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المحتويات

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An Investigation of the Correlation Between the CNTNAP2 rs7794745 gene Polymorphism and Autism in Children from the Middle Euphrates Area of Iraq

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Abstract

Background: Autism is an incurable condition that may be attributed to several factors, such as genetic predisposition and environmental influences. Multiple studies have discovered that a substantial number of genes linked to autism serve as constituents of signaling pathways that regulate the plasticity of synapses and development, hence exerting a notable impact on the origins of the illness. CNTNAP2 is increased during the initial phases of neural tube development. Given the increasing worldwide occurrence of autism, there is a growing demand for effective teaching methods and educational programs.

Methods: A case-control study recorded 90 samples, comprising 50 autistic individuals (males and females, mean age 4.5 ± 2 years) and 40 healthy youngsters (5 ± 2 years). PCR and restriction enzymes are used in polymerase chain reaction-restriction to amplify and analyze DNA sequences. PCR-RFLP genotyped CNTNAP2 at rs7794745. Genomic DNA was isolated from peripheral blood cells of healthy children while buccal cells were swapped from patients to obtain and genotype their DNA.

Results: Our results revealed that the low-frequency distribution (p-value > 0.05) of the rs7794745 SNP is statistically non-significant in ASD patients compared to healthy children.

Conclusion: Our case-control study suggests that rs7794745 polymorphism is unrelated to ASD.

دراسة العلاقة بين تعدد الأشكال الجيني CNTNAP2 rs7794745 والتوحد لدى أطفال منطقة الفرات الأوسط في العراق

صفاء الصفار، حيدر الزبيدي، رؤى حميد العويض

الملخص

التوحد هو حالة غير قابلة للعلاج يمكن أن تُعزى إلى عدة عوامل، مثل الاستعداد الوراثي والتأثيرات البيئية. اكتشفت دراسات متعددة أن عددًا كبيرًا من الجينات المرتبطة بالتوحد تشكل مكونات مسارات الإشارات التي تنظم مرونة وتطور المشابك العصبية، مما يؤثر بشكل كبير على أصل المرض. يزيد مستوى CNTNAP2 خلال المراحل الأولية من تطور الأنبوب العصبي. نظرًا لزيادة انتشار التوحد في جميع أنحاء العالم، هناك طلب متزايد على أساليب تعليمية فعالة وبرامج تعليمية.

الطريقة:

تم تسجيل دراسة حالة-شاهد لـ 90 عينة، تضم 50 فردًا مصابًا بالتوحد (ذكور وإناث، متوسط العمر 4.5 ± 2 سنوات) و40 طفلًا صحيًا (5 ± 2 سنوات). استخدمت تقنية تفاعل البوليميراز المتسلسل والإنزيمات القاطعة (PCR-RFLP) لتضخيم وتحليل تسلسلات الحمض النووي. تم تحديد النمط الجيني لـ CNTNAP2عند الموضع .rs7794745 تم عزل الحمض النووي الجينومي من خلايا الدم المحيطية للأطفال الأصحاء، بينما تم أخذ خلايا من اللعاب للمرضى للحصول على النمط الجيني للحمض النووي الخاص بهم.

النتائج:

كشفت نتائجنا أن التوزيع ذو التردد المنخفض (قيمة الاحتمال > 0.05) للنمط الجيني rs7794745 غير ذي دلالة إحصائية في مرضى اضطراب طيف التوحد مقارنة بالأطفال الأصحاء

الاستنتاج:

تشير دراستنا الحالة-شاهد إلى أن تعدد الأشكال rs7794745 غير مرتبط باضطراب طيف التوحد

1. Introduction

Disruptions in social interaction and limited or repetitive behavior are hallmarks of autism spectrum disorder (ASD), a neurodevelopmental medical condition (Abrahams & Geschwind, 2008). ASD consists of a range of disorders, including autistic disorder, Rett syndrome, Asperger syndrome, and pervasive developmental disorder (Bölte et al., 2019; Taylor et al., 2020). Patients with ASD have difficulties with both verbal and nonverbal forms of social communication in addition to problems with cognitive and physical abilities. These patients also have unusual reactions to sensory experiences, unique passion, and repetitive behaviors (van 't Hof et al., 2021).

A substantial rise in the prevalence of autism has occurred during the last two decades, with an estimated global prevalence of 0.62% (Elsabbagh et al., 2012). Males have about fourfold higher risk of developing the disorder than females (sex ratio 4.2:1) (Fombonne, 2009). Despite experts' best efforts, no one etiological factor has been found. However, some published findings imply that several sets of causal elements, including genetic, environmental, and neurobiological aspects, may contribute to the development of ASD by affecting the developing brain (Hodges et al., 2020). The fact that the frequency of autism spectrum disorder is higher among autistic siblings and the concordance rate is higher in monozygotic twins than in dizygotic twins lends credence to the hypothesis that around 80–90% of autism spectrum disorder may be linked to genetics (Castelbaum et al., 2020).

The formation and functioning of synapses require several vital genes. It has been established that several genes, including CNTNAP2 and NLGN3, NLGN4X, and NRXN1, as well as others, have been associated with the process of adhesion between neurons and glia (Abrahams & Geschwind, 2008). In areas of the brain that are linked with autism spectrum disorder (ASD), the contactin-associated protein-like two gene (CNTNAP2) is expressed. This gene has been investigated for its possible role in the development of ASD (Abrahams et al., 2007; Alarcón et al., 2008). One of the most considerable mammalian genes, CNTNAP2, is a member of a family known as the neurexin superfamily. This family consists of 24 exons, occupies 2.3 megabytes on chromosome 7q, and is one of the most significant genes in mammals (St George-Hyslop et al., 2023).

One gene highly expressed in the growing brain and spinal cord is CNTNAP2, which encodes contactin-associated protein-like 2 (Caspr2) (Zare et al., 2017). The CNTNAP2 protein plays a crucial role in the language impairment that is associated with autism spectrum disorder (ASD) as well as other language-related issues. Early childhood autism spectrum disorder (ASD) children experience difficulties with speech development as a result of reduced CNTNAP2 expression (Rodenas-Cuadrado et al., 2018). Another study revealed that rare and common CNTNAP2 gene variants are associated with ASDs, seizures, and intellectual disability. The CNTNAP2 SNPs rs7794745 and rs2710102 are two frequently occurring non-coding variants located in the 2nd and 13th introns of chromosome 7q, respectively (Uddin et al., 2021).

The results show that A/T in rs7794745 has a more substantial effect on the decrease in the brain's response to sensing human voices(Koeda et al., 2015) . Additionally, it was reported that the A/T genotype in rs7794745 increases the risk of ASD in the Chinese Han group (Lia et al., 2010). The goal of this study was to find out if there was a link between

a widespread change in the rs7794745 of the CNTNAP2 gene and the risk of autism in the Iraqi (middle Euphrates) community.

Aim of the Study: To examine the association of CNTNAP2 gene single nucleotide polymorphism and the risk of autism occurrence in Middle Euphrates Iraqi children.

2. Materials, patients and Methods:

2.1.Patients

Fifty children with autism (males and females, mean age 4.5 ± 2 years) were recruited from the Al-Sibtein Academic Center for autism spectrum disorder, and forty healthy individuals (control group) (mean age 5 ± 2 years) collected from visitors to Al-Zahraa Teaching Hospital were incorporated in our study. The diagnosis was done by well-trained psychiatrists based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria, relying on historical information from interviews and clinical records. All participants and their families were informed about this research. The Ethical Committee Approval: The study protocol received approval from the Ethical Committee (in the Faculty of Pharmacy /Kufa University). Fifty children with autism (males and females, mean age 4.5 ± 2 years) were recruited from the Al-Sibtein Academic Center for autism spectrum disorder, and forty healthy individuals (control group) (mean age 5 ± 2 years) collected from visitors to Al-Zahraa Teaching Hospital were incorporated in our study. The diagnosis was done by well-trained psychiatrists based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria, relying on historical information from interviews and clinical records. All participants and their families were informed about this research. The Ethical Committee Approval: The study protocol received approval from the Ethical Committee (in the Faculty of Pharmacy /Kufa University).

2.2.DNA Extraction and Genotyping

Buccal swaps were obtained from ASD patients due to the difficulties in obtaining blood samples from them; they were preserved in a tube with normal saline. Peripheral blood cells were collected from healthy children. A single nucleotide polymorphism (SNP) in the CNTNAP2 (rs7794745) was targeted and chosen from the National Center for Biotechnology Information SNP database. PCR is a popular method for analyzing genomic variations, and single nucleotide polymorphisms (SNPs) in DNA can serve as genomic character markers in diseases or treatment responses, emphasizing the growing acknowledgment of genomic variations in disease etiology (Mubarak et al., 2020). Genomic DNA was extracted from buccal swaps using (the AddPrep Genomic DNA Extraction Kit). As the manufacturer's instructions stated, the SNP was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

The PCR reaction was performed to amplify a 315 bp fragment containing the aimed SNP using a specified primer. By using a PCR-RFLP, the variants of CNTNAP2 were genotyped. A 315-bp fragment containing the loci was amplified using the following primers: 5' AATACGGACCAAGATACCAAC is the F primer, and 5' TTCACACACAGTGCCTT is the R primer. 50 µl of the following ingredients were added to each reaction:

- 25 µl of the PCR master mix
- 4 µl of each primer
- 13 µl deionized water
- 4 µl of DNA

2.3.The PCR Reaction

The PCR conditions were Initial denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 1 minute, and the final extension at 72°C for 5 minutes. Finally, the PCR products were separated on 1% agarose gel and stained with 5 µl of Safe-Green 100 bp Opti-DNA marker added to the gel's first line pores to measure the PCR products' size. Then, the gel was transferred into the UVP system to visualize the PCR products under a 320nm UV light source. MlucI (ew England Biolabs) restriction enzyme digestion was used to cleave the wild-type sequence into a 315-bp fragment. This A- o-T base pair mutation in the CNTNAP2 gene provides a restriction site. A 1X su table restriction buffer and ten units of the required restriction enzyme were used to digest 1-3 µg of PCR product. The dig sts were then incubated for 3 hours at 37°C (or enzyme-appropriate temperature) before being subjected to electrophoresis to visualize the results. The dig station products were visualized after electrophoresis on a 1% agarose gel stained with a Safe-Green marker.

3. Statistical Analyses

Using (SPSS.v.26.0 software) SPSS Inc. Chicago, IL, the mean levels of each characteristic via genotype were compared using the student t-test and ANOVA. The chi-square test was also used to examine categorical data (alleles and genotypes. A P value could be considered statistically significant if it was less than 0.05)

4. Results

During our research, we looked at a total of ninety participants, the control group consisting of forty healthy children and fifty children diagnosed with autism. According to the findings of the MlucI enzyme, patients and control individuals who do not have a mutation (A→T) in the CNTNAP2 gene (genotype AA) have a fragment of 315 base pairs in size in their PCR products. On the other hand, people with the heterozygous genotype (AT) have three bands in their PCR product: 95 base pairs, 315 base pairs, and 220 base pairs. Furthermore, it is worth noting that persons who are both healthy and patient, who have homozygous genotypes (TT), and who have a mutation (A→T) have two pieces, which are 95 and 220 base pairs Fig.1.

Table 1: Contains all the genotype and allele frequencies data and related ORs (95% CI) for controls and autistic individuals. The frequencies of the genotypes of the rs7794745 A→T are illustrated in Table 1. Thus, a significant association was obtained from genotype distributions of rs7794745 CNTNAP2 gene polymorphism between the cases

of autism and control (95% OR, CI). The ca s' A and T allele frequencies were 48% and 52%, and the healthy group was 39% and 41%, each in order (p = 0.9). There was no significant difference in the frequencies of alleles between the groups.

Table 1*: The Frequency of Each Allele and Genotype of The Rs7794745 Polymorphism in Patients and Controls.

Alleles	**Controls (n = 40)**	**Autism cases (n= 50)**	**OR (95% CI)**	**P**
Alleles (A→T) A	39	48	1.0	0.9
T	41	52	1.03	
Genotypes (A→T) AA	11	12	1.0	0.6
AT	17	24	1.3	
TT	12	12	1.1	
*OR odds ratio; 95% Cl 95% confidence interval; p < 0.05 is statistically significant.				

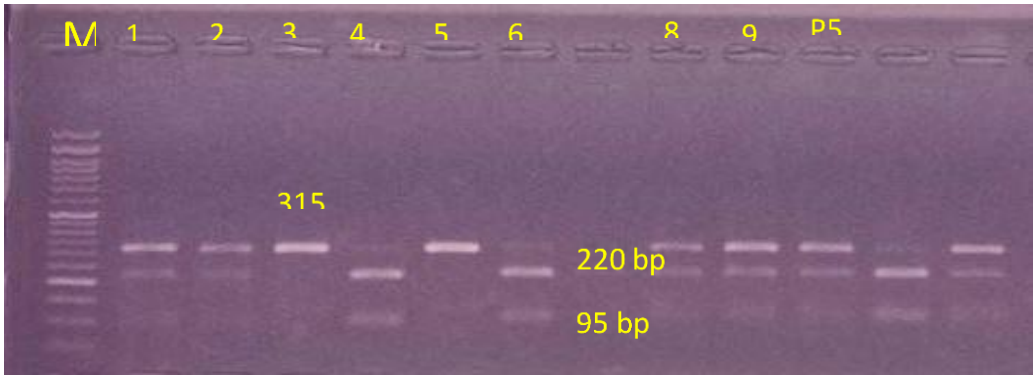

Figure 1: Shows agarose Gel Electrophoresis stained by Redsafe after Pcr-Rflp. Lanes 3, 6, and 9 show the AA, AT, And TT, respectively.

5. Discussion

Autism spectrum disorder (ASD) is a neuropsychiatric developmental condition that is highly heritable and multifactorial. It may rise due to a complex interplay between environmental and genetic risk factors. The genetics of autism spectrum disorder (ASD) are complicated and involve several genes, each of which plays a key role in the development of neural structures in children. Our research attempted to determine if there was an association between polymorphisms in the CNTNAP2 gene and autism. In Iraq children diagnosed with autism, the correlation between the GABRB3, MTR, and MTHFR gene polymorphisms and autism risk has been established (Jabbar & Jebor, 2018; Ma et al., 2005; Muftin et al., 2020). CNTNAP2, also known as NRXN4, is a protein found in the postsynaptic

membrane, which acts as a scaffolding component (Bourgeron, 2009). The CNT AP2 gene is crucial for proper cerebral development, and any disruption to its function significantly increases the likelihood of neurological impairment. Deficits in CNTNAP2 have been associated with ASD-related behaviors such as hyperactivity and epilepsy (Peñagarikano & Geschwind, 2012). An Amis family with autistic traits, cortical dysplasia, and focal epilepsy was found to have a mutation in the CNTNAP2 gene that made it less active (Strauss et al., 2006).

It was also demonstrated that uncommon variations in CNTNAP2 may play a role in the pathogenesis of ASD (Bakkaloglu et al., 2008). The rs7 94745 CNTNAP2 gene polymorphism was studied in a case-control study involving 50 autistic children and 40 controls. Our study's results suggest no significant association of rs7794745 A→T polymorphism to autism (p-value 0.9). like-wi e, no positive association was found between the CNTNAP2 polymorphism and ASD (Jonsson et al., 2014; Poot, 2014; Sampath et al., 2013). Previous research, however, has linked rs7794745 to autism in a variety of populations, including Brazilians and Iranians population (Nascimento et al., 2016). According to a recent study conducted in the Pakistani community, it was discovered that rs7794745 is strongly linked to ASD (Khalid et al., 2020). Additionally, al, research has also shown a direct association between rs7794745 and ASD in the Han Chinese (Lia et al., 2010). A few different factors could have caused these varying findings. Genetic diversity between populations initially had an impact on the results of the association studies. Second, a larger sample size would reduce the sampling error. Third, SD is a highly heterogeneous condition, and the vast majority of prior research has relied on the recruitment of patients with ASD. However, to limit variability, only families with typically developing autistic children were included in this study.

6. Conclusion

rs7794745 CNTNAP2 gene polymorphism was shown to have a non-significant connection with autism in the analyzed community. This is the conclusion that can be drawn from the findings. However, to arrive at a conclusive result, it was necessary to conduct the research with a more significant sample population and diverse ethnic groupings.

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The Effects of Extreme Electrospinning and Environmental Parameters on the Resulted Zein Nanofibers Formulated by the Electrospinning Technique

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Abstract

Objective: The study declares and shows the effect of electrospinning parameters, including the temperature, humidity, applied voltage, syringe gage, the distance between the needle tip and collector, and flow rate on the morphology of the resulted nanofiber using the electrospinning technique in the preparation of Zein polymer nanofibers.

Methods: The Zein nanofiber was formulated as nanofibers using an electrospinning apparatus. The nanofibers were prepared in different conditions, adjusting the parameters in each single run-in various temperatures and humidity. At the same time, the concentration of the zein polymer remained constant in all of the runs. Then, the nanofibers obtained after drying and collecting them from the aluminum foil were transferred to a tightly sealed container. The nanofiber was characterized using Attenuated Total Reflectance Infrared Spectroscopy (FTIR) to detect the presence of Zein polymer in the matrix. Then, the surface morphology of nanofibers was analyzed using the Scanning Electron Microscopy (SEM) technique. To declare the morphologic changes in the resulting nanofibers.

Results: The results indicate that some specific parameters and conditions can lead to perfectly shaped nanofibers, and applying different conditions and parameters can lead to abnormal morphological topography and electro-spraying instead of electrospun nanofibers.

Conclusions: The SEM images provided visual evidence of the change in morphology that resulted in each different run-in with different conditions and parameters.

Aim of the Study: This study aimed to identify the effect of different electrospinning and environmental parameters on the resulting zein polymer nanofibers.

تأثيرات الغزل الكهربائي الشديد والمعلمات البيئية على ألياف الزين النانوية الناتجة التي تم صياغتها بواسطة تقنية الغزل الكهربائي

مختار الجنابي، سرمد الإدريسي

الملخص

تهدف الدراسة إلى توضيح تأثير معلمات الغزل الكهربائي، بما في ذلك درجة الحرارة والرطوبة والجهد المطبق وعيار الحقنة والمسافة بين رأس الإبرة والمجمع ومعدل التدفق على شكل الألياف النانوية الناتجة باستخدام تقنية الغزل الكهربائي في تحضير ألياف النانو بوليمر الزين.

الطريقة:

تمت صياغة ألياف النانو الزين باستخدام جهاز الغزل الكهربائي. تم تحضير الألياف النانوية في ظروف مختلفة، مع ضبط المعلمات في كل تجربة على درجات حرارة ورطوبة متنوعة. في الوقت نفسه، ظل تركيز بوليمر الزين ثابتًا في جميع التجارب. بعد ذلك، تم نقل الألياف النانوية التي تم الحصول عليها بعد التجفيف وجمعها من رقائق الألومنيوم إلى حاوية محكمة الإغلاق. تم تمييز الألياف النانوية باستخدام مطياف الأشعة تحت الحمراء بتحليل الانعكاس الكلي المخفف (FTIR) للكشف عن وجود بوليمر الزين في المصفوفة. ثم تم تحليل شكل سطح الألياف النانوية باستخدام تقنية المجهر الإلكتروني الماسح (SEM) لتوضيح التغيرات المورفولوجية في الألياف النانوية الناتجة.

النتائج:

أشارت النتائج إلى أن بعض المعلمات والظروف المحددة يمكن أن تؤدي إلى تشكيل ألياف نانوية بشكل مثالي، وأن تطبيق ظروف ومعلمات مختلفة يمكن أن يؤدي إلى تضاريس مورفولوجية غير طبيعية ورش كهربائي بدلاً من ألياف نانوية مغزولة كهربائيًا.

الاستنتاجات:

قدمت صور المجهر الإلكتروني الماسح (SEM) دليل بصريًا على التغير في الشكل المورفولوجي الناتج في كل تجربة مختلفة مع ظروف ومعلمات مختلفة

هدف الدراسة:

هدفت هذه الدراسة إلى تحديد تأثير معلمات الغزل الكهربائي والمعلمات البيئية المختلفة على الألياف النانوية لبوليمر الزين الناتجة.

1. Introduction

The electrospinning technique is one of the methods that have been used to prepare different types of nanofibers using high voltage to create surface tension at the droplet of the polymeric liquid at the needle tip, leading to the formation of a Taylor cone. This leads to the release of the solution finely charged polymeric jet forming nanofibers at rotating or adjusted collection surfaces (Abdulhussain et al. 2023). The electrospinning process is affected by several parameters that can change the morphology of the resulting nanofiber. In some cases, it leads to the formation of electro-spraying (Deitzel et al. 2001). Zein is a biopolymer obtained from protein in maize starch (Deitzel et al. 2001; Tortorella et al. 2021; Abdulhussain et al. 2023). It can form nanofibers used in topical and systemic sustained release of accompanied drugs loaded within it (Tortorella et al. 2021). Preparing the nanofibers of this polymer in different types of dosage forms using electrospinning technology made a critical focus on what the specified parameters used to get the perfect nanofibers and what the impact of these parameters on the resulting nanofibers leading to the development of fiber-based drug delivery systems (Tortorella et al. 2021).
The study employed the electrospinning technique in different parameter settings to create nanofiber mats made of zein to explain the effect of parameters on the morphological results of nanofibers (Lasprilla-Botero et al. 2018).

2. Materials and Methods

2.1.Materials: The Zein polymer was acquired from Sigma Aldrich in the United States.

2.2.Methods

2.2.1. Preparation of Nanofiber Solution

A solution was created by dissolving at 1g per 5 ml. The solution comprised 50 ml of 100% ethanol and distilled water (DW), then gentle stirring for one hour to ensure complete dissolution. Subsequently, 3 ml of the polymeric was loaded into a 3 ml syringe.

2.2.2. Preparations of Nanofibers by Electrospinning

At the beginning of the electrospinning process, the electrospinning apparatus, consisting of a syringe pump (New Era pump system, USA) and locally developed and assembled components, was configured to adjust all parameters and introduce one different parameter each run during the entire run. Then, the 3 ml syringe was placed in the syringe pump using different gages, and the applied voltage was adjusted, as well as the distance between the needle tip and collector, the flow rate of polymeric liquid, and the environmental humidity, the temperature recorded these adjustment ware made in each run as described in the Fig.1 (Dai et al. 2014; Abu Owida et al. 2022; Nguyen et al. 2023).

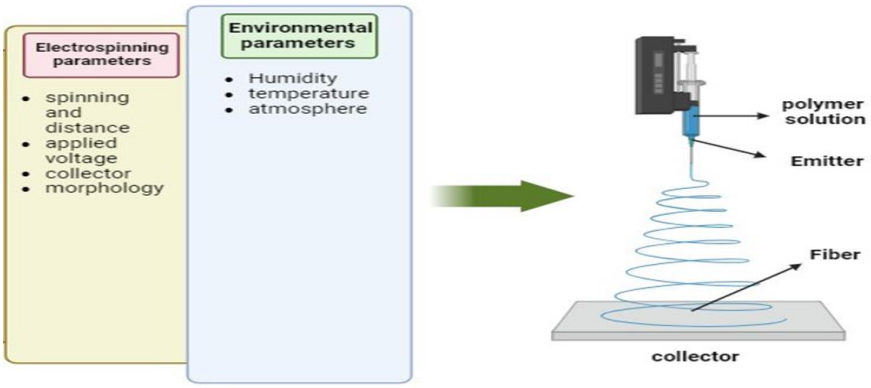

Figure 1: Shows the various parameters influencing the resulting nanofibers, including Electrospinning Parameters and Environmental Parameters.

2.2.3. Fourier Transforms Infrared Spectroscopy (FTIR)

A nanofiber sample was applied onto a petri dish and subsequently examined utilizing FTIR analysis with the assistance of an FTIR spectrophotometer (Bruker-Optic162, Germany). The 10 mg zein nanofibers were placed onto the lens. It was then mixed with 10 mg of potassium bromide (KBr) and fitted into the lens. The chart was created with a resolution of 2 cm-1. FTIR spectrophotometer measured values within the 3500 to 400 cm-1 range (Kamnev et al. 2021). To ensure the precise delivery of the provided formula without any possible interactions (Dai et al. 2014; Kamnev et al. 2021; Nguyen et al. 2023).

2.2.4. FESEM Analysis of Resulted Nanofibers

The morphologic surface topography of the nanofibers was obtained using field emission scanning electron microscopy (FESEM) instrument (FE Axia chem SEM, thermos fisher Holand). The settings of the scanning electron microscope (SEM) were adjusted at an applied voltage of 10 kilovolts (kV) and a surface distance of 5.71 millimeters (mm). A small piece of nanofibers covered with gold (Au) using a sputter-coating technique. Fiber diameter was measured using the Picture J software, applying it to 24 fiber sections derived from a scanning electron microscopy (FESEM) image (Van Roon et al. 2005; Gnanamoorthy et al. 2014).

