ by One way – ANOVA					

3.3.6. Biomarkers Concentration in Patients According to Aeroallergens (CCD Markers)

Table11 shows the effects of Aeroallergen (CCD markers) on the biomarker's concentration of asthmatic patients. The results of the statistical analysis showed that significantly in IL17 (P=0.005), were is it increased in patients that have inhaled (positive) Aeroallergen (CCD markers). While the IL-5 did not show any significant (P=0.169).

Table11: Biomarkers Concentration in Patients According to Aeroallergens (CCD Markers)

Biomarkers/units	Aeroallergen (CCD markers)	N	Biomarker concentration in patients		P value
			Mean	Std. Deviation	
IL-17	Positive	**4**	335.19400	90.970187	**0.005***
	Negative	**96**	218.71934	116.554841	
	Total	**100**	226.74823	117.67214	
IL-5	Positive	**4**	259.32618	408.248584	0.169
	Negative	**96**	556.04075	692.183039	
	Total	**100**	270.19476	421.742178	
* Significant difference $p \leq 0.05$ by One way – ANOVA					

ROC Analysis Receiver operating characteristic (ROC) analysis demonstrated that IL-5 and periostin have high predictive potential for identifying eosinophilic asthma phenotypes in allergen-positive patients, Table12.

Table12: ROC Analysis of Biomarkers in Patients According to S. Ige

Biomarkers/units	AUC	Sensitivity	Specificity	Cut-off	P-value
IL-17	0.536	0.516	0.694	190.837	0.549
IL-5	0.569	0.406	0.75	168.474	0.251
* Significant difference $p \leq 0.05$ by One way – ANOVA					

4. Discussion

Demographic Characteristics and Asthma Prevalence

The current study's demographic analysis highlighted key factors influencing on asthma prevalence and severity in children. The significant proportion of asthmatic patients fall within the (9-12) years age group (58% with p=0.0001) suggesting potential vulnerability in this age group may be due to hormonal changes. The predominant of male patients (72.5%, p=0.0001) aligns with previous study that indicating that boys have tighter airways during early childhood, making them at risk to asthma exacerbation (Ricciardolo *et al.*, 2023). Regarding urban residency was significantly associated with asthma prevalence (65%, p=0.0009). This belongs to urban area had greater air pollutants and allergens. However, passive smoking has been a risk factor for respiratory and allergic conditions in children (Kim, Vazquez and Cubbin, 2023). A strong familial predisposition to asthma (70.2%, p=0.0001) caused by genetic component. This aligns with the findings of Cookson et al., (2011) who recognized genetic polymorphisms associated with IgE levels and airway inflammation. As well as, food allergies were less common among those patients (17.6%, p=0.0001) so that suggesting that aeroallergens rather than ingested allergens play a more important role in triggering asthma.

Biomarkers and Inflammatory Profiles

Elevated IL-5 levels in patients with high eosinophil scores (p=0.0015) explained its role in driving eosinophilic inflammation. IL-5 contributes to eosinophil differentiation and activation, leading to airway hyperresponsiveness and mucus overproduction. These findings confirmed the utility of IL-5-targeted therapies such as mepolizumab in managing eosinophilic asthma (Hammad and Lambrecht, 2021).

Neutrophilic Asthma: In line with its function in neutrophil recruitment and activation, IL-17 levels were considerably greater in individuals with high neutrophil scores (p=0.0072). For severe asthma phenotypes that frequently do not respond to corticosteroid treatment, this discovery is especially pertinent. For these individuals, targeted treatments like anti-IL-17 monoclonal antibodies could provide novel therapeutic options (Yang *et al.*, 2018). Mixed granulocytic asthma had a significantly higher IL-5 concentration (412.86 ± 600.17 units) than non-mixed phenotypes (231.23 ± 350.85 units, p = 0.0021). Although IL-5 is a marker of eosinophilic inflammation, its increased level in individuals with mixed phenotypes could be a reflection of the overlapping inflammatory processes that these patients have. Chu *et al.*, (2015) discovered that a significant decrease in lung activating and chemotactic cytokines, namely IL-17A and IL-5, was linked to the prevention of neutrophilic and eosinophilic inflammation.

Aeroallergen Sensitivity and Biomarker Expression

The study highlighted the significance of environmental triggers in asthma exacerbations by identifying a number of aeroallergens with strong correlations to biomarker levels. IL-5 with Aeroallergens: Patients who were exposed to aeroallergens such white ash (p=0.006), sorrel (p=0.004), and sweet vernal mix (p=0.002) had higher levels of IL-5. According to these results, these allergens are important targets for allergen-specific immunotherapy as they appear to be the main cause of eosinophilic inflammation. IL-17 and Aeroallergens: Patients who were sensitive to firebush (p=0.003) and CCD indicators (p=0.005) had significantly higher levels of IL-17, suggesting that these allergens play a part in neutrophilic or mixed asthma phenotypes. Increased IL-17 in response to these allergens indicates the activation of non-Th2 pathways, especially in instances of severe asthma.

Limitations and Future Directions

The study highlighted the significance of environmental triggers in asthma exacerbations by identifying a number of aeroallergens with strong correlations to biomarker levels. IL-5 with Aeroallergens: Patients who were exposed to aeroallergens such white ash (p=0.006), sorrel (p=0.004), and sweet vernal mix (p=0.002) had higher levels of IL-5. According to these results, these allergens are important targets for allergen-specific immunotherapy as they appear to be the main cause of eosinophilic inflammation. IL-17 and Aeroallergens: Patients who were sensitive to firebush (p=0.003) and CCD indicators (p=0.005) had significantly higher levels of IL-17, suggesting that these allergens play a part in neutrophilic or mixed asthma phenotypes. Increased IL-17 in response to these allergens indicates the activation of non-Th2 pathways, especially in instances of severe asthma. A relatively small sample size may have an impact on the findings' generalizability; the study was cross-sectional, which limited the capacity to establish causal links. Environmental factors that may affect asthma phenotypes, like as pollution and food, were not thoroughly examined.

5. Conclusion

The significance of IL-5 and IL-17 as biomarkers for asthma phenotype in juvenile patients is highlighted by this study. IL-5 plays a crucial role in allergen-induced asthma, as seen by the strong association it has with particular allergens. Asthma treatment could be completely transformed by personalized therapy based on biomarker analysis.

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