2.2.5. The Adjustment of Parameters

An 18-run with an electrospinning device was conducted by selecting the most appropriate formula through its FESEM morphological results run 7. The parameters are fixed in each run depending on the best results, changing only one parameter, as shown in Table 1.

Table 1: The Different Ranges of Electrospinning and Environmental Parameters Were Applied in Each Run, Noticing That the Best Run Was No 7, Fixed in All Runs, Changing Only One Parameter at a Time

Run	Electrospinning parameters				Environmental Parameters	
	Flow rate (ml/h)	Syringe gage	Distance between needle tip and collector (Cm)	Applied voltage (Kv)	Temperature (C°)	Humidity (%)
1	0.5	18	17	15.7	30	26.6
2	0.8	18	17	15.7	30	26.6
3	1	18	17	15.7	30	26.6
4	0.6	15	17	15.7	30	26.6
5	0.6	20	17	15.7	30	26.6
6	0.6	22	17	15.7	30	26.6
7	0.6	18	17	15.7	30	26.6
8	0.6	18	17	15.7	13.8	26.6
9	0.6	18	17	15.7	45	26.6
10	0.6	18	17	24	30	26.6
11	0.6	18	17	19	30	26.6
12	0.6	18	17	15	30	26.6
13	0.6	18	17	15.7	30	71
14	0.6	18	17	15.7	30	47
15	0.6	18	17	15.7	30	20.1
16	0.6	18	15	15.7	30	26.6
17	0.6	18	10	15.7	30	26.6
18	0.6	18	13	15.7	30	26.6

3. Results and Discussion:

3.1.Fourier Transform Infrared Analysis

An analysis of the FTIR spectrum was conducted to identify the specific peaks occurring at different bandwidth intervals in nanofibers-impregnated Zein. To ensure the presence of Zein in each resulting nanofiber without any change. Zein has four distinct bands that accurately reflect its protein composition. 2800 to 3500 cm−1 spectral range corresponds to N-H and O-H bonds vibrational stretching represents the protein amino acids. Amide band spectral characteristic at a wavelength of 1658 cm−1, ascribed to the elongation of the carbonyl (C=O) bonds inside the amide groups of the peptide moieties, namely in the amide I region. At the same time, it represents the amide II at a wavenumber of 1541 cm−1. This specific band is linked to the angular deformation vibrations of the N-H bond. The axial deformation vibrations of the C-N bond appeal at 1238 cm−1 (Gough et al. 2020; Sadat and Joye 2020), as shown in Fig.2.

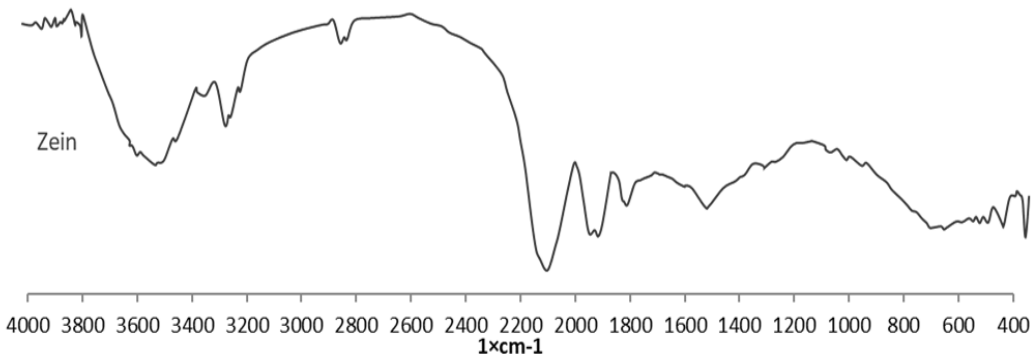

Figure 2: FT-IR spectrum of zein nanofibers, showing distinctive wavelengths corresponding to amide and amine groups within the amino acids that make up the protein moiety of zein

3.2.FESEM Analysis of Different Runs

After the fixation of the run (7) parameters, which is the best-selected formula that has uniform, beadles, transparent fibers at the sizes of around 310nm to 340nm, by changing one parameter at a time in each run, the results show different patterns of nanofibers and electro spraying (no nanofiber result). Results show distinct patterns of morphological aspects in each run when a parameter changes. By changing the flow rate of the syringe pump at different levels (0.5, 0.8, and 1 ml/h), as shown in runs (1, 2, and 3), the resulting nanofibers tend to be more uniform and well-shaped when the flow rate is reduced as shown in Fig.3. That's due to the increased injected liquid from the syringe at the unit time, leading to the deformity of the trajectory jet and Taylor cone. This result shows a correlation to Zergham et al (Yarin, Koombhongse and Reneker, 2001; Jalili, Morshed and Ravandi, 2006).

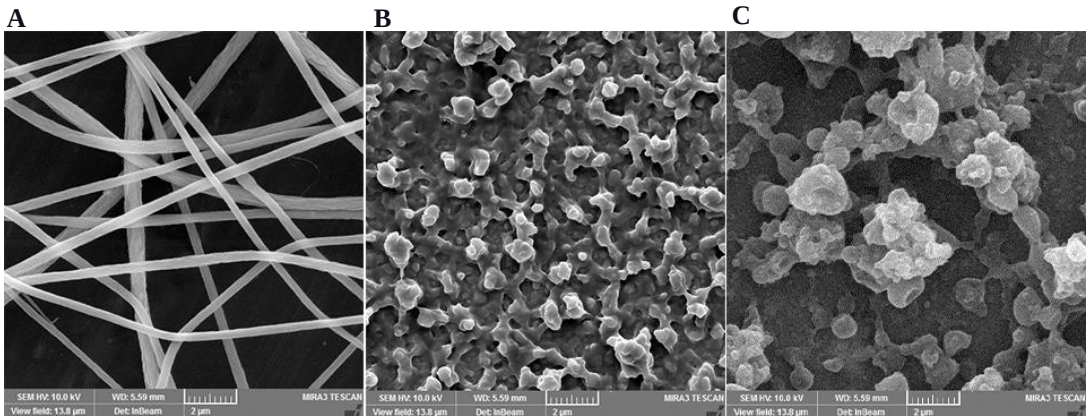

Figure 3: FESEM images depict the morphological changes in nanofibers resulting from adjustments to the syringe pump flow rate: **(A)** 0.5 mL/h, **(B)** 0.8 mL/h, and **(C)** 1 mL/h.

While the adjustment of the syringe gage of runs (4, 5, and 6), the resulting nanofibers show a lack of correlation between the syringe gage and nanofibers morphology. These three runs' nanofibers show excellent characteristics, as shown in Fig.4. These results confirm the results showed by Macossay *et al.* (Macossay et al. 2007)

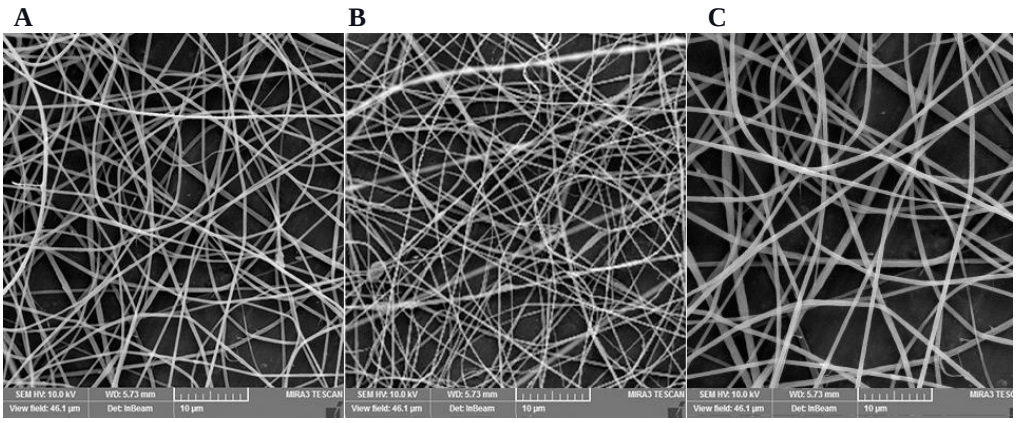

Figure 4: FESEM images show no morphological changes with adjustments in syringe gauge: **(A)** Gauge 15, **(B)** Gauge 20, and **(C)** Gauge 22.

Decreasing the distance between the needle tip and the collector for both the plane and roller collector in runs (16,17, and 18) tends to affect the morphology of the resulting nanofiber. Reducing the distance will lead to bead formation and irregular distribution of the fibers, as shown in Fig.5, which refers to the short time that the fibers take to travel to the collector and, hence, low time for drying before reaching the collector; these results are confirmed by Xue and his colleague (Jalili et al. 2006; Ferrández-Rives et al. 2017; Yousefi et al. 2018).

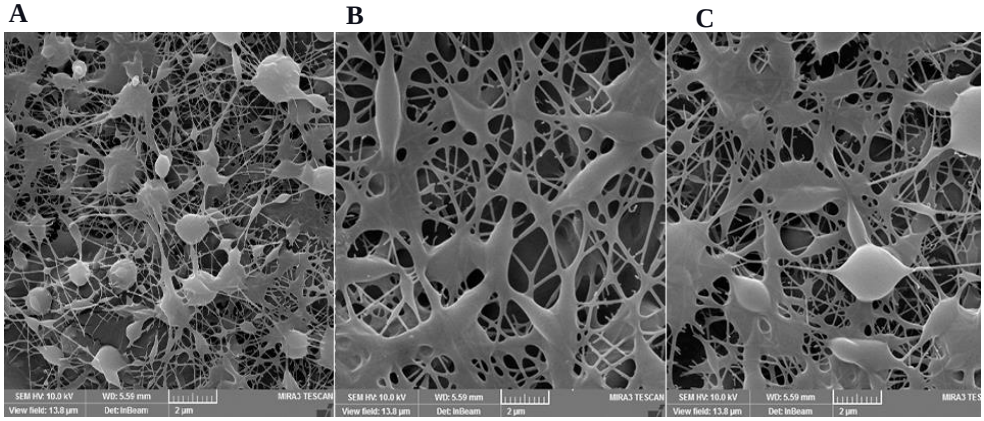

Figure 5: FESEM images display significant morphological changes due to adjustments in the distance between the needle tip and collector: **(A)** 15 cm, **(B)** 10 cm, and **(C)** 13 cm.

An elevation in the applied voltage can result in the formation of beads. With continuous release, the fibers disappear, forming clusters due to electrospraying in run (10, 11, and 12); the differences in the morphology can be seen in Fig.6. These results also appear with previously published data (Ferrández-Rives et al. 2017; Xue et al. 2019a; Xue et al. 2019b).

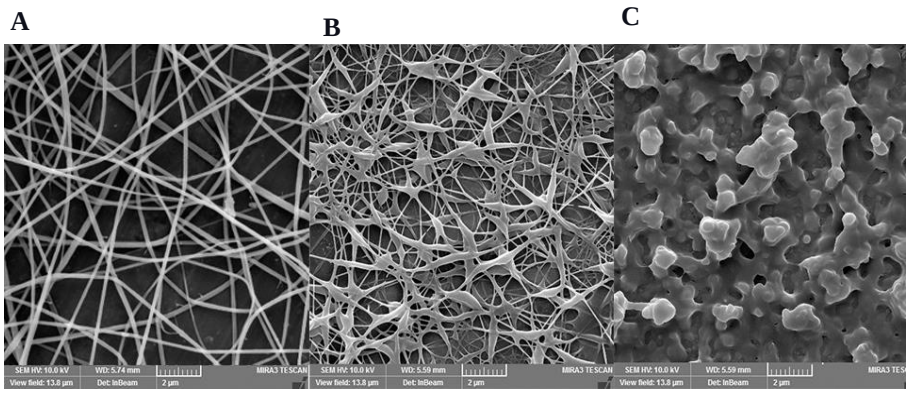

Figure 6: FESEM images reveal significant morphological changes due to adjustments in the applied voltage: **(A)** 15 kV, **(B)** 19 kV, and **(C)** 24 kV.

Decreasing the temperature results in beads due to the decrease in the drying time of the nanofibers plus the increase in viscosity, which can be seen in the run (8). In contrast, the slight elevation in temperature results in an excellent fine nanofiber due to the decrease in viscosity and surface tension, as shown in run (10); very elevated lab temperature led to the premature termination of the fluid jet electrical stretching, as can be seen in run (9) all these results shown in Fig.7. These results are similar to the findings that published by Yang et al. (De Vrieze et al. 2009; Yang et al. 2017; Refate et al. 2023)

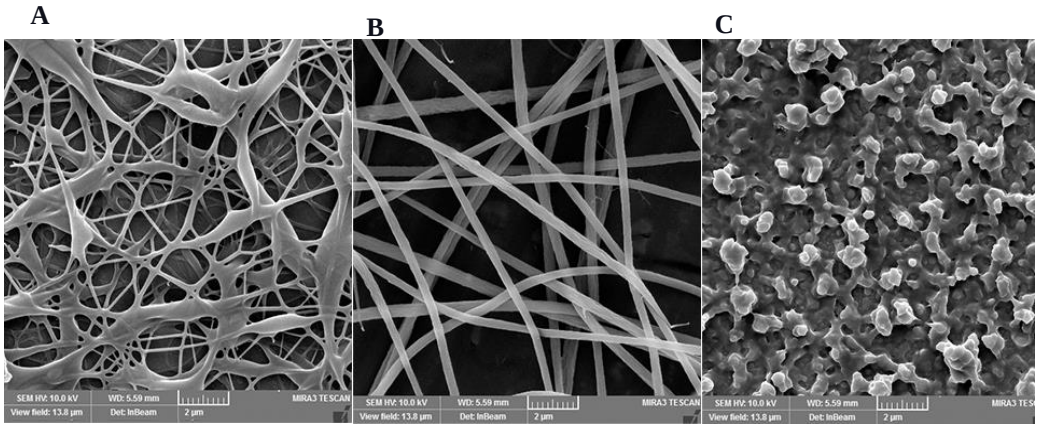

Figure 7: FESEM images show significant morphological changes due to variations in working temperatures and their impact on the resulting nanofibers: **(A)** 13.8°C, **(B)** 30°C, and **(C)** 45°C.

Increased humidity can influence the diameter of electrospun nanofibers. Decreased relative humidity leads to quicker solvent loss, resulting in thicker nanofibers in the run (15 and 14). Conversely, increased humidity hinders the process

of solvent evaporation, leading to the production of thinner nanofibers in the run (13). All these results are shown in Fig.8. These results are highly relative to the findings of Raska et al. (Raksa, Numpaisal and Ruksakulpiwat, 2021; Ura et al., 2021).

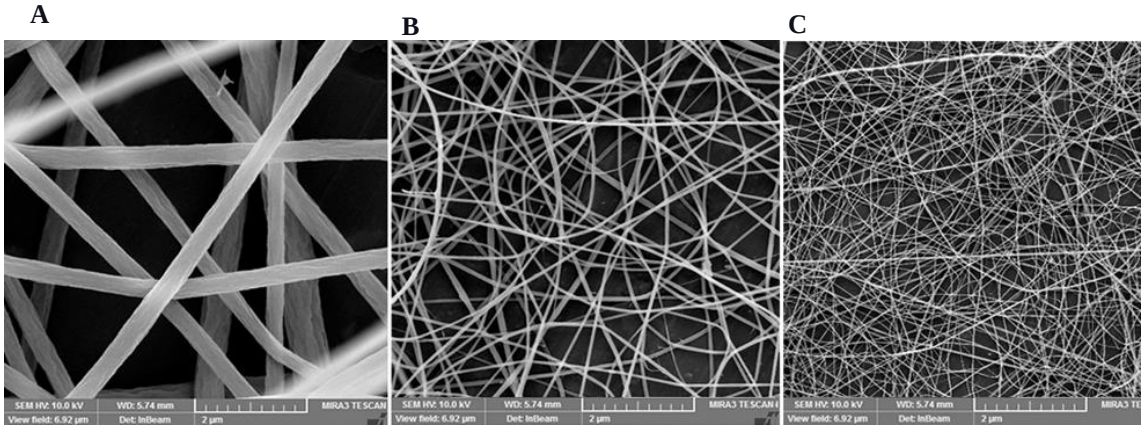

Figure 8: FESEM images reveal significant morphological changes due to variations in working humidity and their impact on the resulting nanofibers: **(A)** 20.1%, **(B)** 47%, and **(C)** 71%.

4. Conclusion

From the results of FESEM analysis, it's clear that electrospinning and environmental parameters have a very severe impact on the morphology of the resulting nanofibers, ranging from a deformity and bead formation to modification on the diameter and size; each modification in each of these factors can result in different results and hence different product, to prepare a specific zein nanofiber for whatever drug loading formula the parameters most adjusted to prepare the desired characteristics.

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Serological Evaluation of Systemic Lupus Erythematosus: Association with Disease Severity in Iraqi Patients

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Abstract

The diagnosis, management and treatment of systemic lupus erythematosus (SLE) in clinical settings often involve serological measures of autoantibodies, specifically the anti-double stranded DNA and generally the anti-nuclear autoantibodies (ANA). It remains to be shown, however, whether these serological measures correspond with the severity classification of SLE, particularly among Iraqi patients. Here, we investigated the relationship between serological measurements of autoantibodies and complement proteins with severity classification of SLE patients. Sixty patients clinically diagnosed with SLE, along with 60 age-matched non-SLE individuals (control) were recruited in the study. Serum levels of ANA, anti-dsDNA, complement C3 and C4 were measured. The SLE patients had significantly higher mean values of ANA, anti-dsDNA, with significantly lower levels of C3 and C4 compared to those of the control (non-SLE). With respect to their SLE disease severity classification, while levels of ANA significantly increased with severity of disease (mild, 7.99±0.65 IU/mL; 9.83±0.93 IU/mL; severe, 13.70±1.60 IU/mL; $p = 0.004$), levels of anti-dsDNA despite increasing (mild, 33.38±2.18 IU/mL; moderate, 39.32±2.28 IU/mL; severe, 42.84±4.80 IU/mL), were not statistically significant ($p = 0.101$). ($p < 0.05$). Conversely, levels of C3 (mild, 1.19±0.05 g/L; moderate, 0.98±0.11 g/L; severe, 0.72±0.17 g/L; $p = 0.009$) and C4 (mild, 0.32±0.02 g/L, moderate, 0.29±0.03 g/L; severe, 0.16±0.03 g/L; $p = 0.002$) significantly decreased with disease severity ($p < 0.05$). Our findings show that although ANA and anti-dsDNA autoantibodies may be important in the diagnosis of SLE, their use in predicting the severity of the disease varies considerably, while also highlighting, the significance of complement components C3 and C4 in monitoring and predicting the severity of the disease in addition to their role in the diagnosis of SLE.

التقييم المصلي لمرض الذئبة الحمامية الجهازية: الارتباط مع شدة المرض لدى المرضى العراقيين

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الملخص

غالبًا ما يتضمن تشخيص وإدارة وعلاج الذئبة الحمامية الجهازية SLE في الحالات السريرية قياسات مصلية للأجسام المضادة الذاتية، وتحديدًا الحمض النووي المضاد المزدوج والأجسام المضادة الذاتية المضادة للنواة (ANA) بشكل عام. ومع ذلك، يبقى أن نوضح ما إذا كانت هذه القياسات المصلية تتوافق مع تصنيف شدة مرض الذئبة الحمراء، خاصة بين المرضى العراقيين. هنا، قمنا بدراسة العلاقة بين القياسات المصلية للأجسام المضادة الذاتية والبروتينات التكميلية مع تصنيف شدة مرضى الذئبة الحمراء. تم تجنيد ستين مريضًا تم تشخيص إصابتهم بمرض الذئبة الحمراء سريريًا، إلى جانب 60 شخصًا غير مصابين بمرض الذئبة الحمراء (الضابطة) في الدراسة. تم قياس مستويات مصلANA ، ومضاداتdsDNA ، ومكمل C3 و .C4كان لدى مرضى الذئبة الحمراء (SLE) قيم متوسطة أعلى بكثير للـANA ، والمضادات للـdsDNA ، مع مستويات أقل بكثير من C3 و C4مقارنة بتلك الموجودة في المجموعة الضابطة (غير المصابين بمرض الذئبة الحمراء). فيما يتعلق بتصنيف شدة مرض الذئبة الحمراء، في حين أن مستويات ANA زادت بشكل ملحوظ مع شدة المرض (خفيف , ;7.99±0.65 IU/mL 9.83±0.93 IU/mL , 13.70±1.6 IU/mL شديد **(**p = 0.004)مستويات مضادات dsDNA على الرغم من الزيادة (خفيف، 33.38±2.18 IU/mL ؛ معتدل، 39.32±2.28 IU/mL ؛ شديد 42.84±4.80 IU/mL)لم تكن ذات دلالة إحصائية(p <0.05 (p = 0.101)) على العكس من ذلك، مستويات C3 (خفيف، 1.19±0.05 جم/لتر؛ معتدل، 0.98±0.11 جم / لتر، شديد، 0.72 + 0.17 جم / لتر)، (p = 0.009) ,C4 (معتدل، 0.32 + 0.02 جم / لتر، معتدل، 0.29 ± 0.03 جم / لتر، شديد، 0.16 0.03 جم / لتر)، **(**p 0.002) بشكل ملحوظ انخفض مع شدة المرض (P <0.05) تظهر النتائج التي توصلنا إليها أنه على الرغم من أن الأجسام المضادة ANA والأجسام المضادة لـ dsDNA قد تكون مهمة في تشخيص مرض الذئبة الحمراء، إلا أن استخدامها في التنبؤ بخطورة المرض يختلف بشكل كبير، مع تسليط الضوء أيضًا على أهمية المكونات التكميلية C3 و C4في مراقبة وتوقع شدة المرض. للمرض بالإضافة إلى دورها في تشخيص مرض الذئبة الحمراء

1. Introduction

Systemic lupus erythematosus (SLE) is a multi-system inflammatory autoimmune disease that affects several organ systems to differing degrees of severity. It has a wide variety of clinical symptoms. Some individuals only experience arthralgias and rashes, whereas others have serious multi-organ involvement, such as vasculitis or nephritis (Connelly and Morand, 2021). SLE is characterized by inefficient apoptotic clearance, deregulation of the immune system, complement activation, immune complexes, and tissue inflammation. Although the exact a etiology of this illness is still unknown, research suggests that a combination of genetic and environmental factors trigger immunological responses, which in turn cause B cells to produce autoantibodies and dysregulate cytokines, both of which harm tissue and organs. Antibodies against nuclear and cytoplasmic antigens are indicative of SLE (Agmon-Levin et al., 2012). Symptoms of SLE can affect one or more organ systems, range in severity, and fluctuate over time and this occasionally makes diagnosing this illness challenging (Ding et al., 2023). Common signs of flare-ups in systemic lupus include skin rashes such as the malar "butterfly rash," arthritis, pleurisy, serositis, alopecia, and lupus nephritis. Unfortunately, patients and doctors alike may become frustrated by the fact that treatment response varies and can be difficult to predict (Lazar and Kahlenberg, 2023). As a result, the clinical signs of SLE vary widely, ranging from minor skin involvement to serious organ damage such kidney failure, pulmonary hypertension, and heart failure, all of which are non-specific (Moutsopoulos and Zampeli, 2021).

Individualized research on SLE patients is complicated by the heterogeneity of SLE severity since it might mediate, confuse, or modify correlations with outcomes; hence, it is best to stratify or account for this severity under different categories. The principle of SLE severity classification takes into account the level of disease activity as well as its duration and in order to quantify SLE activity in the clinical situation, indices for SLE activity take into account both laboratory results and symptoms (Petri et al., 1992). The European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR) are presently using the improved classification criteria for diagnostic decisions for SLE (Ameer et al., 2022).

Despite the difficulties associated with the disease, research has shown that early detection of SLE reduces the risk of flare-ups, prompts use of healthcare services, and lessens the financial burden of the condition. Due to the self-replicating autoimmune phenomena caused by these factors, diagnosis becomes a significant issue that relies on clinical competence as phenomenon manifests itself in a variety of clinical presentations and changes with time. In clinical settings, serological measurements of autoantibodies - generally, the anti-nuclear autoantibodies (ANA) and specifically, the anti-double stranded (ds) DNA are routinely carried out of patients suspected to have SLE and this forms the diagnostic foundation for clinical intervention (Petri et al., 1992, Connelly and Morand, 2021). However, whether these serological measurements correlate with severity classification of SLE, especially among Iraqi patients, remains to be established. Here, we investigated the relationship between serological measurements of autoantibodies and complement proteins with severity classification of SLE patients.

2. Materials, Patients and Methods

2.1.Study Design and Subjects

This study was a cross-sectional study on patients clinically diagnosed with SLE by a specialist and were attending the Al - Imam Husain Medical City in Kerbala Governorate and Marjan Medical City in Babylon Governorate, Iraq, at the Rheumatology and Nephrology clinics in these hospitals. The patients were of both sexes with ages ranging from 16 to 65 years with duration of disease between 1 to 15 years. For the purpose of comparison, age-matched non-SLE individuals (control) were included in the study. These participants had no family history of SLE and were without any apparent medical disease. All subjects included in this study were Arab Iraqis and the study was carried out from November, 2022 to August, 2023.

Ethical approval for human studies was sought and obtained from the research and ethics committee of the College of Medicine, University of Kerbala in Iraq. All subjects (SLE patients and control group) were informed about the study and its aims, and their consent were obtained.

2.2.Classification of SLE Severity

All SLE patients fulfilled the American College of Rheumatology (ACR) criteria for classification of SLE, and were sub-divided into 3 groups based on their SLE disease activity index (SLEDAI) score (mild 0 - 5, moderate 6 - 12, and severe > 12) (Aringer et al., 2019).

2.3.Exclusion Criteria

SLE patients as well as non-SLE participants with autoimmune diseases, inflammation, pregnancy, malignant tumors, neurological disorders and a history of other connective tissue diseases (such as homocystinuria, Marfan syndrome, rheumatic fever, rheumatoid arthritis and osteoarthritis) were excluded from the study.

2.4.Data and Sample Collection

The study data was collected with strict adherence to standard health and safety measures. Demographic and clinical data (such as name, age, sex and medical history) were collected via an interview which was done to patients and /or their parents through a questionnaire.

Blood samples were obtained by venipuncture following disinfection of the antecubital fossa with 70% ethanol. Four milliliters blood was drawn into a gel tube for serum preparation respectively. Serum was prepared by centrifugation at 3000 rpm for 15 minutes and the supernatants were dispensed into Eppendorf tubes and stored at -20^{o}C until use.

2.5.Measurement of Autoantibodies and Complement Proteins

For ANA and anti-dsDNA, enzyme-linked immunoassay testing was performed on the EUROIMMUN analyzer I system (EUROIMMUN, Luebeck, Germany) using the Bio-Rad ANA ELISA (Bio-Rad; Hercules, CA, USA) and anti-dsDNA-NcX enzyme-linked immunoassay (Nunc, Roskilde, Denmark) respectively, as per manufacturer's guidelines.

Following the manufacturer's instructions, serum C3c and serum C4 were evaluated using fully automated turbidimetric immunoassay on a cobas® c502 analyzer (Roche Diagnostics). For C3 and C4, the assay's sensitivity was 0.04 and 0.02 g/l, respectively. For C3 and C4, the reference ranges were 0.9–1.8 g/l and 0.1–0.4 g/l, respectively

3. Statistical Analyses

The statistical software SPSS-25 (Statistical Packages for Social Sciences, version 25) was used for data analysis. Simple frequency, percentage, mean, and standard error of mean were used to present the data. The Pearson's Chi-square test ($\chi 2$-test) was used to determine the significance of the difference in different percentages (qualitative data), independent t test was used to statistical comparison of numerical data between the study groups while One Way ANOVA followed by Tukey's HSD was used to determine the significance of the numerical data differences between the different SLE disease severity classifications of SLE patients. Statistical significance was set at $p < 0.05$.

4. Results

4.1.Demographical and Clinical Characteristics of the Study Subjects

This study recruited a total of 120 individuals comprising of 60 patients clinically diagnosed with SLE and 60 non-SLE individuals (control) with matched demographic features as the SLE patients. Table 1 shows the demography and some clinical features of the study subjects. The SLE patients consisted of 36 females and 24 males while the non-SLE participants consisted of 28 females and 32 males. While the overall age of the subjects ranged from 16 to 67, majority of them (28.3%) were between 41 – 50 years age range, followed by 31 – 40 years age range (i.e. 22.5%), with only 7 subjects aged above 60 years. Comparison between the study groups with respect to age ranges was not significant ($p = 0.41$) as most of the subjects were between the ages of 31 to 50 years in both study groups.

The marital status of the study subjects shows that 43 (71.7%) SLE patients were married, while 17 (28.3%) patients were single and 52 (86.7%) non-SLE participants were married, with 8 (13.3%) single persons in the non-SLE group. The difference between the groups with respect to marital status was statistically significant with $p = 0.04$. Regarding family history of SLE, 44 (73.3%) SLE patients had history of SLE disease, while 16 (26.7%) of them do not. However, all 60 (100%) subjects of the non-SLE group (control) had no family history of SLE disease. The difference between the study groups with respect to family history was statistically significant with $p = 0.01$. Thirty-six (representing 60%) of the patients had mild SLE, while 19 patients (representing 31.7%) had moderate SLE and 5 patients (representing 8.3%) had severe SLE.

Table 1: Demography and Some Clinical Features of the Study Subjects

Features	All (n = 120)	SLE (n = 60)	Non-SLE (n = 60)	p-value
Age range, years (n (%)				
16 - 20	10 (8.3)	4 (6.7)	6 (10.0)	0.41
21 - 30	19 (15.8)	9 (15.0)	10 (16.7)	
31 - 40	27 (22.5)	15 (25.0)	12 (20.0)	
41 - 50	34 (28.3)	13 (21.7)	21 (35.0)	
51 - 60	23 (19.2)	14 (23.3)	9 (15.0)	
> 60	7 (5.8)	5 (8.3)	2 (3.3)	
Sex, n (%)				
Male	56 (46.7)	24 (40.0)	32 (53.3)	0.14
Female	64 (53.3)	36 (60.0)	28 (46.7)	
Marital Status, n (%)				
Single	25 (20.8)	17 (28.3)	8 (13.3)	0.04*
Married	95 (79.2)	43 (71.7)	52 (86.7)	
Family History of SLE, n (%)				
Yes	44 (36.7)	44 (73.3)	0 (0.0)	
No	76 (63.3)	16 (26.7)	60 (100.0)	
SLE Disease Severity, n (%)				
Mild	36 (60.0)	36 (60.0)	-	
Moderate	19 (31.7)	19 (31.7)	-	
Severe	5 (8.3)	5 (8.3)	-	
SLE; Systemic lupus erythematosus, *Statistically significant at $p < 0.05$				

4.2.Laboratory Analysis of Serum Autoantibodies and Complement Proteins

Table 2 summarizes the serum levels of ANA and anti-dsDNA as well as complements C3 and C4 of the SLE patients in comparison to the non-SLE subjects. For ANA, the SLE patients had the mean value of 8.79±2.04 IU/mL which was significantly higher compared to that of the control (non-SLE) which was 0.91±0.03 IU/mL ($p = 0.01$). For anti-dsDNA, the SLE patients had the mean value of 36.55±3.61 IU/mL which was also significantly higher compared to that of the control (non-SLE) i.e. 14.71±0.63 IU/mL ($p = 0.001$). This data is graphically presented in Fig.1.

Table 2: Mean Serum Levels of ANA and Anti-Dsdna As Well as Complements C3 And C4 Of the SLE Patients in Comparison to the Non-SLE Subjects

Variable	SLE (Mean ± SEM)	Non-SLE (Mean ± SEM)	p – value
Age, years	41.53±1.71	39.30±1.55	0.194
ANA, IU/mL	8.79±2.04	0.91±0.03	0.01*
Anti-dsDNA, IU/mL	36.55±3.61	14.71±0.63	0.001*
C3, g/L	1.09±0.19	2.86±0.09	0.04*
C4, g/L	0.3±0.02	1.10±0.04	0.02*
SLE; systemic lupus erythematosus, SEM; standard error of mean, ANA; anti-nuclear autoantibodies, Anti-dsDNA; anti-double stranded DNA, C; complement *Statistically significant at $p < 0.05$			

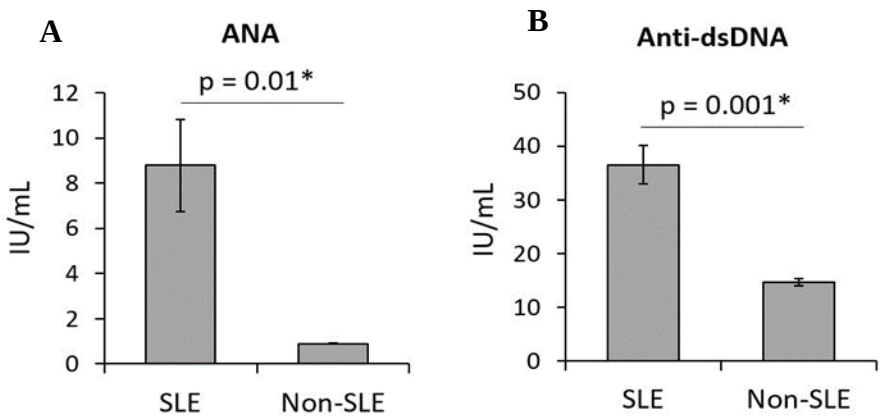

Figure 1: Mean Levels of Serum Autoantibodies. **(A)** The mean ANA level was significantly higher in the serum of SLE patients compared to the non-SLE group. **(B)** Similarly, the mean anti-dsDNA level was significantly higher in the serum of SLE patients compared to the non-SLE group ($p < 0.05$). *Statistically significant at $p < 0.05$. Error bars represent the standard error of the mean (SEM).

For C3, the SLE patients had the mean value of 1.09±0.19 g/L which was significantly lower compared to that of the control (non-SLE) which was 2.86±0.09 g/L ($p = 0.04$). For C4, the SLE patients had the mean value of 0.30±0.02 g/L which was significantly lower compared to that of the control (non-SLE) which was 1.10±0.04 g/L ($p = 0.02$). This data is graphically presented in Fig.2.

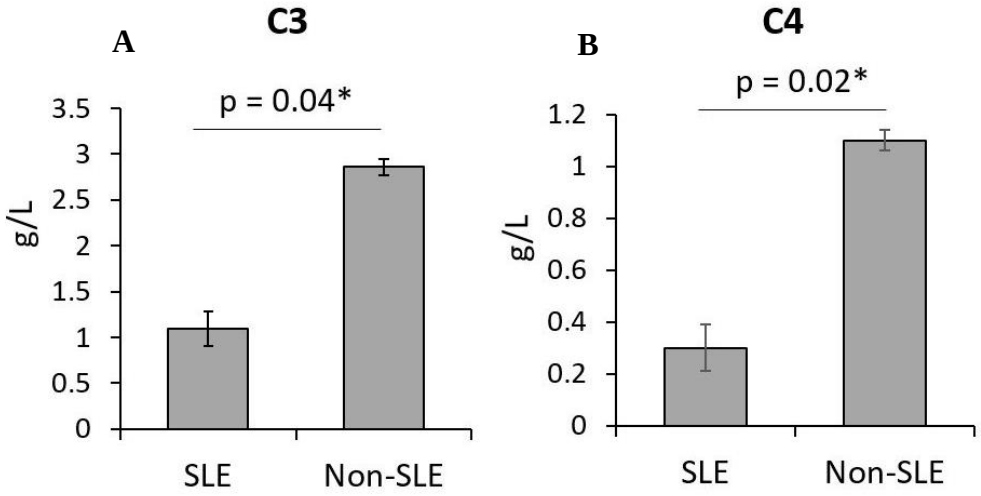

Figure 2: Mean Levels of Serum SLE-associated Complement. **(A)** Mean C3 level was significantly lower in the serum of SLE patients compared to the non-SLE group. **(B)** Similarly, the mean C4 level was significantly lower in the serum of SLE patients compared to the non-SLE group ($p < 0.05$). *Statistically significant at $p < 0.05$. Error bars represent the standard error of the mean (SEM).

4.3.Serum Autoantibodies and Complement Proteins Levels Based on SLE Disease Severity

Table 3 summarizes the serum levels of ANA and anti-dsDNA as well as complements C3 and C4 of the SLE patients with respect to their SLE disease severity classification. Levels of ANA increased with severity of disease as the patients with mild SLE had the mean value of 7.99±0.65 IU/mL, those with moderate SLE had 9.83±0.93 IU/mL and those with severe SLE had 13.70±1.60 IU/mL, the differences of which were statistically significant ($p = 0.004$). For anti-dsDNA, serum levels also increased with disease severity as the patients with mild SLE had the mean value of 33.38±2.18 IU/mL, those with moderate SLE had 39.32±2.28 IU/mL and those with severe SLE had 42.84±4.80 IU/mL, however, the observed differences were not statistically significant ($p = 0.101$). This data is graphically presented in Fig.3.

Table 3: The SLE Patients' Mean Serum Levels Of ANA, Anti-Dsdna, and Complements C3 And C4 in Relation to their SLE Severity Classification

Variable	SLE Disease Severity			p – value
	Mild (Mean ± SEM)	Moderate (Mean ± SEM)	Severe (Mean ± SEM)	
Age, years	42.50±1.36	41.21±1.99	46.40±2.77	0.455
ANA, IU/mL	7.66±0.65	9.83±0.93	13.70±1.60	0.004*
Anti-dsDNA, IU/mL	33.38±2.18	39.32±2.28	42.84±4.80	0.1
C3, g/L	1.19±0.05	0.98±0.11	0.72±0.17	0.009*
C4, g/L	0.32±0.02	0.29±0.03	0.16±0.03	0.002*
SLE; systemic lupus erythematosus, SEM; standard error of mean, ANA; anti-nuclear autoantibodies, Anti-dsDNA; anti-double stranded DNA, C; complement, *Statistically significant at $p < 0.05$.				

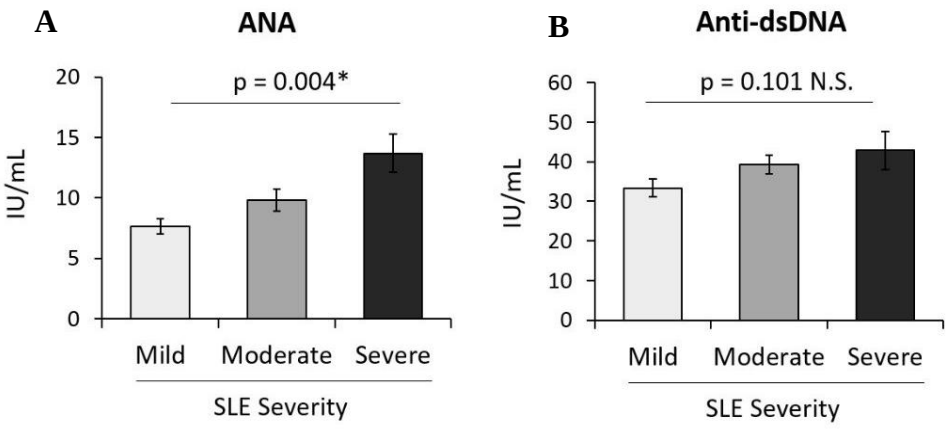

Figure 3: Mean Serum Autoantibodies of SLE Patients Based on Disease Severity Classification. **(A)** Mean ANA levels based on disease severity classification. **(B)** Mean anti-dsDNA levels based on disease severity classification.

In SLE patients, levels of ANA increased as the disease progressed; these differences were statistically significant ($p = 0.004$). For anti-dsDNA, serum levels also increased with disease severity as the SLE patients however, the observed differences were not statistically significant ($p = 0.101$). *Statistically significant at $p < 0.05$. Error bars indicated standard error of mean (SEM). Conversely, levels of C3 and C4 decreased with severity of disease as the patients with mild SLE had the mean C3 value of 1.19±0.05 g/L, those with moderate SLE had 0.98±0.11 g/L and those with severe SLE had 0.72±0.17 g/L, the differences of which were statistically significant ($p = 0.009$). For C4, serum levels also decreased with disease severity as the patients with mild SLE had the mean C4 value of 0.32±0.02 g/L, those with moderate SLE had 0.29±0.03 g/L and those with severe SLE had 0.16±0.03 g/L; the observed differences were statistically significant ($p = 0.002$). This data is graphically presented in Fig.4.

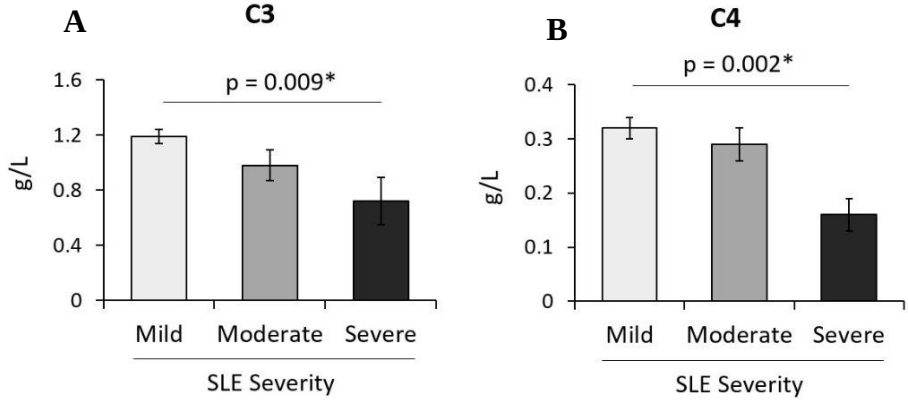

Figure 4: Mean Serum Complement Levels of SLE Patients Based on Disease Severity Classification. **(A)** Mean serum C3 levels declined with increasing disease severity (mild > moderate > severe), with a statistically significant difference ($p = 0.009$). **(B)** Mean serum C4 levels also declined with increasing disease severity, with a statistically significant difference ($p = 0.002$). *Statistically significant at $p < 0.05$. Error bars represent the standard error of the mean (SEM).

5. Discussion

The complexity and heterogeneity of SLE make it challenging to assess the disease's severity using a single serological indicator. One of the hallmarks of SLE is increased apoptosis along with impaired clearance of apoptotic cells, resulting in the emergence of high levels of a myriad of autoantibodies. Autoantibody synthesis also leads to tissue harm through the formation and deposition of autoantibody-autoantigen immune complexes (Ding et al., 2023). The EULAR/ACR SLE classification criteria for SLE have been developed and updated regularly to aid diagnosis, monitoring and management of SLE (Aringer et al., 2019). However, factors such as race, locality, and sex affect the severity, risk, and clinical manifestation of SLE, with women and some non-European-derived populations having a higher prevalence (Bertsias et al., 2010). To relate the severity classification of SLE with levels of some serological indicators, this study measured the serum levels of SLE-associated autoantibodies (ANA and anti-dsDNA), complement components (C3 and C4), of SLE patients in comparison to non-SLE subjects (control). The serum levels of the patients' autoantibodies complement components were the evaluated based on their disease severity classification.

To achieve homogeneity in the study population, we ensured that the SLE patients as well as the non-SLE participants recruited in the study were Iraqi Arabs. Also, statistical comparisons between the study groups with respect to mean age and age range, were not significant ($p < 0.05$). However, as reported in previous studies (Margery-Muir et al., 2017, Nusbaum et al., 2020), female preponderance (60%) was observed in the present study. Interestingly, marriage status of the SLE patients and the non-SLE group differed significantly, according to the results ($p < 0.05$). A crucial observation to make is that, while the difference between the groups in married versus single was marginally significant ($p = 0.04$), this finding contradicts the findings of Qu et al.'s study, which found that these differences were not statistically significant (i.e. $p = 0.058$, p-value set at > 0.05) (Qu et al., 2019). On the other hand, our finding suggests that the marital status bears some responsibility for SLE, and may have an assessment role in the condition in future studies. The role of family history of SLE in the predisposition to development of SLE has been extensively evaluated in previous studies. In 2002, Cooper et al identified family history of lupus or other systemic autoimmune diseases in a parent or sibling as a risk factor for development of lupus (Cooper et al., 2002). Similar results have been reported in more recently conducted studies. Here, we observed that 73% of the SLE patients had family history of the disease while none of the non-SLE subjects had family history of the disease. Moreover, a meta-analysis reported deleterious impact of familial history on the clinical manifestations and laboratory disorders in SLE patients (Chen et al., 2018).

Categorization of the SLE patients based on SLEDAI severity scores, indicated that majority of the patients (60%) had mild SLE, with only 5 patients having severe levels of the disease. This finding is consistent with the reports of many SLE severity classification studies (Fanouriakis et al., 2020, Nikolopoulos et al., 2020).

The majority of lupus patients have a mild form of the disease that is typified by flares, which are periods of time when symptoms worsen for a while, then get better or perhaps go away entirely. However, it has been highlighted that

some cases may become more severe over time, with mild, moderate, and severe cases eventually making up one-third of each group (Fanouriakis et al., 2020).

SLE patients are characterized by the present of autoantibodies, which can form immune complexes and for this reason are considered harmful. The development of antibodies to components of the cell nucleus, also known as anti-nuclear antibodies, or ANA, is a common serological finding among these SLE manifestation. With antibodies to DNA and Sm, a compound of proteins and uridine-rich RNA molecules that is strongly linked to SLE, these antibodies target DNA, RNA, proteins, and protein-nucleic acid complexes (Pisetsky et al., 2019).

In this study, patients with SLE had mean serum ANA and anti-dsDNA levels significantly higher than those without SLE. This is consistent with the findings of previous studies (Elessawi et al., 2019, González et al., 2015).

Positive ANA was initially not a prerequisite for admission in earlier SLE classifications; it was seen as equally significant as anti-dsDNA and other autoantibodies. With anti-dsDNA autoantibodies listed as one of the classification criteria for SLE, they are relatively effective indicators for monitoring disease activity and treatment response (Aringer et al., 2021). Additionally, this study demonstrates a significantly lower level of C3 and C4 cytokine markers in the SLE patients when compared to Control ($p = 0.02$ and 0.04, respectively). These results are in conflict with those of Troldborg et al, who found that SLE patients had higher plasma concentrations of C3 than controls ($p < 0.05$) (Troldborg et al., 2018). However, they are in line with Qu et al. (2019), who found that SLE was associated with lower levels of C4 and C3 (0.19±0.08 g/L and 0.58±0.24 g/L, respectively), when compared to a healthy control (0.29±0.11 g/L and 0.97±0.15 g/L) ($p < 0.001$). In general, Sandhu and Quan noted that complement activation is important in SLE and that blood levels of C3 and C4 should be continuously monitored to determine whether the disease is active (Sandhu and Quan, 2017).

Serum levels of the autoantibodies and complement components may play significant role in the diagnosis of SLE, however due to the heterogenous nature of the disease, their predictive usefulness in monitoring disease severity and progression remains to be established. In our findings, elevations in ANA levels along with decrease in C3 and C4 levels were observed to occur significantly in relation to SLE disease severity as it worsens from mild to moderate and to severe. However, while there were observable elevations in anti-dsDNA with increase in disease severity, these differences were not statistically significant. This observation highlights the significance of ANA evaluation in the diagnosis and management of SLE and supports the inclusion of a positive result in the ANA detection test as a required entry condition for the SLE classification, which is one of the primary modifications made to the EULAR/ACR 2019 classification criteria in comparison to earlier SLE classification systems (Aringer et al., 2021). Some authors have even referred to this as a strategic change, calling it the most relevant change made by the EULAR/ACR 2019 (Damoiseaux and van Beers, 2023, Serra-García et al., 2022). Anti-dsDNA on the other hand was initially regarded the most prominent immunological criterion in the EULAR/ACR 2019 classification once the positive ANA entry criterion is satisfied. However, recent studies have raised questions on the specificity of anti-dsDNA as a pathogenic factor and biomarker for SLE. This resulted from reports of anti-dsDNA antibodies among patients with bacterial, viral or parasitic infections (Wozencraft and Staines, 1990, Hamilton et al., 2006, Rekvig et

al., 2006) as well as cancers (Lv et al., 2005) and even healthy individuals (Rekvig, 2015). The result we obtained here supports the growing realization that the anti-dsDNA is not a distinct SLE-specific molecule and hence the usefulness of anti-dsDNA determination in the diagnosis and classification of SLE should be investigated further and followed up.

There is contradiction in the relationship between SLE and the complement system. It has long been established that SLE exacerbations stimulate the complement system, which is thought to be mostly due to nephritic activity. Whether this complement activation has a role in the pathophysiology of SLE or is just a harmless event has been discussed (Weinstein et al., 2021). As a modulator of inflammation, complement insufficiency increases the risk of developing SLE. While C3 deficit is infrequently linked to SLE-like illness, inherited complement C4 deficiency, whether partial or total, carries a substantial risk of developing SLE (Walport et al., 1997). Nevertheless, these complement components play a crucial role in avoiding immune complex-mediated tissue damage, as evidenced by the correlation found between complement deficits and SLE (Narayanan et al., 2010). Importantly, this study underscores the potential significance of complement components C3 and C4 determination not only in the diagnosis of SLE, but also in management of the disease.

6. Conclusion

In conclusion, we have demonstrated that although ANA and anti-dsDNA autoantibodies may be important in the diagnosis of SLE, their use in predicting the severity of the disease varies considerably, with ANA having a higher specificity than anti-dsDNA. Additionally, we emphasized the significance of complement components C3 and C4 in monitoring and forecasting the severity of the disease in addition to their role in the diagnosis of SLE.

Since the SLE autoimmune response is heterogenous, it may never be possible to develop a diagnostic and severity evaluation approach that is 100% sensitive and specific. Consequently, it is important to define reasonable goals that can be applied universally in all clinical settings. Further research is required to enhance the diagnostic and disease management efficacy of anti-dsDNA assays in diverse populations.

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Studying the Role of L-Thyroxine in Long-Term Management of Hypothyroidism: A Comparative Analysis with Healthy Control

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Abstract

Background: Hypothyroidism, characterized by insufficient thyroid hormone production by the thyroid gland, which may lead to significant challenges to metabolic health and overall well-being. Hence, studying the intricate correlation between thyroid biological functions, metabolic pathways and treatment outcomes are crucial for patients care management. Although, recent researches have clarified the complexity between thyroid hormone levels and metabolic pathways, there are still unanswered questions about the specific mechanisms and practical implications. **Patients and methods:** Across sectional study was conducted in Iraq from September 2023 to July 2022 involved 100 females with hypothyroidism undergoing L-thyroxine therapy for at least four months and 50 healthy subjects as control. Blood samples was collected after an overnight fast for plasma extraction. Various biochemical and hormonal assays were performed including TSH, TT4, FT4, TT3, FT3, TSH, fasting plasma glucose, insulin levels, HOMA-IR, cholesterol, triglycerides, HDL, LDL, VLDL, and BMI calculations for categorizations into weight groups.

Results: there were notable differences in weight, blood pressure, and BMI but not in age between patients with hypothyroidism and healthy controls. In addition, patients with hypothyroidism showed decreased FT4 levels, which suggested problems with the regulation of free thyroxine, along with increased TSH and FT3 levels, which indicated thyroid dysfunction and

hyperactivity. Furthermore, patients had lower HOMA-IR indices and impaired glucose metabolism, highlighting the complex relationship between thyroid function and metabolic parameters. Patients had dyslipidemia indicated by elevated total cholesterol and triglyceride levels, and altered lipid metabolism was suggested by lower VLDL levels. Long-term L-thyroxine therapy resulted in a tendency toward lower TSH levels, with TT3 levels fluctuating over the course of treatment.

Conclusion: The aforementioned results offer fresh perspectives on the intricate relationships among thyroid function, metabolic markers, and treatment results in hypothyroidism. They underscore the necessity for additional investigation to clarify underlying mechanisms and enhance clinical management approaches.

دراسة دور الليفوثيروكسين في الإدارة طويلة الأمد لقُصور الغدة الدرقية: تحليل مقارن مع المجموعة الضابطة السليمة

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الخلاصة:

المقدمة: قصور الغدة الدرقية، الذي يتميز بإنتاج هرمون الغدة الدرقية بشكل غير كافٍ، والذي قد يؤدي إلى تحديات كبيرة في الصحة الأيضية والعامة للفرد. لذا، فإن دراسة الارتباط المعقد بين وظائف الغدة الدرقية البيولوجية ومسارات الأيض ونتائج العلاج أمر أساسي لإدارة رعاية المرضى. على الرغم من أن الأبحاث الحديثة قد أوضحت تعقيد العلاقة بين مستويات هرمون الدرقية ومسارات الأيض، إلا أن هناك أسئلة لم يتم الإجابة عنها بعد حول الآليات الخاصة والتبعات العملية.

المرضى وطرق العمل: تم إجراء دراسة مقطعية في العراق من سبتمبر ٢٠٢٣ إلى يوليو ٢٠٢٢ شملت ١٠٠ أنثى مصابة بالنقص الدرقي تخضع لعلاج الإلثيروكسين لمدة لا تقل عن أربعة أشهر و ٥٠ شخصاً سليماً كمجموعة ضابطة. تم جمع عينات الدم بعد الصيام الليلي لاستخراج البلازما. تم إجراء مجموعة من التحاليل الكيميائية والهرمونية بما في ذلك TSH و TT4 و FT4 و TT3 و FT3 و TSH وسكر البلازما الصائم ومستويات الأنسولين ومؤشر HOMA-IR والكوليسترول والتريجليسريدات و HDLو LDLو VLDLوحسابات BMI للتصنيف في مجموعات الوزن.

النتائج: كانت هناك فروق بارزة في الوزن وضغط الدم ومؤشر كتلة الجسم ولكن ليس في العمر بين المرضى الذين يعانون من النقص الدرقي والأشخاص الأصحاء. بالإضافة إلى ذلك، أظهر المرضى الذين يعانون من النقص الدرقي انخفاضًا في مستوياتFT4 ، مما يشير إلى مشاكل في تنظيم الثايروكسين الحر، إلى جانب زيادة في مستويات TSH وFT3 ، مما يشير إلى اضطراب الغدة الدرقية وزيادة النشاط. علاوة على ذلك، كانت لدى المرضى مؤشرات HOMA-IR أقل واضطراب في استقلاب الجلوكوز، مما يسلط الضوء على العلاقة المعقدة بين وظيفة الغدة الدرقية والمعايير الأيضية. كان لدى المرضى دايسليبيديميا مشيرة إلى زيادة في مستويات الكوليسترول الكلي والتريجليسريدات، واقترحت اضطرابات في استقلاب الدهون من خلال خفض مستويات .VLDL أدى العلاج بالإلثيروكسين على المدى الطويل إلى انخفاض في مستويات TSH، مع تذبذب مستويات TT3 على مدى العلاج.

الاستنتاج: تقدم النتائج المذكورة أفاقًا جديدة حول العلاقات المعقدة بين وظيفة الغدة الدرقية والمؤشرات الأيضية ونتائج العلاج في النقص الدرقي. إنها تؤكد على ضرورة إجراء المزيد من البحوث لتوضيح الآليات الأساسية وتعزيز النهج السريري للإدارة.

1. Introduction

Hypothyroidism is a common endocrine dysfunction that characterized by inadequate production of thyroid hormones by the thyroid gland (Chiovato et al., 2019; Gottwald-Hostalek and Schulte, 2022; Leng and Razvi, 2019). This condition impacts people of all ages and both genders, with significant implications for metabolic health, cardiovascular function, and overall well-being. Understanding the intricate relationship between thyroid function, metabolic markers, and long-term treatment outcomes is essential for enhancing patient care and refining clinical management approaches (Staff, 2021).

Recently, there has been increased attention toward investigating the correlation between thyroid function and metabolic biomarkers in patients with hypothyroidism. Numerous studies have uncovered the complex interplay between thyroid hormone levels and various metabolic pathways, such as glucose metabolism, lipid regulation, and body weight control (Bensenor, 2019; Deniz et al., 2022; Fotakis et al., 2022). However, the precise mechanisms underlaying these associations and their clinical implications for patients with hypothyroidism still need to be unmasked (Al-Fatlawi, 2022; Chiu et al., 2023; Kemkem et al., 2020; Liu et al., 2019; Luo et al., 2022; Xu and Zhong, 2022).

Aim of the study: this current study aims to address gaps between thyroid function, metabolic functions and treatment outcomes in patients with hypothyroidism by exploring a comprehensive analysis of thyroid hormones and metabolic parameters compared to the healthy control population. Additionally, the study looks to address the impact of hypothyroidism on metabolic health and assess the efficacy of long-term L-thyroxine treatment in managing thyroid dysfunction and optimizing metabolic biomarkers. Furthermore, the study intends to explore the role of L-thyroxine, the primary medication for hypothyroidism patients in thyroid hormone levels and normalize metabolic parameters, which may provide valuable insights into the efficacy and safety of this treatment approach in clinical practice.

2. Materials, Patients and Methods

2.1.Patients' Constant and Enrollment

This cross-sectional study was conducted from July 2022 to September 2023. The study involved 150 female individuals included 100 patients had been diagnosed with hypothyroidism and 50 healthy control population were recruited from a private clinic in Iraq, where they sought medication and advice for their condition. The patients have been selected based on clinical assessment by specialist doctor which may include physical exam, laboratory tests and thyroid hormone levels. Patients received treatment according to the specialist endocrinologist and thyroid specialist. The study included patients had been receiving levothyroxine therapy for at least four months.

2.2.Inclusion Criteria:

1. Female patients aged 40 years or older.
2. Patients receiving levothyroxine therapy for at least four months for thyroid hormone replacement.
3. Exclusion Criteria:
4. Patients with a treatment duration of less than four months.
5. Patients under 40 years old.
6. Patients taking medications affecting THRA1 expression or levothyroxine activity.
7. Patients who had undergone thyroidectomy.

2.3. Blood Sample Collection

After an overnight fast, 5 ml of blood was drawn from each patient via vein puncture. The samples were collected in EDTA tube for plasma extraction.

2.4. Biochemical and Hormonal Assay Methods

The following techniques were used to measure each different parameter following the commercial instructions:

1. Estimation of Serum Thyroid Stimulating Hormone (TSH): Electrochemiluminescence immunoassay (ECLIA) using the Cobas e immunoassay analyzer.
2. Estimation of Serum Total Thyroxine (T4), Estimation of Serum Free Thyroxine (FT4) and Estimation of Serum Total Triiodothyronine (T3) and Free Triiodothyronine (FT3): Competitive chemiluminescence immunoassay.
3. Estimation of Fasting Serum Glucose Level: Glucose oxidase method.
4. Estimation of Fasting Serum Insulin Level: ELISA kit.
5. Estimation of Insulin Resistance (HOMA-IR): Calculated using the formula: HOMA = (Fasting serum insulin × Fasting serum glucose) / 405.
6. Estimation of Cholesterol, Triglycerides, HDL, LDL, and VLDL: Enzymatic assays.
7. Measurement of Body Mass Index (BMI): BMI was calculated using the formula: BMI = Weight / (Height)^2, and individuals were categorized as normal weight, overweight, or obese based on BMI values (Asil et al., 2014; Dewi et al., 2021).

2.5.Statistical Analysis

The present data was analyzed using Social Sciences (SPSS version 26). The results were presented as mean±SD, frequencies and percentages in appropriate tables and graphs. Independent t-test, and post hoc analysis were used where is appropriate to find out the possible association between the related variables of the current study as LSD. Tukey's method was used to calculate 95% confident interval, which used for either

pairwise comparison or comparing multiple groups. Besides, a statistical Pearson correlation test was also used to determine the relationship between the parameters under study. Statistical association was considered significant when p value equal or less than 0.05 or 0.01 (P value ≤ 0.05 , 0.01).

3. Results

3.1. Demographic Characteristics of Control and Patient Groups

The demographic data of the study's participant was presented in Table 1 and Fig.1A-E. For age, the difference between means is 1.360 ± 1.588 years with a non-significant p-value of 0.3932. interestingly, we observed a significant difference in weight (13.22 ± 2.470 kg, p < 0.0001), BMI (4.872 ± 0.9133 kg/m^2, p < 0.0001), systolic blood pressure (9.210 ± 2.361, p < 0.0001), and diastolic blood pressure (3.610 ± 1.152, p < 0.0021) between patient's population comparing to the healthy control population.

Table 1: Demographic Analyses of The Study Participants

Parameters	Difference between means ± SEM	P value
Age/Year	1.360 ± 1.588	0.3932
Control N (50)		
Patients N (100)		
Weight/Kg	13.22 ± 2.470	<0.0001
Control N (50)		
Patients N (100)		
BMI/(kg/m2)	4.872 ± 0.9133	<0.0001
Control N (50)		
Patients N (100)		
Systolic blood pressure SBP	9.210 ± 2.361	<0.0001
Control N (50)		
Patients N (100)		
Diastolic blood pressure DBP	3.610 ± 1.152	<0.0021
Control N (50)		
Patients N (100)		
Data was presented as mean±SEM, SEM refers to standard error of the mean, P<0.05 considered significant, BMI refers to body mass index.		

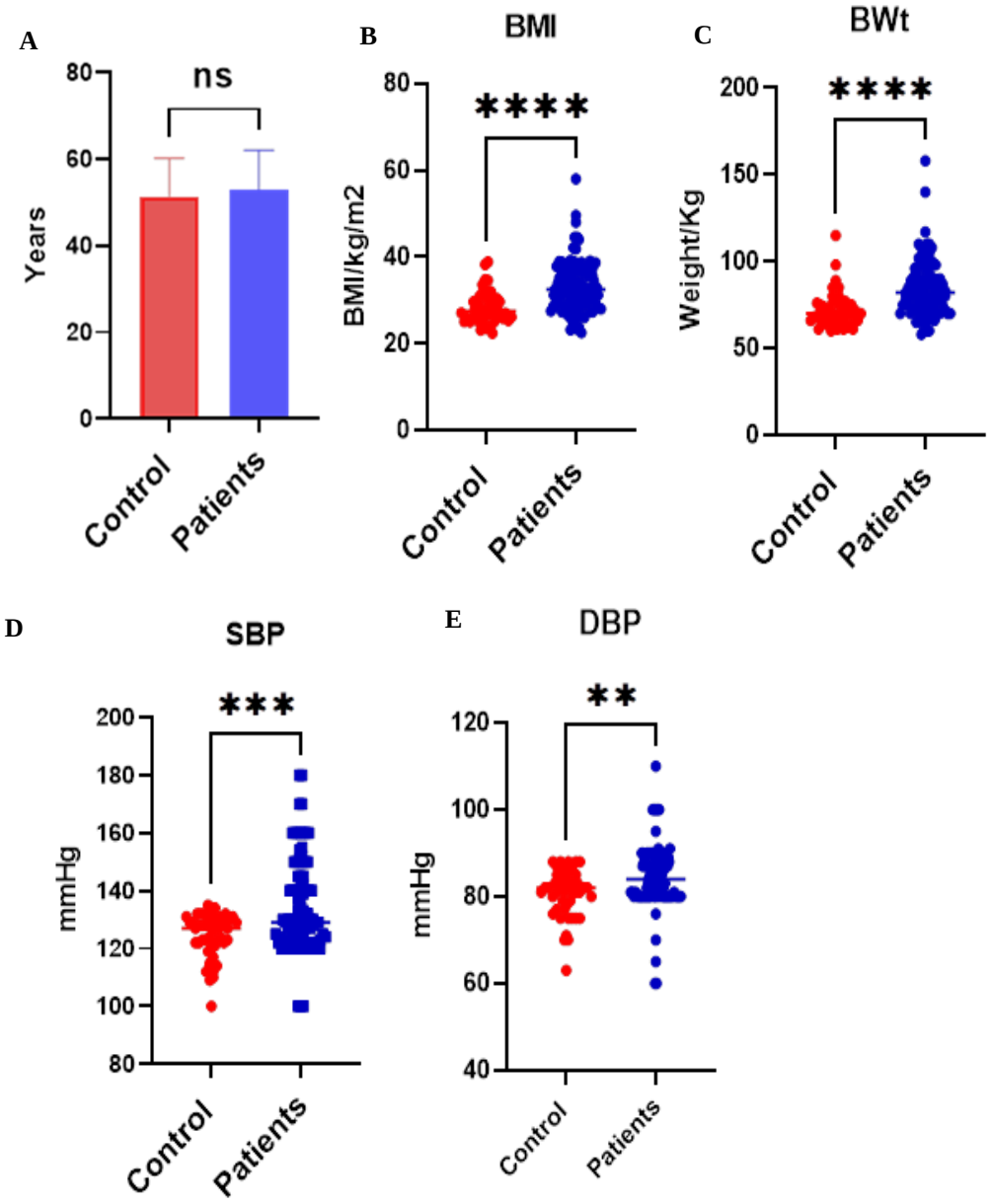

Figure 1: Demographic Analyses of The Study Participants. **A)** Refers to The Difference of The Age Means Between Control and Patients. **B)** Refers To Body Mass Index BMI. **C)** Demonstrates Differences of The Body Weight/Kg Means Between Control and Patients. **D)** Refers to The Differences of The Systolic Blood Pressure SBP Mmhg Between Control and Patients. **E)** Refers to The Differences of The Diastolic Blood Pressure SBP Mmhg Between Control and Patients. * P<0.01, ** P<0.001, *** P<0.0001, Ns=Non-Significant.

3.2. Analyzing of Age, BMI and Comorbidities in Patient and Healthy Control Groups

Table 2 compares different variables between control healthy people (N=50) and patients with hypothyroidism (N=100). it spans a broader age range in patients, up to 89 years, which may indicate disease prevalence or diagnoses in older age. Interestingly, hypothyroidism patients showed a significantly higher proportion of obese individuals (68%) compared to the control group (30%), and a higher prevalence of Diabetes Mellitus (DM) (37% in patients vs. 16% in controls). The majority of patents under the category of 1-6 years treatment, followed by 34% with 7-12 years, while 11% of the patients defined under the category of more than 12 years duration of treatment.

Table 2: Distribution of Variables in Control and Patient Groups

Parameters	Categories	Control N (50)	Patients N (100)
Age (Year), (N)%	40-49	(22), 44	(35), 35
	50-59	(16), 32	(38), 38
	60-69	(12), 24	(22), 22
	70-79	-	(4), 4
	80-89	-	(1), 1
BMI/ (kg/m^2), (N)%	Underweight	-	-
	Normal	(5), 10	(5), 5
	Overweight	(30), 60	(27), 27
	Obese	(15), 30	(68), 68
Duration of treatment (Year), (N)%	1-6	-	(55), 55
	7-12	-	(34), 34
	13-19	-	(6), 6
	20-25	-	(5), 5
DM, (N)%	Yes	(8), 16	(37), 37
	No	(42), 84	(63), 63
Data was presented as number of patients (N) and percentage.			

3.3. Analyzing Thyroid Hormones and Metabolic Markers in Patients and Healthy Control Population

Patients exhibit a marked increase in TSH (4.300 ± 4.708 μU/ml) compared to the control group (2.130 ± 0.8125 μU/ml, $p<0.0015$), indicating potential thyroid dysfunction. While FT3 levels is slightly elevated in patients (5.450±0.997 ng/ml) compared to controls (5.108±0.979 ng/ml), the differences are statistically significant ($p<0.0483$). FT4 is significantly lower in patients (13.38±2.283 pmol/L) than in controls (14.24±1.901 pmol/L, $p<0.0229$), indicating potential disruptions in free thyroxine regulation. Moreover,

patients exhibit higher insulin levels (16.90±9.827 µU/ml) and fasting plasma glucose (117.5±42.49 mg/dl, p<0.0001 and p<0.0044, respectively), suggesting impaired glucose metabolism. Surprisingly, the HOMA-IR index is significantly lower in patients (0.9815±3.186, p<0.0001), indicating potential compensator mechanisms or nuanced metabolic interactions as described in Table 3.

Table 3: Plasma Thyroid Hormone and Metabolic Markers in Patients and Control Groups**.**

Parameters	means±SD		P<0.05	95% CI
	Control N=50	Patients N=100		
TSH, µU/ml	2.130 ± 0.8125	4.300±4.708	<0.0015	0.8421 to 3.497
TT3, ng/ml	1.726± 0.4442	1.772±1.753	0.8546	-0.234 to -0.0214
FT3, pmol/L	5.108±0.979	5.450±0.997	0.0483	0.05292 to 0.6709
TT4, nmol/L	141.8±14.56	138.3±24.71	0.3480	-11.06 to 3.921
FT4, pmol/L	14.24±1.901	13.38±2.283	<0.0229	-1.280 to 0.3973
Insulin, µU/ml	9.023±4.019	16.90±9.827	<0.0001	5.012 to 10.74
FPG, mg/dl	99.70±12.86	117.5±42.49	<0.0044	5.639 to 29.96
HOMA-IR	2.120±5.166	0.9815±3.186	<0.0001	2.133 to 3.959

TSH=thyroid stimulating hormone, TT3=total thyroid hormone, FT3=free thyroid hormone, TT4=total thyroid hormone, FT4=free thyroid hormone, FPG=fasting plasma glucose. Data presented as mean±SD, P value≤0.05 considered significant.

3.4. Comparing Blood Lipid Profile in Patients and Healthy Control Participants

Table 4 represents a comprehensive of plasma lipid profiles between healthy control and hypothyroidism patients. Hypothyroidism patients exhibit substantially elevated total cholesterol (184.93±42.299 mg/dl) and triglyceride (141.91±63.952 mg/dl) levels compared to the control group (160.28±29.449 mg/dl and 111.22±34.797 mg/dl, respectively), indicating dyslipidemia and altered lipid metabolism in the patient cohort. Clearly, VLDL levels are significantly lower in patients (28.15±13.136 mg/dl) than in controls (33.81±11.799 mg/dl), suggesting potential differences in lipid composition or metabolism. While LDL levels are modestly elevated in patients (110.08±35.414 mg/dl) compared to controls (101.91±24.445 mg/dl), and HDL levels show no significant difference.

Table 4: Plasma Blood Lipid Profiles in Patients and Control Groups

Parameters	means±SD		P<0.05
	Control N=50	Patients N=100	
Cholesterol	160.28±29.449	184.93±42.299	0.001
TG	111.22±34.797	141.91±63.952	0.002
HDL	48.14 ±12.884	50.46±11.213	0.257
LDL	101.91±24.445	110.08±35.414	0.145
VLDL	33.81±11.799	28.15±13.136	0.011
Data presented as mean±SD, P value≤0.05 considered significant.			

3.5. Duration of L-Thyroxin Treatment

Table 5 shows the distribution of L-thyroxin treatment across different time intervals in hypothyroidism patients. The data shows that the majority of patients fall within the 1-5 years duration category, constituting 42% of the total. The second most prevalent duration range is 6-10 years, encompassing 44% of the patients. A smaller proportion of patients have been on L-thyroxin treatment for longer periods, with 8% in the 11-15 years range, and 3% each for 16-20 years and 21-25 years.

Table 5: Represents Duration of L-Thyroxin Treatment/Year

Duration of treatment/ (years)	Patients%
1-5	42
6-10	44
11-15	8
16-20	3
21-25	3
Data was presented as percentage.	

3.6. Plasma Hormone Levels Following L-Thyroxine Treatment

Table6 demonstrates the plasma hormone levels according to the duration of L-thyroxin treatment. The data represented as Mean ±SD for different hormones: TSH, TT3, FT3, TT4, and FT4. The data suggesting that the levels of all five hormones change over time with L thyroxin treatment. TSH levels decrease over time, while TT3, FT3, TT4, and FT4 levels increase over time. The changes in hormone levels are generally small, but they are statistically significant.

Table 6: Represents Plasma Hormone Levels According to the Duration of L Thyroxin Treatment, Mcg /Year

Duration of treatment (years)	TSH Mean ±SD	TT3 Mean ±SD	FT3 Mean ±SD	TT4 Mean ±SD	FT4 Mean ±SD
1-5	5.817±5.912	1.634±0.2191	5.475±0.8589	134±26.45	13.01±2.487
6-10	3.647±3.546	1.573±0.2129	1.573±0.2129	138.7±23.06	13.61±2.151
11-15	1.913±1.140	1.544±0.1111	5.621±0.9615	144.5±26.55	14.07±1.796
16-20	1.08±1.195	7.54±9.929	5.44±0.8073	146.5±11.48	12.86±1.985
21-25	2.223±1.348	1.477±0.125	5.293±0.7422	165.8±9.362	14.01±3.081
Data was presented as Mean ±SD, SD refers to standard deviation of the mean.					

3.7. Plasma TSH Levels Across L Thyroxin Treatment Durations

Table 7 demonstrates Long-term use of L-thyroxine led to a trend towards lower thyroid-stimulating hormone (TSH) levels, but statistically significant differences were only observed between individuals who had been on L-thyroxine for 6-10 years and those treated for 21-25 years; the latter group showed slightly lower levels. TSH levels in all other treatment time periods were comparable to those of individuals receiving treatment for 1-5 years. These results indicate that the observed decrease in TSH levels may not be consistent across treatment periods.

Table 7: Tukey's Multiple Comparisons Test of Plasma TSH Levels According to the Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
6-10 vs. 1-5	2.170	-0.5817 to 4.921	0.5212
11-15 vs. 1-5	3.904	-1.016 to 8.825	0.1721
16-20 vs. 1-5	4.737	-2.885 to 12.36	0.3372
21-25 vs. 1-5	3.594	-4.029 to 11.22	0.6945
11-15 vs. 6-10	1.734	-3.168 to 6.637	0.6470
16-20 vs. 6-10	2.567	-5.044 to 10.18	0.6868
21-25 vs. 6-10	1.424	-6.187 to 9.035	0.9501
16-20 vs. 11-15	0.8330	-7.802 to 9.468	0.9958
21-25 vs. 11-15	-0.3103	-8.946 to 8.325	>0.9999
21-25 vs. 16-20	-1.143	-11.56 to 9.271	0.9930
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.8.Plasma TT3 levels across L thyroxin treatment durations

Tukey's multiple comparisons test was run to analyze the values of plasma TT3 levels across L-thyroxin treatment/year. A comparison of various treatment duration groups is shown in each row, with the mean difference in TT3 levels, a 95% confidence interval, and an adjusted p-value provided. In some comparisons, such as 16–20 years vs. 1–5 years, statistically significant differences (adjusted p-value <0.05) are noted, indicating significant differences in TT3 levels between individuals with these particular durations of thyroxine treatment as described in the Table 8.

Table 8: Tukey's Multiple Comparisons Test of Plasma TT3 Levels According to The Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Adjusted P Value
6-10 vs. 1-5	-0.06085	-0.9339 to 0.8122	0.9997
11-15 vs. 1-5	-0.09006	-1.651 to 1.471	0.9998
16-20 vs. 1-5	5.906	3.488 to 8.325	<0.0001
21-25 vs. 1-5	-0.1571	-2.576 to 2.261	0.9998
11-15 vs. 6-10	-0.02920	-1.585 to 1.526	>0.9999
16-20 vs. 6-10	5.967	3.552 to 8.382	<0.0001
21-25 vs. 6-10	-0.09629	-2.511 to 2.319	>0.9999
16-20 vs. 11-15	5.996	3.256 to 8.736	<0.0001
21-25 vs. 11-15	-0.06708	-2.807 to 2.673	>0.9999
21-25 vs. 16-20	-6.063	-9.368 to -2.759	<0.0001
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.9.Plasma FT3 Levels Across L thyroxin Treatment Durations

Table 9 shows a significant variation in free triiodothyronine (FT3) levels were not observed between any groups when different L-thyroxine treatment durations (1–25 years) were analyzed, indicating that FT3 may not be significantly impacted by treatment duration, at least not during the investigated timeframe.

Table 9: Tukey's Multiple Comparisons Test of Plasma FT3 Levels According to The Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
6-10 vs. 1-5	3.902	3.508 to 4.297	<0.0001
11-15 vs. 1-5	-0.1460	-0.8513 to 0.5593	0.9783
16-20 vs. 1-5	0.03524	-1.057 to 1.128	>0.9999
21-25 vs. 1-5	0.1819	-0.9108 to 1.275	0.9904
11-15 vs. 6-10	-4.048	-4.751 to -3.346	<0.0001
16-20 vs. 6-10	-3.867	-4.958 to -2.776	<0.0001
21-25 vs. 6-10	-3.720	-4.811 to -2.629	<0.0001
16-20 vs. 11-15	0.1812	-1.057 to 1.419	0.9941
21-25 vs. 11-15	0.3279	-0.9099 to 1.566	0.9473
21-25 vs. 16-20	0.1467	-1.346 to 1.640	0.9988
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.10. Plasma TT4 Levels Across L-Thyroxin Treatment Durations

The plasma TT4 levels Tukey's multiple comparisons test in Table 10 reveals varying lengths of L thyroxin treatment per year. rows denote a distinct comparison between two duration groups and includes the adjusted p-value, the corresponding 95% confidence interval, and the mean difference in TT4 levels. Notably, after correcting for multiple comparisons, the adjusted p-values are used to assess whether the observed differences are statistically significant. Given that all adjusted p-values in this dataset are greater than 0.05, it is possible that there are no appreciable variations in mean TT4 levels amongst the treatment duration groups that are being compared. Comparing 16-20 years of treatment to 1-5 years revealed an average difference of -12.51 in plasma TT4 levels. However, the wide range of uncertainty (-53.29 to 28.26) and the high adjusted p-value (0.9122) suggest that this difference is not statistically meaningful.

Table10: Tukey's Multiple Comparisons Test of Plasma TT4 Levels According to The Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95% CI of diff.	Adjusted P Value
6-10 vs. 1-5	-4.702	-19.42 to 10.02	0.8997
11-15 vs. 1-5	-10.43	-36.75 to 15.89	0.8036
16-20 vs. 1-5	-12.51	-53.29 to 28.26	0.9122
21-25 vs. 1-5	-31.75	-72.52 to 9.028	0.2000
11-15 vs. 6-10	-5.731	-31.95 to 20.49	0.9733
16-20 vs. 6-10	-7.810	-48.52 to 32.90	0.9835
21-25 vs. 6-10	-27.04	-67.76 to 13.67	0.3506
16-20 vs. 11-15	-2.080	-48.27 to 44.11	>0.9999
21-25 vs. 11-15	-21.31	-67.50 to 24.88	0.7003
21-25 vs. 16-20	-19.23	-74.94 to 36.47	0.8712
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.11. Plasma FT4 Levels Across L-Thyroxin Treatment Durations

In most comparisons that represented in the Table 11 (1-5 years vs. 6-10, 11-15, 16-20), the length of L-thyroxine treatment did not significantly affect FT4 levels; however, potential trends of higher FT4 in the 21–25-year group and lower FT4 in the 16–20-year group compared to reference (1-5 years) call for additional research using larger datasets to confirm and explore influencing factors.

Table 11: Tukey's Multiple Comparisons Test of Plasma FT4 Levels According to The Duration of L Thyroxin Treatment/Year

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Adjusted P Value
6-10 vs. 1-5	-0.6024	-1.114 to 2.069	0.7428
11-15 vs. 1-5	-1.061	-3.646 to 2.047	0.7532
16-20 vs. 1-5	0.1476	-6.333 to 2.486	>0.9999
21-25 vs. 1-5	-0.9990	-1.813 to 7.006	0.9497
11-15 vs. 6-10	-0.4587	-4.113 to 1.559	0.9852
16-20 vs. 6-10	0.7500	-6.804 to 2.001	0.9821

21-25 vs. 6-10	-0.3967	-2.284 to 6.521	0.9984
16-20 vs. 11-15	1.209	-6.120 to 3.871	0.9367
21-25 vs. 11-15	0.06208	-1.600 to 8.391	>0.9999
21-25 vs. 16-20	-1.147	-1.505 to 10.54	0.9730
Mean Diff. refers to the differences between the two means, CI of diff refers to the confident interval of the mean. P<0.05 considered significant.			

3.12. Study the Correlation Between Thyroid Hormones and Metabolic Parameters

The heatmap in the Fig.4, display correlation coefficients and p-values for a range of treatment-related parameters. Among the noteworthy correlations is the one that shows a positive relationship between Insulin and Fasting Blood Glucose (FBG)—that is, higher Insulin levels are associated with higher FBG levels. Furthermore, there is a positive correlation between the length of treatment and the Body Mass Index (BMI), suggesting a relationship between higher BMI and longer treatment periods. On the other hand, a negative correlation has been observed between BMI and Thyroxine (TT4) levels, indicating a negative relationship between higher BMI and lower TT4 levels. Additionally, the data show a positive correlation between TT4 levels and treatment duration, suggesting that longer treatment may have an impact on higher levels of Thyroxine. Notably, there is no discernible relationship between age and treatment duration. The aforementioned results highlight the intricate connections between thyroid hormones, metabolic parameters, and treatment duration, underscoring the diverse range of clinical associations.

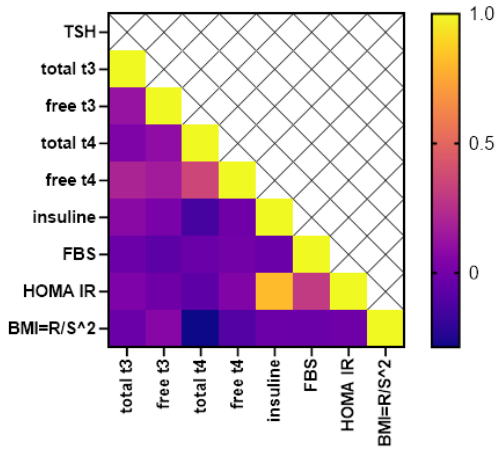

Figure 4: Heat Map Correlation Matrix of the Analysis of Health Parameters in Treatment: Thyroid Function, Insulin Levels, Blood Sugar, And BMI.

3.13. Study Treatment Related Correlations: Thyroid Hormones, Metabolic Factors and Duration in the Clinical Context

Table 12 presents the correlation coefficient (r values) and corresponding P values for various L-thyroxine treatment parameters, including TSH, FT4, Insulin, FBG, TT3, FT3 and TT4. The correlation coefficient (r) indicates the strength and direction of the relationship between two variables. The p-values help determine the statistical significance of these correlations. Clearly, we found a statistically significant positive correlation between BMI and the duration of treatment (r = 0.277, p = 0.005**), indicating that individuals with a longer treatment duration tend to have a higher BMI. Additionally, a significant negative correlation is observed between TT4 and BMI (r = -0.2865, p = 0.0039), suggesting that higher BMI is associated with lower levels of Thyroxine. The data also indicates significant positive correlations between Insulin and FBG, and a notable negative correlation between TT3 and FBG.

Table 12: Treatment-Related Correlations: TSH, Free T4, Insulin, FBS, BMI, Duration, and Age

Parameter	Value	FBG	Insulin	HOMAIR	BMI	Duration of treatment	Age
TSH	r	0.006	- 0.024	- 0.48	0.277	- 0.227	- 0.085
	P value	0.954	0.810	0.637	0.005**	0.023*	0.399
FT4	r	0.007	- 0.016	0.045	- 0.102	0.076	0.073
	P value	0.942	0.874	0.656	0.312	0.454	0.469
Insulin	r	-0.0321		0.812	-0.0257	-0.05789	0.0423
	P value	0.7509		0.0001***	0.7993	0.5672	0.6760
FBG	r		-0.0321	0.306	-0.0314	-0.1583	-0.01965
	P value		0.7509	0.002**	0.7564	0.1157	0.8461
TT3	r	-0.0273	0.07177	0.03486	-0.0305	0.1688	-0.0632
	P value	0.7868	0.4779	0.7306	0.7630	0.0431	0.5320
FT3	R	-0.07560	0.01750	-0.01412	0.06097	-0.00252	0.1153
	P value	0.4548	0.8628	0.8891	0.5468	0.9801	0.2534
TT4	R	-0.03350	-0.1444	-0.07232	-0.2865	0.2314	0.0122
	P value	0.7408	0.1517	0.4746	0.0039	0.0206	0.9034
r refers to the Pearson correlation coefficient, P<0.05 considered significant.							

4. Discussion

There were no statistical differences in age between patients with hypothyroidism and healthy control population (1.360 ± 1.588 years, p-value = 0.3932), but significant differences in weight (13.22 ± 2.470 kg, $p < 0.0001$), Body Mass Index (BMI) (4.872 ± 0.9133 kg/m², $p < 0.0001$), systolic blood pressure (9.210 ± 2.361, $p < 0.0001$), and diastolic blood pressure (3.610 ± 1.152, $p < 0.0021$). These results suggest that various factors such as lifestyle, diet, and genetics might contribute. Furthermore, we compared health parameters between a control group of healthy individuals (N=50) and patients with hypothyroidism (N=100). The hypothyroidism patients showed a wider age range up to 89 years, possibly indicating disease prevalence or diagnoses in older age. Interestingly, there is a significantly higher proportion of obese individuals (68%) in the hypothyroidism group compared to the control group (30%), and a higher prevalence of DM (37% in patients vs. 16% in controls. The majority of patients receive treatment for 1-6 years, then 34% receive treatment for 7–12 years, and 11% receive treatment for more than 12 years. These results point to possible connections between age, DM, and obesity and hypothyroidism; however, more investigation is required to validate these connections and fully comprehend their implications. These findings align with previous publications in which scientists suggested that the frequency and incidence of hypothyroidism rise with people aged, and with women experiencing a two-fold higher prevalence than men according to a population-based study in the Piedmont Region, Italy (Caputo et al., 2020). In general, there are a limited study that explored the effects of hypothyroidism on specific demographic groups (Kumar and Gupta, 2021; Natarajan and Prakash, 2015). Thus, the results of this study regarding the significant variations in blood pressure, BMI, and weight among patients with hypothyroidism may offer insightful new information to the field.

Hypothyroidism patients showed a significant difference in the biochemical parameters comparing to the control group. A significant increase in the plasma patients TSH comparing to the healthy control people, indicating potential thyroid dysfunction. Additionally, elevated levels of hypothyroidism patient's plasma FT3 plasma levels indicated hyperactivity of thyroid biological activity. Unlikely, FT4 was dramatically lower in the patients, signaling potential disruptions in free thyroxine regulation. The observed higher insulin levels and fasting plasma glucose in patients imply impaired glucose metabolism. Remarkably, patients have a lower Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index, suggesting compensatory pathways or complex metabolic interactions. Interestingly these findings agree with the other studies in which they reported that increase in TT3 and FT3 plasma levels in hypothyroidism patients. furthermore, there appears to be a strong positive relationship between serum TG and serum TSH/FT3-FT4, while FT4 showed a negative association (Forero-Saldarriaga et al., 2020; Huang et al., 2022; Paczkowska et al., 2020; Selmer et al., 2012). According to the American Thyroid Association, patients who are hypothyroid will have an

elevated T3 level. In some individuals with a low TSH, only the T3 is elevated and the FT4 or FT3 is normal (Forero-Saldarriaga, Puerta-Rojas and Correa-Parra, 2020; Paczkowska et al., 2020).Recently, scientists have found that children in the upper baseline TSH tertile showed higher concentrations of FT3, FT3/FT4 ratio, and TSH, which agree with our data for increased TSH and FT3 levels in patients with hypothyroidism (Carreras-Badosa et al., 2023). These results highlight the intricate relationship between thyroid function and metabolic parameters and hypothyroidism. Contextual factors, such as genetic and lifestyle factors, are important, though, and drawing conclusions about causality based only on correlations found in this study should be done with caution. However, the findings of this study suggest a link between higher insulin levels, elevated fasting plasma glucose, and impaired glucose metabolism in patients, and also indicates signs of potential compensatory mechanisms through a lower HOMA-IR index. These findings are novel and provide significant value in increasing our knowledge of this area. Patients with hypothyroidism showed dramatic elevated of total cholesterol and triglyceride levels compared to the control group, indicating dyslipidemia and altered lipid metabolism. Interestingly, VLDL levels are significantly lower in patients than in controls, suggesting potential differences in lipid composition or metabolism. LDL levels are modestly elevated in patients compared to controls, while HDL levels show no significant difference. These findings align with recent studies in which scientists reported that hypothyroidism significantly associated with blood lipid alterations mainly concerning total cholesterol and LDL (Paczkowska et al., 2020; Ramulu et al., 2016). Additionally, these data agree with previous studies suggested that perturbations in the actions of T3 and T4 influence the normal metabolic pathways (Duntas and Brenta, 2018; Tan et al., 1998). Recently, scientists have found that TSH was high in 5.2% of the 49.5% of patients who were diagnosed with hyperlipidemia, which strongly agree with the findings of this current study (Iqbal et al., 2022a, 2022b, 2021). Hence, these data represent valuable insight in to the relationship between hypothyroidism and lipid profiles.

A trend toward reduced plasma TSH levels with L-thyroxine use. Those who received treatment for 6-10 years and those who received treatment for 21-25 years showed statistically significant differences, with the latter having slightly lower TSH levels. However, TSH levels in other treatment periods were similar to those who received treatment for 1-5 years, indicating that the observed decrease over time is not consistent across treatment periods. Similarly, scientists suggested that Scientists recognized the crucial role of TSH in FT3 conversion, and more studies have shown that temporarily stopping oral levothyroxine increases plasma TSH levels and alters FT3/FT4 ratios significantly within three days (Carlwe et al., 2013; Duntas and Jonklaas, 2019). This suggests a direct correlation between TSH levels and the conversion of FT3 and FT4. The lower TSH levels observed in hypothyroidism patients undergoing therapy across treatment duration may be attributed to the TSH-lowering effect of the treatment.

Plasma TT3 levels are examined across L-thyroxine treatment durations using Tukey's multiple comparisons test. The results show statistically significant differences in some comparisons, such as 16–20 years vs. 1–5 years, indicating significant variations in TT3 levels for these particular treatment durations as described previously. The FT3 levels for various L-thyroxine treatment durations (1–25 years) and does not reveal any significant variation between groups. This suggests that FT3 may not be significantly affected by treatment duration within the investigated timeframe. Multiple comparison test indicates that plasma TT4 levels did not change statistically among the treatment duration groups that were being compared. Not surprisingly, these data agree with various studies, firstly, scientists have explored the effects of thyroid hormone therapy on the level of quality of life and thyroid related symptoms in patients with hypothyroidism, with intervention duration ranging from 3 to 18 months. More studies investigated the duration of L-thyroxine therapy and its impact on thyroid function status and metabolic pathways aiming to optimize levothyroxine therapy (Emerson, 2018; Feller et al., 2018; Hennessey, 2017; Johnson, 2019; Skelin et al., 2018).

5. Conclusion

Our study reveals that a significant difference between patients with hypothyroidism and healthy controls, indicating potential lifestyle and genetic influences. patients with hypothyroidism showed disrupted thyroid function, with elevated plasma TSH and FT3 levels, and lower FT4 levels, alongside dyslipidemia characterized by increased total cholesterol and triglycerides. Trends in plasma TSH levels during L-thyroxine therapy suggest treatment's impact on thyroid hormone regulation. While significant variations occur in plasma TT3 levels across treatment durations, FT3 and TT4 levels remain relatively stable. Hence these data unmask the complexity between hypothyroidism, metabolic pathways and treatment outcomes, necessitating for further research for optimizing treatment strategies and improve patient outcomes.

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Microsponges: An Innovative Instrument in Pharmaceutical Research

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Abstract

A microsponge delivery system is a novel and distinctive method of administering medications organizationally. The use of microsponge medication delivery enables efficient and prompt regulation of drug administration.

Microsponge delivery system consists of porous microspheres that vary in sphere size range (5 to 300 µm). These microspheres have a significant porous frame and a small spherical form. Microneedle drug delivery systems are typically used for drug administration via the skin, although they lately show potential for drug transfer through the mouth, eyes, and injection routes. Microsponge delivery systems can readily alter the pharmacological release pattern and enhance the stability of the formulation while simultaneously reducing the adverse effects of the medicine.

The primary objective of microsponge medication delivery is to achieve the greatest peak plasma concentration in the bloodstream. The most notable characteristic of Microsponge delivery systems is their inherent ability to self-sterilize.

In conclusion, the microsponge delivery system has been extensively studied and shown to have antiallergic, antimutagenic, and nonirritating properties. This study covers the formulation, criteria for medication inclusion in microsponge delivery systems, formulation techniques, evaluation parameters, and the function of microsponge delivery systems in treating different illnesses. This study will be valuable for examining the usage of microsponge delivery systems in various diseases.

الاسفنج المايكروي: أداة مبتكرة في البحوث الصيدلانية

مصطفى رعد عبد الباقي ، فرقان محمد عبدالاله

الخلاصة

المقدمة: يعد نظام توصيل الادوية باستخدام الاسفنج المايكروي طريقة جديدة ومميزة لإعطاء الأدوية بطريقة منتظمة. إن استخدام الاسفنج المايكروي لتوصيل الدواء يتيح التنظيم الفعال والسريع لإدارة الدواء.

يتكون نظام التوصيل الاسفنج المايكروي من كريات مجهرية مسامية تختلف في نطاق حجم الكرة (5 إلى 300 ميكرومتر). تحتوي هذه الكرات المجهرية على إطار مسامي كبير وشكل كروي صغير. تُستخدم أنظمة توصيل الدواء باستخدام الإبر الدقيقة عادةً لإعطاء الدواء عبر الجلد، على الرغم من أنها أظهرت مؤخرًا إمكانية نقل الدواء عبر الفم والعينين وطرق الحقن. يتمتع نظام التوصيل الاسفنج المايكروي بالقدرة على تغيير نمط الإطلاق الدوائي بسهولة وتعزيز استقرار التركيبة، مع تقليل الآثار الضارة للدواء في نفس الوقت.

الهدف الأساسي من توصيل الأدوية باستخدام الإسفنج المايكروي هو تحقيق أعلى تركيز في البلازما في مجرى الدم. الميزة الأكثر بروزًا لنظام التوصيل الاسفنج المايكروي هي قدرتها الكامنة على التعقيم الذاتي.

في الختام، تمت دراسة نظام توصيل الاسفنج المايكروي على نطاق واسع وأظهر أنه يتمتع بخصائص مضادة للحساسية ومضادة للطفرات وغير مهيجة. تغطي هذه الدراسة التركيبة، ومعايير إدراج الدواء في نظام توصيل الاسفنج المايكروي، وتقنيات التركيب، ومعايير التقييم، ووظيفة نظام توصيل الاسفنج المايكروي في علاج الأمراض المختلفة. ستكون هذه الدراسة ذات قيمة كبيرة في المستقبل لفحص استخدام نظام توصيل الاسفنج المايكروي في أمراض مختلفة.

1. Introduction

Medication delivery systems with precise control over release rates and targeted medication distribution to particular body areas significantly influence the healthcare system. Traditional formulations of topical medications target the superficial skin layers. Consequently, upon application, they discharge their active components and provide a concentrated amount of active drug ingredient that is promptly assimilated (Cheng et al., 2023). Nevertheless, they result in an extreme buildup of substances inside the outermost layer of the skin and the layer underneath it. Hence, it is necessary to possess a system that prolongs the active component's duration on the skin's surface or inside the epidermis.

Meanwhile, the system must reduce the permeation of active ingredients through the skin and into the body (Raina et al., 2023). A novel medication delivery method known as "Microsponge" was recently identified. The Microsponge Delivery System is a proprietary polymeric system composed of porous microspheres that are extensively cross-linked. These particles are tiny, spongy spheres containing many interconnected empty spaces inside a non-collapsible framework (Biharee et al., 2023). This expansive permeable surface regulates the rates at which the active substance is released. The diameter of microsponges ranges between 5 and 300 μm, and distinctive spheres with a diameter of 25 μm may include as many as 250,000 holes. This leads to a substantial reservoir filled with the active agent up to its maximum capacity. The substance can encapsulate various active substances, including emollients, perfumes, sunscreens, essential oils, and anti-fungal, anti-infective, and anti-inflammatory compounds. It is used as a carrier system for topical application (Wani et al., 2022).

The microsponge technology was developed by Won in 1987, and the earliest patents were granted to Advanced Polymer Systems, Inc. This Company created many versions of the technique and applied them to various cosmetic, over-the-counter (OTC), and prescription pharmaceutical products. Cardinal Health, Inc. has acquired a license to use this fascinating technology to develop topical therapies (Kumar and Kumar Kataria, 2022).

2. Advantages of Microsponges Compared to Other Formulations

Microsponges provide several benefits in contrast to alternative preparations now available on the market.

2.1.Advantages Compared to Traditional Formulations

Conventional formulations of topical drugs are specifically formulated to target the outermost layers of the skin. Upon application, these products release their active components. The product offers a high concentration of active ingredients that are rapidly assimilated. This leads to an excessive buildup of components in both the epidermis and the dermis (Supe and Takudage, 2021). Microsponges can prevent this from happening. The microsponge system has the potential to effectively reduce the adverse effects of drugs, such as irritation, without compromising their effectiveness. This is achieved by gradually releasing the active component to the skin, similar to the microsponge Benzoyl peroxide preparations that demonstrate high effectiveness with low irritation (Nora Zawar. Yousif and Zeina D Salman, 2023).

2.2.Advantages in Comparison to Liposomes and Microencapsulation

Microsponges offer superior features compared to other technologies, such as microencapsulation and liposomes. Controlling the rate of actives' release is often challenging with microcapsules. The encapsulated actives will be released upon the rupture of the microcapsule wall. Liposomes are characterized by their restricted capacity, challenging formulation, limited chemical stability, and susceptibility to microbial contamination (Antonijoan et al., 2007; Biharee et al., 2023).

2.3.Advantages in Comparison to Ointments

Patient adherence to ointment is diminished due to its visually unappealing, thick, and oily consistency. Ointments have limited efficacy as drug delivery methods, hence inducing irritation and sensitization due to the need for a large quantity of active drug ingredients for optimal therapeutic outcomes. Another disadvantage of topical preparations is the unpleasant smell, unregulated evaporation of the active component, and the possibility of medications being incompatible with the vehicles used. On the other hand, the microsponge system prolongs the duration in which an active ingredient remains either inside the epidermis or on the skin surface (Rahman et al., 2022).

3. The Benefits of the Microsponge Medication Delivery Method Can Be Summarized as Follows (Shailaja and Ashok, 2022; Tiwari et al., 2022)

1. It provides uninterrupted activity and sustained release for 12 hours.
2. Improves the overall functionality and effectiveness of the product.
3. Reduces discomfort and enhances patient adherence.
4. Enhances the product's elegance.
5. It can be integrated into many compositions.
6. Exhibits excellent thermal, physical, and chemical stability.
7. This substance does not cause irritation, mutations, toxicity, or allergies.
8. Permits the incorporation of immiscible substances.
9. Transforms liquids into solid particles to enhance the management of substances.
10. Enhances the absorption and availability of drugs.
11. Enhances therapeutic efficacy.
12. It can absorb skin secretions up to 6 times its weight without changing its outward appearance, reducing skin oiliness.

4. Required Characteristics for Microsponge Drug Delivery Systems

(Borawake et al., 2021; Choudhary and Akhtar, 2022)**:**

1. Exhibit a pH range of 1 to 11 with consistent stability.
2. Exhibit thermal stability up to 130°C.
3. These products possess self-sterilization capabilities due to their pore size of 0.25μm, which effectively blocks the entry of germs. Consequently, there is no need to add any preservatives to these products.
4. Possess a substantial loading capacity, often ranged 50% - 60%.
5. This product has excellent fluidity and is cost-effective.
6. Provide excellent compatibility with various vehicles and substances.

5. Properties of Materials Suitable for Encapsulation in Microsponges (Mandal et al., 2024; Yehia et al., 2022)

The microsponge technology allows for the encapsulation of active chemicals, enabling their incorporation into various products like powders, creams, lotions, gels, and soaps.

Material intended for entrapment in microsponges must meet certain conditions, including:

1. It should have low water miscibility or limited solubility.
2. It must exhibit inertness towards monomers.
3. The formulation mustn't increase mixture viscosity.
4. It must not induce the collapse of the spherical architecture of the microsponges.

5. The substance should have complete miscibility with the monomer. It should also have the ability to enhance its miscibility by adding a tiny quantity of an immiscible solvent, such as water.
6. Resistant to polymerization of catalysts and circumstances.
7. The materials confined in the vehicle must have limited solubility to prevent issues in cosmetic preparations. If solubility is not limited, the cars may absorb micro sponges before the application.
8. The optimization of microsponge capacity and polymer design is necessary to achieve the required release rate over a specific period.

6. Techniques for the Production of Microsponges

The microsponge drug delivery device may be manufactured using two methods. The procedure may be either a single-step liquid-liquid suspension polymerization or a two-step procedure of the quasi-emulsion solvent diffusion approach. The choice between the two methods depends on the medicine's physico-chemical characteristics that must be loaded.

6.1.Liquid-Liquid Suspension Polymerization

The microsponges are produced using a one-step process called liquid-liquid suspension polymerization, as illustrated in Fig.1. This approach involves dissolving the monomers, together with the active medication (which is non-polar), in a suitable solvent for the monomer. The resulting mixture is then dispersed in an aqueous phase with simultaneous agitation. To create a suspension, suspending agents and surfactants are introduced into the aqueous phase. The suspension is made by forming droplets of a specific size, which are then subjected to polymerization by adding a catalyst or raising the temperature. A polymerization-induced reservoir system that opens at the surface via pores. A non-reactive liquid that does not mix with water but mixes with the monomer is used to create a network of pores. Once polymerization is finished, the liquid is filtered from the microsponge and infused into a premade microsponge. Afterward, different active substances are incorporated into the microsponge, serving as topical application carriers. Solvents might be used to facilitate the prompt and effective incorporation of functional compounds. Two-step methods are employed, and polymerization is conducted using porogen, which the functional group replaces if the medicine is sensitive to polymerization (Jyoti and Kumar, 2018; Shukla and Niranjan, 2022).

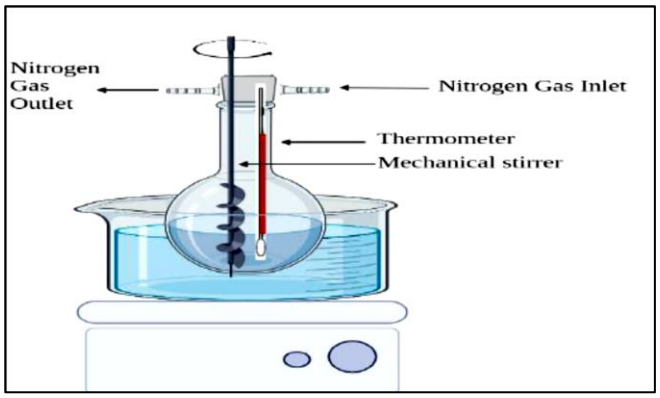

Figure 1: Liquid-Liquid Suspension Polymerization Method for Microsponge Preparation (Mantry Et Al., 2022)

6.2. Microsponges May be Formed Using a Quasi-Emulsion Solvent Diffusion Procedure Involving

Several polymers. Two stages are involved in this process: an inner phase and an exterior phase. Eudragit type RS 100, located within the inner phase, was liquified in ethyl alcohol. Then, the drug is incorporated into the solution and dissolved using ultrasonication at a temperature of 35°C. The PVA solution is combined with water in the outer phase. An inner phase was then transferred into the outer phase and stirred for 60 minutes. After the stirring operation, the solution was filtered to separate the microsponge. The microsponges are then dehydrated in a high-temperature oven at 40°C for 12 hours, after which their weight is determined as described in Fig.2. (Pokharel et al., 2023).

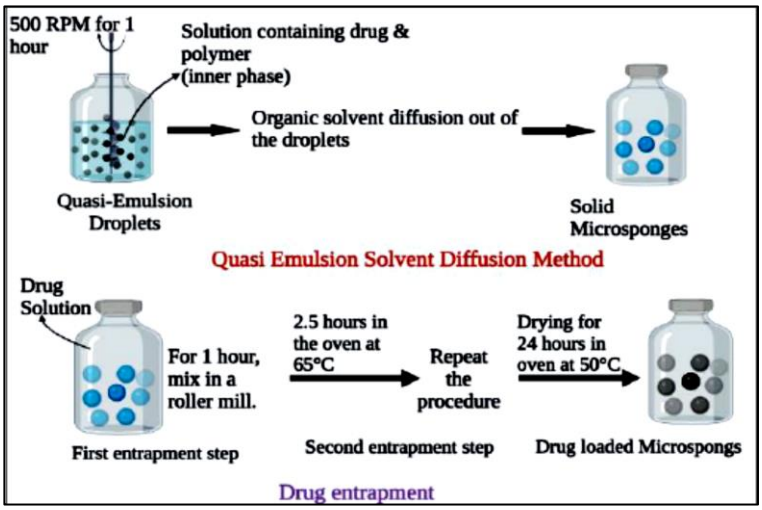

Figure 2: Quasi-Emulsion Solvent Diffusion Method for Microsponge Preparation (Wani et al., 2022)

7. Assessment of Microsponge

Particle size determination: the upper limit for the particle size of microsponge spheres is 30 µm, but the typical range falls between 10-25 µm. The particle size of the microsponge is determined using laser light diffraction or a similar technique, whether it is loaded or empty. Larger particles, measuring 30 µm in diameter, result in a gritty texture. Therefore, utilizing smaller particles, measuring 10-25 µm in diameter, in topical formulations is preferable.

1. Morphological examination of microsponges (Surface topography) to depict the microsponge's internal structure and scanning electron microscopy (SEM) may be used to examine a shattered microsponge particle.
2. The actual density of microsponges may be measured by using an ultra-pycnometer with helium gas and computing the mean of many observations.
3. Compatibility testing may be performed using several methods, including thin layer chromatography (TLC), powder X-ray diffraction (XRD), Fourier Transform infrared spectroscopy (FT-IR), and Differential Scanning Colorimetry (DSC).
4. The composition of the polymer and monomer in the microsponge may influence the drug's partition coefficient inside the microsponge system. Consequently, this directly impacts the rate at which the medicine is released from the carrier.

5. A porosity study may be conducted to analyze the pore structure and assess the dimensions of nanocavities formed in molecular dynamics simulations. A mercury intrusion porosimetry device may determine the pore structure, diameter, and volume.
6. The resiliency of microsponges may be analyzed and improved by examining how they release substances over time based on their cross-linking characteristics.
7. Evaluation of stability: the stability examinations comply with the standards established by the International Council of Harmonization (ICH).
8. Studies on dissolving were conducted in a controlled laboratory setting. The process of in vitro dissolution may be performed using the USP XXIII method. This involves using a dissolving assembly that contains 900 mL of saline phosphate buffer solution with a certain pH. The solution is heated to a temperature of 375 °C and spun at a predetermined rotation rate per minute.
9. Study of the release and deposition of drugs from microspheres. The Franz cell oriented with static diffusion is often used to facilitate the release of medication from the semisolid dosage form. This technique involves subjecting the outside layer of the skin, known as the epidermis, to a suitable temperature. In contrast, the inner layer of the skin, known as the dermis, is exposed to the receptor part of the solution, while the receptor compartment is filled with 20 mL buffer media. The buffer medium should be agitated at 600 rotations per minute and kept at 32 ±0.5 °C. Subsequently, the skin was immersed in the diffusion medium for 1 hour before the test sample was applied. A certain quantity of the sample is placed in the donor compartment. Diffusion cells evaluate the amount of medication applied externally and then analyze it at specific intervals. Afterward, the skin is meticulously detached, and the drug-affected region of the skin is purified with distilled water (Pethappachetty et al., 2022; Shirodkar and Pissurlenkar, 2022)[A1] [u2].

8. Applications of Microsponge

8.1.Microsponge Technology Has Applications in The Field of Cosmetics

Microsponge technology in oral cosmetics is particularly fascinating since it allows for the controlled release of volatile compounds, prolonging the sensation of freshness. Microsponges or volatile chemicals may be readily included in dental pastes or mouthwashes. Microsponge can prolong the lifespan of various colored cosmetic products, such as rouge or lipsticks, by capturing and retaining the color inside the microsponge. Microsponge facilitates the breakdown of homogeneity and enhances the coverage capacity. Therefore, the cosmetics created with microsponge will be gorgeous (Dutta et al., 2022).

8.2.Drug Delivery Systems Using Microsponges For Controlled Release

Microsponge delivery methods are being investigated because of the enhanced efficacy of medications, higher safety, and improved aesthetic appearance of formulae used in topical treatments, OTC pharmaceuticals, and personal care products. This delivery strategy is specifically developed to gradually release active moiety over a prolonged period, minimizing adverse effects while retaining therapeutic effectiveness. They have several applications. Primarily, it is used for topical applications, but it has also been lately exploited for administering drugs orally. It provides a formulator with many options for creating pharmaceutical or cosmetic products. These products are designed to effectively distribute the active ingredient with little dose, enhancing product stability, minimizing side effects, and adjusting medication release (Patil et al., 2022).

To provide treatments, the microsponge delivery method combines over-the-counter goods, such as various moisturizers, sunscreens, and particular rejuvenating things. Microsponge, as a delivery tool, can be used for topical administration in three different manners in items that are now being developed or are already available in social pharmacies:

1. The reservoir of the microsponge sphere releases active moieties over an extended period.
2. To serve as a receptacle for absorbing undesirable substances, such as surplus skin oils.

3. Microsponge spheres are enclosed containers that effectively isolate the components from direct contact with the skin, allowing them to operate on the surface.

Microporous drug delivery systems are used for topical application and recreational administration of bioactive substances. Microsponge aims to optimize medication delivery by minimizing the required dosage, enhancing product stability, minimizing side effects, and modifying drug release (Khattab and Nattouf, 2022).

8.3.Utilization of Microsponge for Transdermal Administration

Traditional topical medication formulations are believed to affect the superficial layers of the skin primarily. When applied to the skin, these conventional products release their pharmacologically active medication, forming a highly concentrated film of pharmacologically active drug that is quickly absorbed. To prevent the accumulation of excessive active components within the epidermis and dermis layers of skin, it is possible to use a microsponge drug delivery tool to package the medication. Microsponge technology may significantly reduce medication irritation while still preserving its efficacy. The porous structured microsponge can also be formulated into various topical dosage forms, including gels, creams, lotions, and ointments (Patole et al., 2023).

8.4.Oral Drug Delivery Using Microsponges

Although the oral route offers convenience and security, it is not universally acceptable for all delivered medications. This includes medications with a brief half-life that is rapidly excreted from the body, medicines metabolized by the acidic conditions of the stomach or bile produced in the intestine, or medications that need delivery to the colon to treat certain medical disorders (Sen et al., 2023). Consequently, this resulted in the development of methods for controlling the dispensation of the drug. Microsponges were created as pharmaceuticals, providing notable advantages compared to other delivery methods, such as nanoparticles, microspheres, and liposomes. Microsponges possess a porous configuration that enables effective colon targeting in conjunction with their extended delay period. This is accomplished by protecting the medicine from the acidic environment of the stomach, and the release of bile in the small intestine, and the drug is only released when the pH level in the colon is attained (Gade et al., 2022; Sammour et al., 2023).

8.5.Ocular Microsponge Drug Delivery

Pharmaceuticals that dissolve in water may be used as ointments or suspensions applied directly to the skin. On the other hand, drugs that do not dissolve in water can also be used externally as ointments or suspensions in water. Pharmacokinetics pertains to the complex mechanism via which drugs are absorbed and distributed throughout the eye. Subsequently, the medicine is transported into the front part of the eye by crossing the barrier between the blood and the fluid in the eye. The medicine is conveyed from the anterior segment of the eye to the Schlemm's canal and trabecular meshwork, where the replenishment of the intraocular fluid eliminates it. The medicine is eliminated from the aqueous humor and enters the systemic circulation by crossing the blood-aqueous barrier. Ultimately, a pharmaceutical component circulating in the body traverses the blood-retina barrier and arrives in the eye's posterior chamber (Dhyani and Kumar, 2022).

8.6.Cutaneous Administration of Medication Via Microsponge

The microsponge medicine delivery technology functions as a foundation for many topical formulations. The microsponge consists of polymers that can bind or retain a substantial quantity of medicine. This is attributed to its porous structure, with a pore count of around 250,000 pores per milliliter/gram. This leads to its superior capacity to transport drugs compared to alternative drug delivery systems, particularly in topical formulations, since it prolongs the duration of drug presence in the dermis or epidermis region. This facilitates reducing its lengthy use, frequency, absorption into the bloodstream, and its adverse effects on the skin, such as eczema, hypersensitivity, and rash. An alternative method to mitigate these adverse reactions is using a suitable, physiologically inert polymer that eludes recognition by the body as an external entity. The polymers

must possess non-irritation, non-mutagenicity, non-allergenicity, and non-toxicity (Dhyani and Kumar, 2024).

8.7.Microsponge Is Used in The Field of Tissue and Bone Engineering.

The admixture of liquid methyl methacrylate monomer, polymethyl methacrylate powder, tricalcium phosphate grains, and calcium-deficient hydroxyapatite powders in water led to the creation of bone-like substances that closely resembled natural bone. The final composites displayed holes and had a resemblance to microsponges. Upon the breakdown of the collagen sponge sheet, the essential fibroblast growth factor (bFGF) was released into the subcutaneous tissue of the mouse animal (Ma et al., 2022). The bFGF exhibited localized angiogenic activity in a manner that was dependent on the dosage. Collagen microsponges containing bFGF were injected into a blood-deprived animal's hind leg. This resulted in a substantial augmentation in blood circulation, a feat that could not have been accomplished by administering the bFGF bolus. The findings illustrate the importance of using type I collagen as a source of bFGF. A novel biodegradable graft material, including collagen microsponges, has been created for cardiovascular tissue grafting, aiming to enhance tissue regeneration. A novel patch, consisting of a biodegradable polymer and collagen microsponge, has been made to mend the circulatory system. This patch can function as an innovative surgical material (Bhattacharjee et al., 2014).

9. Factors Must Be Taken into Account While Formulating A Microsponge Product

(Bhattacharjee et al., 2014; Jayasawal et al., 2022; Zhang et al., 2021)

1. The microsponge drug delivery system can dispense medications in many forms, such as lotions, creams, soaps, and powders. Several factors are considered to get the desired product characteristics throughout the vehicle development process. The solubility of the active substances in the vehicle should be reduced to a minimum. Failure to meet this requirement will decrease the vehicle's microsponge drug delivery activity and reduce cosmetic problems; the automobile should not include more than 10-12% w/w of microsponge medicine delivery system. The polymer designs and payloads for the active microsponge system must be adjusted to achieve the desired release rate throughout the defined duration. When constructing vehicles for a given use, it is essential to consider certain factors to achieve the desired product features.
2. To prevent cosmetic problems, the amount of powder loaded into the automobile must be enough but not exceed 10-12%. To preserve the integrity of the polymer, limiting the solubility of the active ingredient in the vehicle is necessary, thereby preventing the skin from degrading before delivery. To achieve the desired gradual release of the active component within the specified timeframe, it is essential to accurately determine the polymer composition and loading quantities.

10. Factors Related to Ensuring Safety

Microsponge systems comprise commonly used polymers containing minimal amounts of prominent monomers. Furthermore, their cross-linking makes them biologically inactive since the body cannot break them down or replicate them into other molecules.

These macroscopic particles are of significant size and may penetrate the outermost layer of the skin due to their dimensions. They are located under the epidermis and release their substance gradually over time. This release pattern helps reduce the buildup of active substances on the skin's surface, which may improve the safety of topical treatments (Aguero et al., 2021; Gusai et al., 2021).

11. Recent Developments in Microsponge Formulation

Technological advancements have been achieved by modifying procedures used to produce nanosponges and porous micro pellets. An example is the manufacturing of nanosponges using Cyclodextrin (CD) to deliver

drugs. These sophisticated drug delivery systems are used for administering several medications, including flurbiprofen, dexamethasone, doxorubicin hydrochloride, and itraconazole (Ghourab et al., 2020; Kaur et al., 2015). The β-CD molecule is cross-linked by a reaction with biphenyl carbonate, creating nanosponges as a sophisticated formulation. Scientists have noted that including a cytotoxic component in the formulation as a carrier, system may enhance the effectiveness of the medicine. Cancer targeting and gas delivery systems were also approaches that can be utilized using microsponge (Jain et al., 2020).

The latest advancement in the field focuses on the creation and laboratory analysis of betamethasone microsponges incorporated into a gel for topical applications to reduce inflammation. The use of the Quasi-emulsion technique achieves this. A recent study has developed a microsponge formulation that contains controlled-release risperidone, which is used for treating schizophrenia and schizoaffective disorders (Rahman et al., 2022).

Microsponges were effectively synthesized using the quasi-emulsion solvent diffusion technique, employing Eudragit Rs 100 and PVA with distilled water and the liquid-liquid suspension polymerization method. These substances possess the capacity for increased stability, better ability to be formulated in various ways, and decreased occurrence of adverse reactions while preserving their effectiveness in treating medical conditions (Choudhary and Akhtar, 2022; Jayasawal et al., 2022; Wani et al., 2022).

12. Conclusive Results

The market for microsponge technology has great potential due to the need for innovative and highly effective medicinal and cosmetic products. The microsponge delivery technology has excellent potential for the accurate and controlled release of an encapsulated active chemical. This approach has the advantage of reducing the adverse effects of pharmaceuticals while ensuring treatment effectiveness. Furthermore, it demonstrated significant improvements in the stability of the formulation, as well as an increase in the refinement and flexibility of the formulation. Multiple investigations have shown they are also considered harmless, non-allergenic, and non-mutagenic. This drug delivery method is primarily used in over-the-counter (OTC) skincare products, prescription drugs, cosmetics, and sunscreens. Owing to its multiple applications in pharmaceutical administration, this technology demonstrates tremendous promise and is projected to undergo intense attention in the following years via many research programs.

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Molecular Docking Study of Five Novel 1,2,3-Triazole Linked Metronidazole Derivatives as Cytotoxic Agents

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Abstract

Background: Five novel synthetic 1,2,3-triazole-linked metronidazole compounds that target the tyrosine kinase of the epidermal growth factor receptor belong to the ErbB receptor family, which includes Her1 (EGFR), Her2 (erb-B2), Her3 (erb-B3), and Her4 (erb-B4). Certain human carcinomas, such as lung and breast cancer, feature cells that overexpress EGFR. This causes the anti-apoptotic Ras signaling cascade to be incorrectly activated, resulting in uncontrolled cell growth. Inhibiting EGFR TK (PDB code: 1M17) is an important target in the development of anticancer drugs since it can help prevent tumor growth and metastasis.

Materials and Methods: Using the molecular operating environment to evaluate the binding affinity of new design compounds against targeting proteins (EGFRTK). The molecular docking process predicts how molecules interact with the target enzyme, and criteria such as S-score and RMSD evaluate the docking outcomes by comparing estimated and experimental structures. It is an extremely useful tool for drug discovery and studying molecular interactions.

Results: The newly synthesized compounds (I-V) demonstrated improved binding energy (S.score) ranging from -7.5733 to -8.4456 Kcal/mol and reduced rmsd values ranging from 0.8752 to 1.6182 with the enzyme active site, as compared to erlotinib's binding energy of -7.7359 Kcal/mol and rmsd value of 1.720.

Conclusion: The docking results demonstrated that all synthesized compounds (I-V) had higher energy of binding (S-score) and lower Root Mean Square Deviation (RMSD) values, indicating theoretical potential as effective EGFR inhibitors when compared to the reference ligand (erlotinib)

دراسة الإرساء الجزئي لمشتقات جديدة من ١,٢,٣- تريازول المرتبطة بالميترونيدازول كمركبات مضادة للسرطان

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الخلاصة

المقدمة: تنتمي خمس مركبات جديدة من الميترونيدازول المرتبطة بالتريازول 1,2,3 إلى عائلة مستقبلات ErbB التي تستهدف كيناز التيروزين لمستقبل عامل نمو البشرة، والتي تشمل Her1 (EGFR)، Her2 (erb-B2)، Her3 (erb-B3)، وHer4 (erb-B4). تتميز بعض أنواع السرطان البشري، مثل سرطان الرئة والثدي، بخلايا تعبر عن EGFR بشكل مفرط. يؤدي هذا إلى تنشيط غير صحيح لمسار الإشارة المضاد للاستماتة Ras، مما ينتج عنه نمو غير منضبط للخلايا. يعتبر تثبيط EGFR TK (الرمز PDB: 1M17) هدفًا مهمًا في تطوير الأدوية المضادة للسرطان لأنه يمكن أن يساعد في منع نمو الأورام وانتشارها.

المواد والطرق: تم استخدام بيئة التشغيل الجزيئي لتقييم قوة ارتباط المركبات الجديدة المصممة ضد البروتينات المستهدفة .(EGFRTK) يتنبأ إجراء الربط الجزيئي بكيفية تفاعل الجزيئات مع الإنزيم المستهدف، وتقييم المعايير مثل S-score و RMSDنتائج الربط من خلال مقارنة الهياكل المقدرة والتجريبية. يُعد هذا أداة مفيدة جدًا لاكتشاف الأدوية ودراسة التفاعلات الجزيئية.

النتائج: أظهرت المركبات الجديدة المصنعة (I-V) طاقة ارتباط محسّنة (S.score) تتراوح من -7.5733 إلى -8.4456 كيلو كالوري/مول وانخفاض قيم rmsd تتراوح من 0.8752 إلى 1.6182 مع الموقع النشط للإنزيم، مقارنة بطاقة ارتباط إرلوتينيب البالغة -7.7359 كيلو كالوري/مول وقيمة rmsd البالغة 1.7200.

الاستنتاج: أظهرت نتائج الربط الجزيئي أن جميع المركبات المصنعة (I-V) لديها طاقة ارتباط أعلى (S-score) وقيم انحراف جذر تربيعي متوسط (RMSD) أقل، مما يشير إلى إمكانية نظرية كمثبطات فعالة لـ EGFR مقارنة بالليجند المرجعي (إرلوتينيب).

1. Introduction

Cancer is a disease characterized by uncontrolled cell development that does not meet the parameters for normal, healthy cells(Paulmurugan, 2012). Cancer spreads and impairs healthy cells' regular biological functions through the invasion of nearby tissues and metastasis to other tissues(Al-Sowayan et al., 2020). If cancer cells spread, which is known as metastasis, they may cause death. A variety of environmental and internal variables promote cancer formation, including exposure to nicotine, chemicals, radiation, and infectious agents(Alibeg et al., 2020a), as well as genetic mutations, hormone imbalances, immune system abnormalities, and random genetic variations(Kifah Abbas et al., 2023). Cancer's causes are numerous, complex, and only poorly understood(Blackadar, 2016). According to a World Health Organization (WHO) estimate from 2018, 18.1 million people globally had cancer, with 9.6 million dying from it(Siegel et al., 2022).

Some metronidazole derivatives, including metronidazole acid acyl sulfonamide(Luo et al., 2011) and cinnamic acid metronidazole ester derivatives(Qian et al., 2010), are produced to block epidermal growth factor receptor (EGFR) tyrosine kinase. These compounds use metronidazole's structure, which consists of a nitroimidazole ring, to target EGFR, potentially for therapeutic uses in cancer treatment, utilizing its capacity to limit cancer cell proliferation by interfering with EGFR signaling pathways. Nitroimidazoles are frequently used as antibacterial chemotherapeutics and antiangiogenic hypoxic cell radiosensitizers(Liew et al., 2023; Synthesis, 2015).

Nitroimidazole derivatives have sparked widespread interest because they have the potential to infiltrate and accumulate in malignancies, as well as undergo bioreduction to form electrophilic chemicals that may disrupt proteins and nucleic acids(Mizumoto et al., 2002)(Naji et al., 2023). Importantly, the toxicity and metabolism of nitroimidazoles, notably metronidazole, have been investigated(Saeed et al., 2019). As a result, nitroimidazoles may present an appealing opportunity to use these chemicals as carriers for targeted delivery in cancer therapy(Rashed et al., 2021). The epidermal growth factor receptor belongs to the tyrosine kinase family. Because of its widespread distribution in cells and crucial role in cell survival, it is now recognized as an anticancer therapy target. EGFRs are abundant in mammalian epithelial cell membranes and play a role in cell proliferation, death, and differentiation. They are junctions that relay extracellular growth impulses within cells. EGFR belongs to the ErbB receptor family, which also comprises Her1 (EGFR), Her2 (erb-B2), Her3 (erb-B3), and Her4 (Erb-B4)(Pao et al., 2004). The EGFR tyrosine kinase-mediated cell growth signaling pathway is critical in the formation and progression of many solid cancers, including non-small cell lung cancer, head, neck, and breast cancer(Gijtenbeek et al., 2022). Overexpression of EGFR family receptors has always been identified in these malignancies, accounting for roughly 60% of all cancers, and their overexpression or aberrant activation frequently results in cell malignant transformation(Herbst, 2004a). EGFR TK (Epidermal Growth Factor Receptor Tyrosine Kinase) is a common target in anticancer drug development since it is essential for cell growth, proliferation, and survival. Dysregulation of EGFR signaling is common in malignancies, making it a promising target for therapeutic intervention. Inhibiting EGFR TK can help suppress tumor growth and spread, making it an important target in the development of anticancer medicines(Stamos et al., 2018).

The active site of the epidermal growth factor receptor is made up of a region that binds substrate and an area that binds adenosine triphosphate (ATP), which is the cofactor that all kinases employ to phosphorylate their substrates. It has been demonstrated that inhibitors with the ability to bind to the cofactor binding site are more successful in blocking protein kinase. The knowledge of the connection between ATP and the kinase active site has been extremely helpful in the development of potent and targeted inhibitors. Whatever Within the active site, in a region referred to as the hinge area, the purine base of adenosine triphosphate makes two major hydrogen bonding contacts with the protein backbone. This region is named for the way it connects the two distinct lobes of the enzyme—the sheet-rich N-terminal lobe and the helix-rich C-terminal lobe(Herbst, 2004b).

2. Methodology

2.1.Chemical synthesis

The overall synthetic methods are intended to be as follows:

1. Synthesis of the propargylic ether derivative of metronidazole (IIa). It was produced using Williamson ether synthesis from propargyl bromide and metronidazole under basic conditions(Leadbeater and McGowan, 2013).
2. Synthesis of aryl azides from aniline derivatives. The diazotization-azidation process was used to create the aryl azide derivatives from aniline derivatives(Gribanov et al., 2016).
3. Synthesis of 1,2,3-triazole derivatives of metronidazole. The reaction between compound IIa (terminal alkyne) and aryl azide derivatives in the presence of sodium ascorbate and CuSO4.5H2O Leads to the synthesis of desirable hybrid molecules (I-V); this method is known as the copper-catalyzed azide-alkyne cycloaddition(Portal et al., 2019).

The following Fig.1. presents the synthesis procedures for the final compounds and their intermediates.

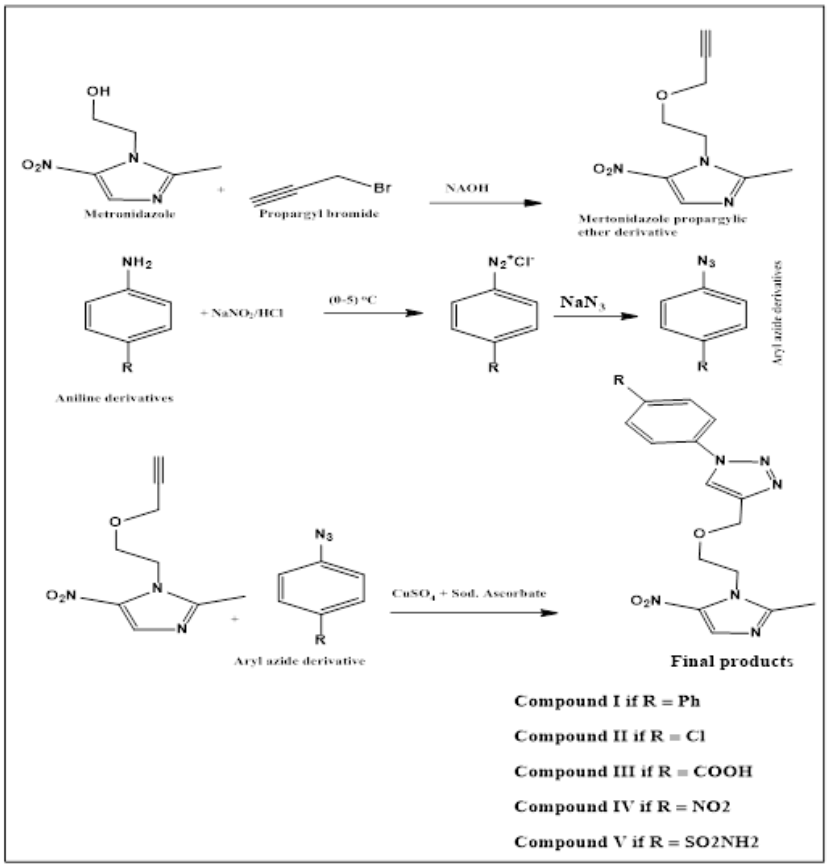


Figure 1: Synthesis of Final Compounds and Their Intermediates

2.2.The Software and System of The Computer

Chem Draw Professional Software Pro 12.0 and Molecular Operating Environment (MOE 2015) were utilized. Through using a laptop, that has the following features: a 256GB solid-state drive (SSD), an Intel or AMD CPU, an integrated or dedicated GPU, 8GB of RAM, and the Windows operating system.

2.2.1. Preparing Receptor and Ligands with The Molecular Docking Method

The docking process involves two steps:

1. Ligand preparation: Using Chem Draw Professional (12.0), the ligand molecular structures were precisely constructed. Following that, the ligand was protonated in a three-dimensional shape, the partial charge was added, then energy was minimized, and the results were saved.
2. Protein preparation: The epidermal growth factor receptor's crystal structure (PDB code: 1M17) is downloaded into the Molecular Operational Environment (MOE 2015) via the PDB website to prepare the protein(Izzaty et al., 1967). The following steps are taken to prepare the target protein:

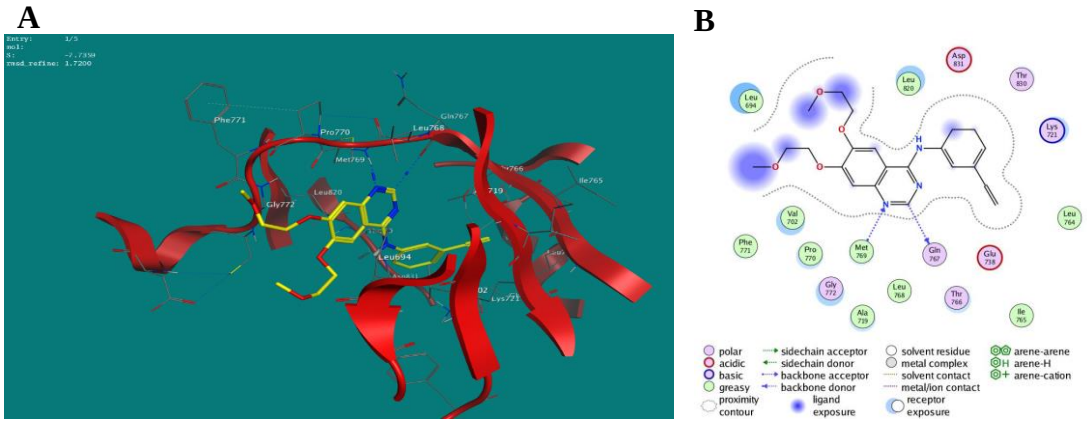
Only the chain sequences involved in the protein action were kept; the other chains were eliminated. The tiny molecules were eliminated. Additionally, water molecules were eliminated. Bonds are hidden by the addition of hydrogen; next, fix the protein's atoms' potential and locate its active site. Lastly, the docking procedure is carried out once the previously synthesized ligand has been imported into MOE from saved data(Jereva et al., 2021)(Synthesis, 2015). Analyzing the compound's inhibitory effect and the degree of similarity between amino acids that interact on identical active sites is necessary; these criteria can be found in S. scores and the root mean square deviation (rmsd) values when the results show improved energy of binding (Score) and lower RMSD values mean optimum posture.

3. Result

The Molecular Operating Environment (MOE), which facilitates the visualization, characterization, and assessment of protein interactions with ligands, is utilized in this study to investigate the best possible way for a ligand to bind to an active site on a target(Mahdi et al., 2013). This method produces an excellent graphical depiction of the findings by illustrating the positions and interactions of ligands with receptor-binding residue(Jakhar et al., 2019)(Alibeg et al., 2020b). The molecular operating environment confirmed the binding selectivity of proposed drugs for epidermal growth factor receptor tyrosine kinase (PDB code:1M17). This demonstrated that the newly synthesized compounds (I-V) bind selectively to the epidermal growth factor receptor tyrosine kinase in the same primary active region as the reference ligand (erlotinib), producing the findings presented in Table 1, Fig.2, Fig.3, Fig.4, Fig.5, Fig.6. and Fig.7.

Table 1: Binding Properties of Newly Synthesized Compounds with EGFR Tyrosine Kinase (PDB Code: 1M17).

Compound	S. score (Kcal/mol)	rmsd	No. of binding sites	Binding amino acids
Erlotinib	-7.7359	1.7200	2	Gln767, Met769
Compound I	-8.1576	0.9570	4	MET769, LYS721, ASP831, THR766.
Compound II	-7.5733	0.8752	5	MET769, LYS721, ASP831, THR766, LYS721.
Compound III	-8.2570	1.4705	5	Two MET 769, Two LYS721, LEU820.
Compound IV	-8.1581	1.4606	4	Two MET769, LYS721, LEU694.
Compound V	-8.4456	1.6182	4	MET769, LYS721, TWO CYS773



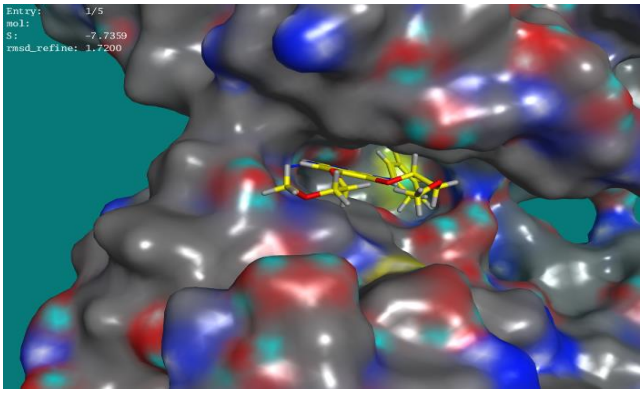
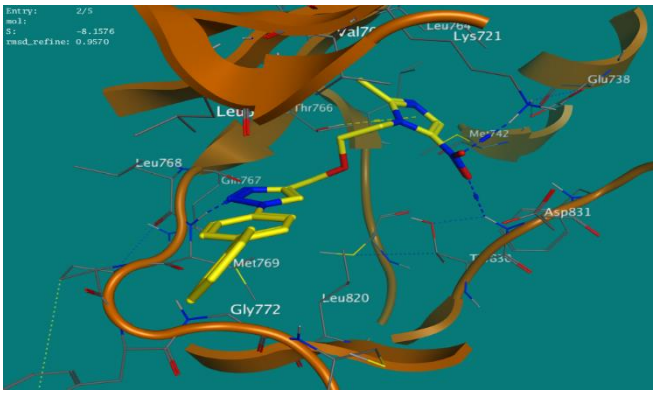
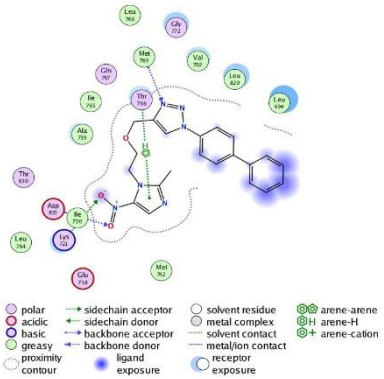


Figure 2: Docking Result of Reference Ligand (Erlotinib) With Epidermal Growth Factor Receptor (PDB Code: 1M17). Where **A**) Represents 3D Structure, **B)** Represents 2D Structure and **C**) Explains The 3D Picture of Entrance and Binding with Whole Protein.

A



B



C

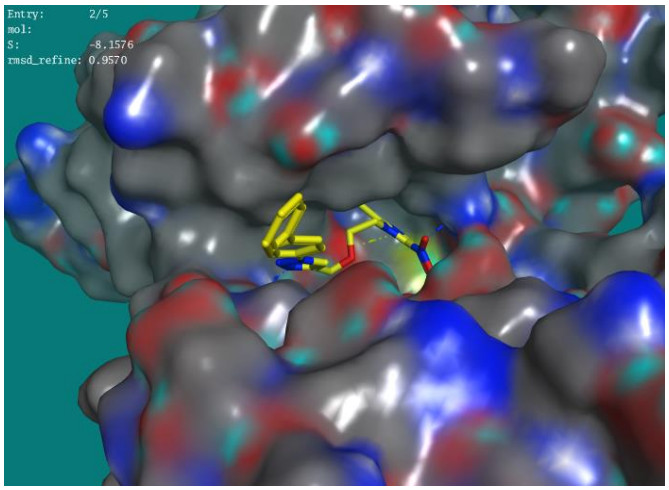
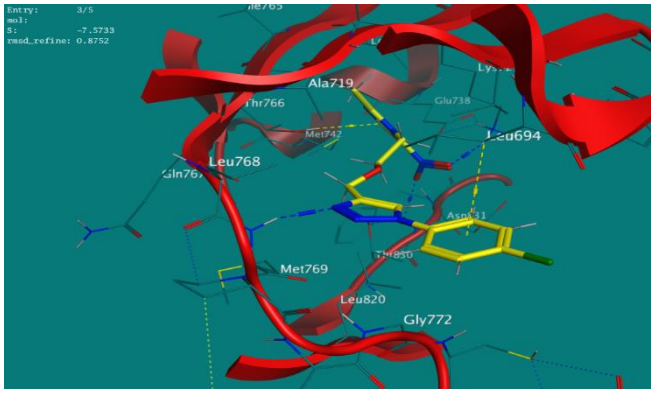
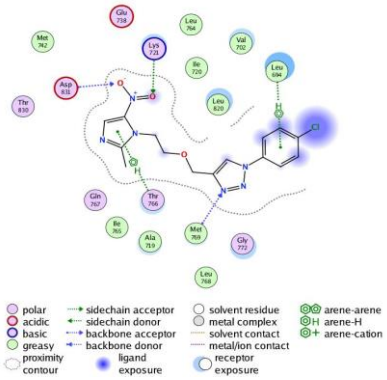


Figure 3: Docking Result of Compound I With Epidermal Growth Factor Receptor (PDB Code: 1M17). Where **A**) Represents 3D Structure, **B**) Represents 2D Structure and **C**) Explains The 3D Picture of Entrance and Binding with Whole Protein.

A



B



C

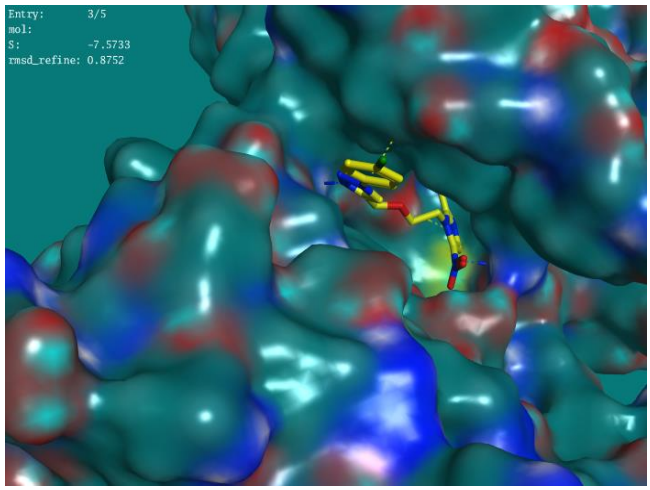
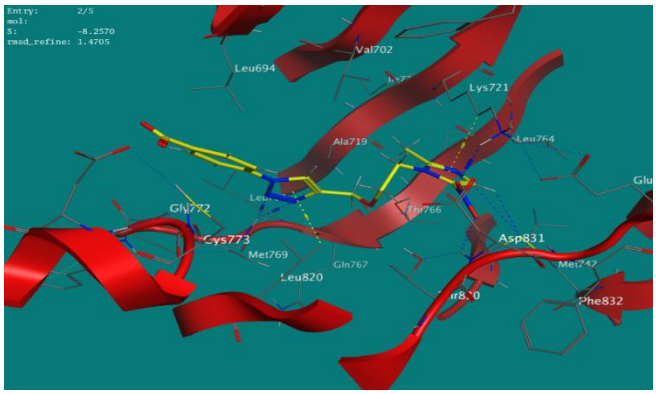
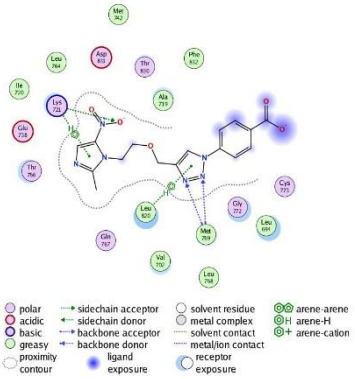


Figure 4: Docking Result of Compound II with Epidermal Growth Factor Receptor (PDB Code: 1M17). Where **A)** Represents 3D Structure, **B)** Represents 2D Structure and **C)** Explains The 3D Picture of Entrance and Binding with Whole Protein**.**

A



B



C

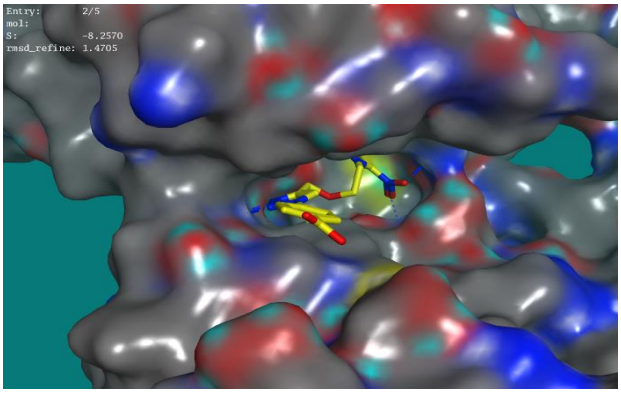
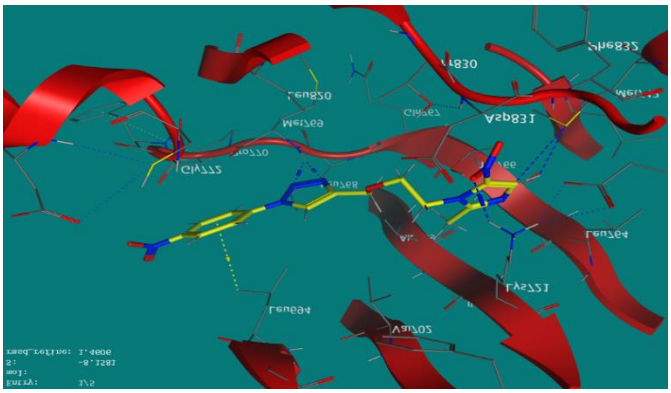
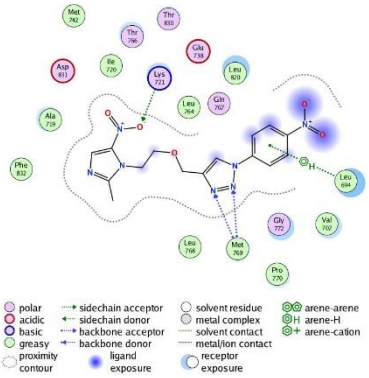


Figure 5: Docking Result of Compound III with Epidermal Growth Factor Receptor (PDB Code: 1M17). Where **A** Represents 3D Structure, **B** Represents 2D Structure and **C** Explains The 3D Picture of Entrance and Binding with Whole Protein.

A



B



C

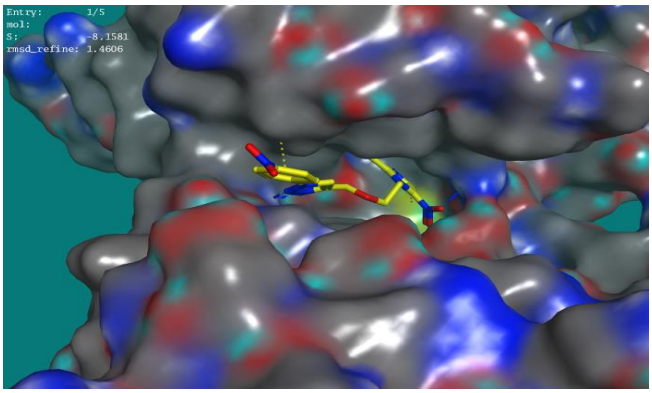


Figure 6: Docking Result of Compound IV with Epidermal Growth Factor Receptor (PDB Code: 1M17). Where **A** Represents 3D Structure, **B** Represents 2D Structure and **C** Explains The 3D Picture of Entrance and Binding with Whole Protein.

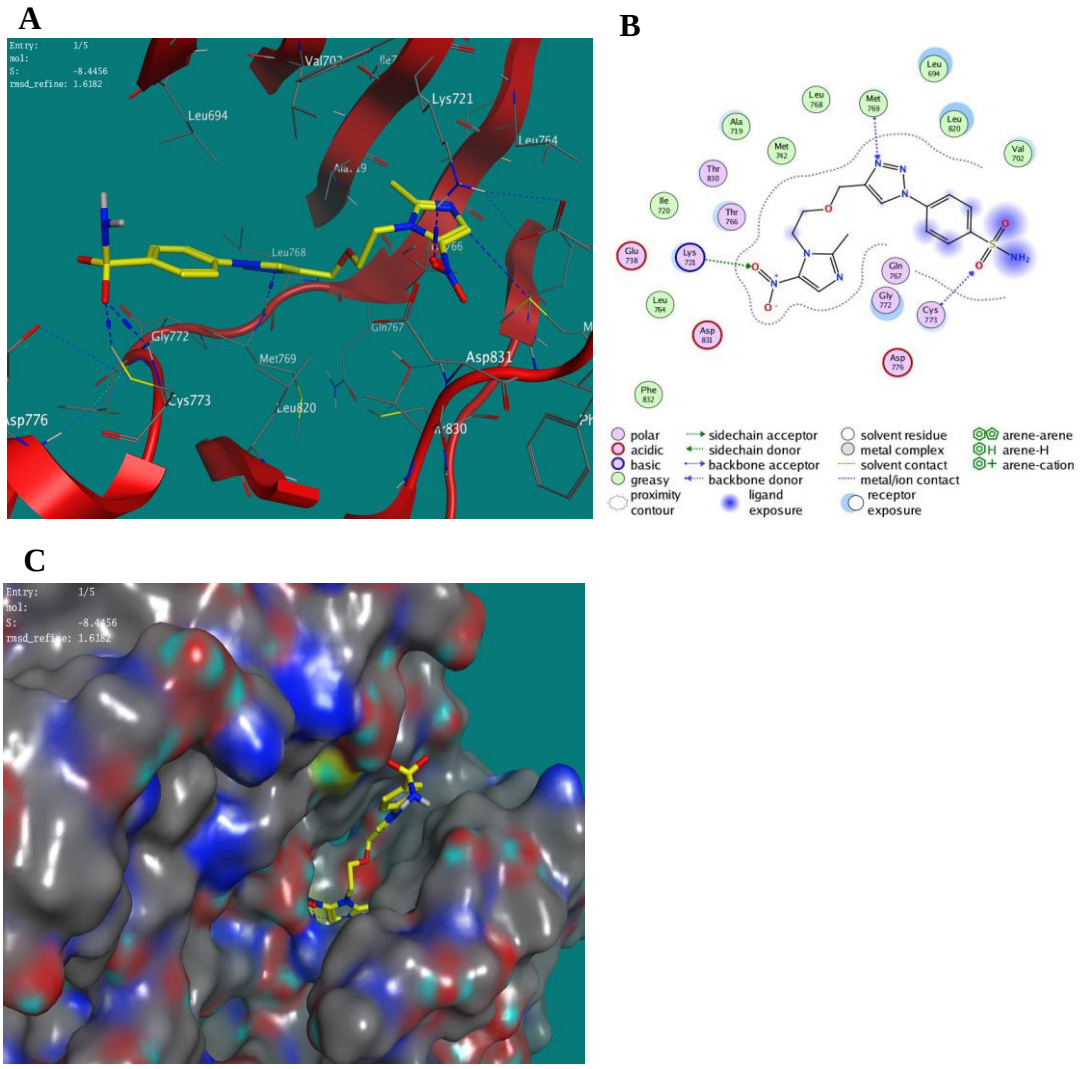


Figure 7: Docking Result of Compound V with Epidermal Growth Factor Receptor (PDB Code: 1M17). Where **A)** Represents 3D Structure, **B)** Represents 2D Structure and **C)** Explains The 3D Picture of Entrance and Binding with Whole Protein.

4. Discussion

Since adenosine triphosphate (ATP) is the cofactor used by all kinases to phosphorylate substrates, the active site consists of an ATP-binding area and a substrate-binding region. Inhibitors that can bind to the cofactor-binding site have been shown to be more effective in inhibiting protein kinase. The design of powerful and specific medicines has greatly benefited from understanding how ATP is linked to the kinase active site. Deep within the active site, the purine base of adenosine triphosphate forms two significant hydrogen bonding interactions with the protein backbone in a region known as the hinge region. This region is named for the way it connects the two distinct lobes of the enzyme—the sheet-rich N-terminal lobe and the helix-rich C-terminal lobe. In comparison to the reference ligand

(erlotinib), the suggested compounds' overall structure, which includes a nitroimidazole ring, ether linkage, triazole ring, and benzene ring with para substitution, enabled them to achieve high binding affinity and more interactions within the enzyme's ATP binding site. In contrast to erlotinib, which only displays two hydrogen bonds, newly produced compounds (I-V) reveal additional hydrogen bonds with amino acid moieties of the enzyme, with four or five contacts. The newly synthesized compounds (I-V) demonstrated improved binding energy (S.score) ranging from -7.5733 to -8.4456 Kcal/mol and reduced rmsd values ranging from 0.8752 to 1.6182 with the enzyme active site, as compared to erlotinib's binding energy of -7.7359 Kcal/mol and rmsd value of 1.7200. This suggests that the newly developed compounds have a higher ability to bind to the target protein, which is desired for medication efficacy and provides more accurate structural representation than the reference ligand.

5. Conclusion

The study used the Molecular Operating Environment (MOE) tool to determine the efficacy of newly synthesized compounds as EGFR tyrosine kinase inhibitors. The docking results demonstrated that all synthesized compounds (I-V) had higher energy of binding (S-score) and lower root mean square deviation (RMSD) values, indicating theoretical potential as effective EGFR inhibitors when compared to the reference ligand (erlotinib).

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Attitudes on Diabetes Among Type 2 Diabetic Patients in Karbala City

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Abstract

Background: Diabetes is a serious health problem that has reached an alarming scale, with over 500 million individuals affected globally. Type 2 diabetes is the most common type of diabetes, accounting for over 90% of all diabetes worldwide. The Middle East and North Africa (MENA) region has the greatest regional prevalence of diabetes (16.2%) and the second-largest expected increase (86%) in the number of people with diabetes.

Objective: This study aims to assess the attitude toward diabetes among type 2 diabetic patients in Karbala City and the factors associated with their attitude.

Patients and methods: A cross-sectional study was conducted on 252 type 2 diabetic patients in multiple health institutes in Karbala City through face-to-face interviews using a questionnaire developed by the University of Michigan Diabetes Research and Training Center (MDRTC). SPSS version 22.00 was used to perform statistical analysis. The means of the groups were compared using independent samples t-test and ANOVA. Pearson's rank correlation coefficient test was used to show relationships between diabetic attitude scores, glycated hemoglobin, fasting blood sugar, and random blood sugar.

Results: The mean age of the patients was 56 ± 9.83 years. 64.3% of the patients were female. The mean duration of diabetes was 10.1 ± 6.82 years. The mean attitude score for the patients was 31.55 ± 6.18 out of 50. The majority of patients (72.6%) had a moderate attitude level.

Conclusion: Continuous education programs and healthcare attention are needed to enhance patients' attitudes toward the disease and its complications, especially female patients, those with low educational levels, housewives, those not working, those with lower economic status, those with longer diabetes duration, those who take insulin as treatment, and those who have complications.

المواقف من مرض السكري بين مرضى السكري من النوع الثاني في مدينة كربلاء

بشار خلدون غني، علي عبد الرضا أبو طحين، حميد حسين الجميلي

الخلاصة

المقدمة: مرض السكري هو مشكلة صحية خطيرة وصلت إلى نطاق ينذر بالخطر، حيث تأثر أكثر من 500 مليون فرد على مستوى العالم. داء السكري من النوع 2 هو أكثر أنواع مرض السكري شيوعا، حيث يمثل أكثر من 90٪ من جميع أنواع مرض السكري في جميع أنحاء العالم. تشهد منطقة الشرق الأوسط وشمال أفريقيا أكبر انتشار إقليمي لمرض السكري (16.2٪) وثاني أكبر زيادة متوقعة (86٪) في عدد المصابين بالسكري.

الهدف:

تهدف هذه الدراسة إلى تقييم الموقف تجاه مرض السكري بين مرضى السكري من النوع 2 في مدينة كربلاء والعوامل المرتبطة بموقفهم.

طرائق العمل:

أجريت دراسة مقطعية مستعرضة على 252 مريضا بالسكري من النوع 2 في معاهد صحية متعددة في مدينة كربلاء من خلال مقابلات وجها لوجه باستخدام استبيان طوره مركز أبحاث وتدريب السكري بجامعة ميشيغان (MDRTC). تم استخدام SPSS الإصدار 22.00 لإجراء التحليل الإحصائي. تمت مقارنة وسائل المجموعات باستخدام اختبار (ت) للعينات المستقلة و ANOVA. تم استخدام اختبار معامل ارتباط الرتب لبيرسون لإظهار العلاقات بين درجات موقف مرضى السكري، والهيموجلوبين السكري، وسكر الدم الصائم، وسكر الدم العشوائي.

النتائج:

كان متوسط عمر المرضى 56 ± 9.83 سنة. 64.3٪ من المرضى كانوا من الإناث. كان متوسط مدة مرض السكري 10.1 ± 6.82 سنة. كان متوسط درجة الموقف للمرضى 31.55 ± 6.18 من 50. غالبية المرضى (72.6٪) لديهم مستوى موقف معتدل.

الاستنتاجات:

هناك حاجة إلى برامج التعليم المستمر والاهتمام بالرعاية الصحية لتعزيز مواقف المرضى تجاه المرض ومضاعفاته، وخاصة المرضى الإناث، وذوي المستويات التعليمية المنخفضة، وربات البيوت، وأولئك الذين لا يعملون، وذوي الوضع الاقتصادي المنخفض، والذين يعانون من فترة أطول لمرض السكري، وأولئك الذين يتناولون الأنسولين كعلاج، وأولئك الذين يعانون من مضاعفات.

1. Introduction

Diabetes is a serious health problem that has reached an alarming scale, with over 500 million individuals affected globally. According to estimates from the International Diabetes Federation (IDF), the number of persons with diabetes has increased alarmingly, more than tripling from 2000 to an estimated 537 million in 2021 (International Diabetes Federation, 2021). Its prevalence increased from 108 million in 1980 to 422 million in 2014 (World Health Organization, 2022). 8.5% of persons who were 18 years of age and older had diabetes in 2014. A total of 1.5 million deaths were directly related to diabetes in 2019, and 48% of these deaths occurred among those under the age of 70. It contributed to an additional 460,000 renal disease deaths, and high blood glucose is responsible for 20% of cardiovascular fatalities (Institute for Health Metrics and Evaluation, 2019). It is a significant contributor to renal disease, heart attacks, strokes, blindness, and lower limb amputation (Al-Zubaidi and Abbas, 2017). An estimated 2 million people died in 2019 from diabetes-related renal damage (World Health Organization, 2022). The number of deaths due to this disease increased by as great as 70% in the whole world from 2000-2019 (World Health Organization, 2021).

Type 2 diabetes is the most common type of diabetes, accounting for over 90% of all diabetes worldwide (International Diabetes Federation, 2021). Over the past few decades, there has been a consistent rise in both the incidence and prevalence of diabetes. Over the last three decades, the prevalence of type 2 diabetes has risen substantially in countries of all income levels (Al Mousawi, 2018; Mustafa Murtadha, 2013). The vast majority of diabetics reside in low- and middle-income countries (Hasan et al., 2023). A global goal is to halt the rise in diabetes and obesity by 2025 (World Health Organization, 2023). Type 2 diabetes can be averted or delayed through regular exercise, having a healthy eating plan, keeping to the ideal weight, and being free from tobacco. Eating a proper diet, exercising, use of medications, as well as frequent screening and timely treatment for complications, will treat diabetes, delay its effects, and prevent them (World Health Organization, 2022).

Diabetes is a significant public health issue in the Eastern Mediterranean Region, where it affects around 14% of people. In this region, the burden of diabetes could affect more than 100 million people by 2045. This region has the highest rates of diabetes worldwide and it contains six of the ten nations with the highest rates of diabetes in the world (World Health Organization, 2021). The Middle East and North Africa (MENA) region has the greatest regional prevalence of diabetes (16.2%) and the second-largest expected increase (86%) in the number of people with diabetes, with an estimated 136 million individuals by 2045. One in six adults (73 million) in the MENA region has diabetes, which is the highest proportion among the International Diabetes Federation (IDF) Regions. The majority (24.5%) of deaths in adults of working age from diabetes occur in this region. Even though this region is home to 13.6% of the world's diabetics, only 32.6 billion USD, or 3.4% of the entire global budget, was spent there (International Diabetes Federation, 2023, 2021). Diabetes is one of Iraq's top ten causes of death (Iraqi Ministry of Health, 2022).

Health education is crucial in regions with limited resources like ours, where diabetes mellitus (DM) causes a significant financial burden and necessitates the immediate involvement of clinicians at all levels, particularly primary care physicians, who are typically the first to provide care and frequently encounter both newly diagnosed and known diabetics. For diabetic patients to achieve glycemic control and avoid the onset of complications from their disease, adjustments in their attitudes are therefore essential (Chawla et al., 2019).

The study of diabetics' attitudes toward their condition is regarded as a fundamental initial step in the educational process (Khurshid and Othman, 2014). The first step of a plan that aims to promote diabetes prevention and reduce complications and expenditure is to do research that helps to identify the baseline attitude level of the target group (Karbalaeifar et al., 2016). A person's attitude towards a health disorder has a direct impact on his or her motivation to adhere to the prescribed treatment plan (Wan et al., 2016). Generally, the attitude studies about type 2 diabetes bring up several important points. They are a key element in determining diabetic patients' level of attitude. Also, they can examine the patients' attitudes towards diabetes and its complications, which will contribute to the detection of any common errors or beliefs that may negatively impact self-care (Gani et al., 2023). Attitude studies also help to identify factors that impact the patients' attitude such as age, the duration of diabetes, and glycemic control (Mousavi and Shojaei, 2021). Through the process of highlighting the current gaps in patients' attitudes, stakeholders and healthcare workers can devise specific interventions and programs and thus they can improve diabetes management and ensure that complications are of minimal risk. These surveys are also useful in the design of healthcare services targeted at locally specific demographic factors that include age, educational level, and residence (Asante et al., 2023). Hence, attitude studies are one of the key elements in better diabetes management and prevention as they help healthcare workers understand the problems that their patients face. One of the values of this research is that it will uplift our understanding of patients' attitudes toward this illness. So future education programs could target the weak points, false beliefs, or practices. The aims of this study are: to assess the attitudes of diabetes among type 2 diabetic patients in Karbala city, and to assess the factors associated with their attitude.

2. Patients and Methods

2.1. Study Design and Selection of Patients

A cross-sectional study that was conducted on type 2 diabetic patients in Karbala City in Al-Imam Al-Hussein Medical City, Al-Imam Al-Hassan Center for Endocrinology and Diabetes (HMEDC) which is the only specialized diabetes center in the governorate, Al-Imam Al-Hassan Al-Mujtaba Teaching Hospital, two Primary Health Care Centers which included Al-Ghadeer and Al-Abbasiya Al-Gharbiya and three Medical Public Clinics that provide their medical services in the afternoon period which included Al-Ghadeer, Al-Iskan and Al-Abbasiya Al-Gharbiya on 252 patients. The sample size was calculated according to the equation, $n = Z^2 P (1 – P)/d^2$ where n is the sample size, Z is the statistic corresponding to 95% confidence (1.96), P is the prevalence of diabetes (16.2%) according to the Middle East and North Africa (MENA) region of the International Diabetes Federation (International Diabetes Federation, 2021), and d is precision (0.05). The minimal sample size required is 209 patients.

2.2. Data Collection

The data collection was conducted using convenience sampling over eight months duration from 1/2/2023 to 1/10/2023 through face-to-face interviews with the patients 5 days per week using a questionnaire as the data collection instrument.

2.3. Inclusion Criteria

All patients who had type 2 diabetes, aged more than 18 years, and diagnosed with diabetes for more than 6 months were included.

2.4. Exclusion Criteria

The study excluded patients who were pregnant, who had a history of mental illness or had psychiatric disorders.

2.5. Instruments

The questionnaire consisted of two parts. The first part of the questionnaire consisted of 21 questions regarding sociodemographic information and clinical data about diabetes including gender, date of birth, marital status, educational level, job type, residence, economic status, smoking, duration of diabetes, type of treatment, complications due to diabetes, family history of diabetes, source of advice about diabetes, past medical history, hemoglobin HbA1c, fasting blood sugar, and random blood sugar levels. The second part consisted of 10 questions (statements) on the attitude of the patients regarding their disease and was taken from the diabetic care profile, which was developed by the University of Michigan (Fitzgerald et al., 1996). English was the questionnaire's original language, and two bilingual translators (Google Translate and Britannica English-Arabic Translator) were used to translate English to Arabic. A pilot study was done on 20 patients in 3 weeks duration extended from 1/2/2023 to 22/2/2023 and the duration of each patient interview was about 30 minutes.

2.6. Assessment of Economic Status

Two variables were used for patients' Economic Status; The first was according to the patients' own assessments and the second was according to the Crowding Index Level. The Crowding Index Level was calculated from The Crowding Index which is the proportion of all the individuals living in the house and all the house rooms apart from the kitchen and bathroom (World Health Organization, 2018). Patients with a Crowding Index of (less than 1) were considered as having a Low Crowding Index Level, whereas patients with a Crowding Index of (1-2.99) and (more than or equal to 3) were considered as having a Medium and High Crowding Index Levels respectively (Iraqi Ministry of Planning, 2014). The Low, Medium and High Crowding Indexes were converted to Good, Average and Weak Economic Statuses respectively.

2.7. Assessment of Attitude Scores

Each item was assessed using one of five subscales: 1 = strongly disagree (SD), 2 = disagree (DA), 3 = neutral (NE), 4 = agree (A), and 5 = strongly agree (SA). The maximum score was 50. The scores were reversed for negatively-worded items before data analysis.

2.8. Assessment of Attitude Level

Three categories were created based on the respondents' scores. The following cut-off points were used to determine the attitude scoring level: 75%–100%, 50%–74%, and 0%–49% for good, moderate, and poor levels, respectively (Al-Mutawaa et al., 2022).

2.9. Ethical Approval

Ethical approval was taken from the Medical Research Bioethical Committee at the University of Kerbala – College of Medicine (Number: 13 at 6/3/2023), and approval was taken from the Karbala Health Directorate. Verbal consent was taken from the participants.

2.10. Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 22.00 and Microsoft Excel 16 were utilized to compute descriptive statistics. The significance level for all statistical tests was set at 0.05. Mean ± standard deviation (St.D) was the interpretation used for continuous variables and frequencies and percentages were used to represent categorical data. The means of two groups were compared using an independent samples t-test and the means of three or more groups were compared using ANOVA and Tukey's post hoc tests. Using Pearson's rank correlation coefficient test, relationships between diabetic attitude scores, glycated hemoglobin, fasting blood sugar, and random blood sugar were investigated.

3.Results

3.1. Sociodemographic Characteristics of The Patients

The patients' sociodemographic details are shown in (Table 1). The mean age of the patients was 56 ± 9.83 years. 64.3% of the patients were females. 79.0% of the patients were married. Regarding the educational level, 42.1% of the patients completed the primary level and 24.6% of them were classified as illiterate (Neither reads nor writes).

Regarding the job type, 11.9% of the patients were government employees, 16.3% of them were retired and 56.7% of them were housewives. For the males, 35.6% of them were retired, 31.1% of them were classified as free business and 26.7% as government employees. For the females, the majority of them (88.3%) were housewives.

Regarding the Residence, 91.3% of the patients lived in urban areas. 55.2% of the patients lived in Owned houses, 17.1% lived in Rented houses, 17.1% lived in houses built in Agricultural areas and 10.7% lived in houses built in Slum areas.

Regarding economic status according to the patients' assessments, 127 (50.4%) patients considered themselves as having weak economic status, 111 (44.0%) as having average economic status and 14 (5.6%) as having good economic status. Regarding economic status according to the Crowding Index Level, 64 (25.4%) patients were considered as having weak economic status, 167 (66.3%) as having average economic status and 21 (8.3%) as having good economic status. 74.2% of the patients were non-smokers.

Table 1: Sociodemographic Characteristics of The Patients

Variables	Categories	Frequency	Percentage (%)
Gender	Male	90	35.7
	Female	162	64.3
Age Groups / years	Under 45	30	11.9
	45-54	87	34.5
	55-64	80	31.7
	65-74	46	18.3
	75 and older	9	3.6
Marital Status	Single	3	1.2
	Married	199	79
	Widower / Widow	46	18.3
	divorced	4	1.6
Educational level	Neither reads nor writes	62	24.6
	Primary	106	42.1
	Intermediate	34	13.5
	Preparatory	20	7.9
	University \ Institute	25	9.9
	Postgraduate	5	2
Job type	Government employee	30	11.9
	Retired	41	16.3
	Free Business	32	12.7
	Not working	6	2.4
	Housewife	143	56.7
Residence	Urban	230	91.3
	Rural	22	8.7
House type	Owned	139	55.2
	Rented	43	17.1
	Slum	27	10.7
	Agricultural	43	17.1
Economic Status according to the patients' assessments	Weak	127	50.4
	Average	111	44
	Good	14	5.6
Economic status according to Crowding Index Level	Weak	64	25.4
	Average	167	66.3
	Good	21	8.3
Smoking	Yes	31	12.3
	No	187	74.2
	Ex-smoker	34	13.5

3.2. Clinical Data About Diabetes Mellitus

The patients' clinical data about diabetes mellitus is shown in (Table 2).

The mean duration of diabetes was 10.1 ± 6.82 years. 250 patients (99.2% of the patients) took treatment, including any one or more of these types (diet-only, oral pills-only, insulin-only, oral pills and insulin, herbals-only). 71.0% of the patients had their treatment included oral pills-only, 70.6% included diet-only, 15.1% included oral pills and insulin, 15.1% included herbals-only and 9.9% included insulin-only. 2 patients (0.8%) didn't take any kind of treatment, not even diet-only treatment. 40.9% of the patients reported that their disease was not under control and 34.5% reported that it was somewhat under control. 96.0% of the patients had complications due to diabetes. The percentage of complications among type 2 diabetic patients is shown in (Fig.1). 217 (86.1%) of the patients reported that they suffer from diabetic neuropathy, 122 (48.4%) from hypoglycemia, 92 (36.5%) from cataract, 70 (27.8%) from sexual dysfunction (all were males), 60 (23.8%) from diabetic retinopathy, 54 (21.4%) from coronary heart disease, 37 (14.7%) from diabetic foot, 11 (4.4%) from peripheral vascular disease, 10 (4.0%) from stroke, 8 (3.2%) from diabetic nephropathy and 59 (23.4%) reported that they suffer from other complications. Concerning other complications that were mentioned by the patients, 17 (29%) of them reported they suffered from weight loss, 15 (25%) from non-alcoholic fatty liver disease, 12 (20%) from glaucoma, 5 (8%) from recurrent urinary tract infection, 4 (7%) from joint pain, 2 (3%) from amputation in the foot and gingivitis, weight gain, dry eye and paronychia with abscess formation were reported by one patient (2%) for each of them.

77.0% of them reported that they had a first-degree family member with type 2 diabetes (father, mother, brother, sister, son, daughter). The sources of information about diabetes are shown in (Fig.2). 231 patients (91.7%) took their information from doctors, 190 (75.4%) from family members, 168 (66.7%) from social media, 147 (58.3%) from relatives and friends, 111 (44.0%) from nurses, 101 (40.1%) from TV, radio and newspapers and 2 (0.8%) from other source which was from pharmacists. 90.9% of them said that they had other diseases besides type 2 diabetes. 180 (71.4%) of them had dyslipidemia, 152 (60.3%) had hypertension, 71 (28.2%) had heart diseases, 28 (11.1%) had thyroid diseases, 11 (4.4%) had stroke, 7 (2.8%) had kidney diseases and 46 (18.3%) had other types of diseases.

The glycemic control values of the patients are shown in (Table 3). The Hemoglobin HbA1c was recorded for 147 patients (58.3%) and the mean was 9.44 ± 2.10%. The Fasting Blood Sugar (FBS) was recorded for 120 patients (47.6%) and the mean was 176.11 ± 65.26 mg/dL. The Random Blood Sugar (RBS) was recorded for 97 patients (38.5%) and the mean was 257.15 ± 87.37 mg/dL.

Table 2: Clinical Data About Diabetes Mellitus

Variables	Categories	Frequency	Percentage (%)
Diabetes duration groups	5 years or less	79	31.3
	6-10 years	74	29.4
	11-15 years	50	19.8
	16-20 years	32	12.7
	More than 20 years	17	6.7
Take treatment	Yes	250	99.2
	No	2	0.8
Type of treatment	Diet only	178	70.6
	Oral pills only	179	71
	Insulin only	25	9.9
	Oral pills and insulin	38	15.1
	Herbals only	38	15.1
Disease under control	Yes	62	24.6
	Somewhat	87	34.5
	No	103	40.9
Complication	Yes	242	96
	No	10	4
Family history	Yes	194	77
	No	58	23
Other chronic diseases	Yes	229	90.9
	No	23	9.1

Table 3: Glycemic Control Values of The Patients (N = 252)

Glycemic control values	Mean ± St.D	Frequency	Percentage (%)
Hemoglobin HbA1c in %	9.44 ± 2.10	147	58.3
Fasting Blood Sugar in mg/dL	176.11 ± 65.26	120	47.6
Random Blood Sugar in mg/dL	257.15 ± 87.37	97	38.5
St.D = Standard Deviation			

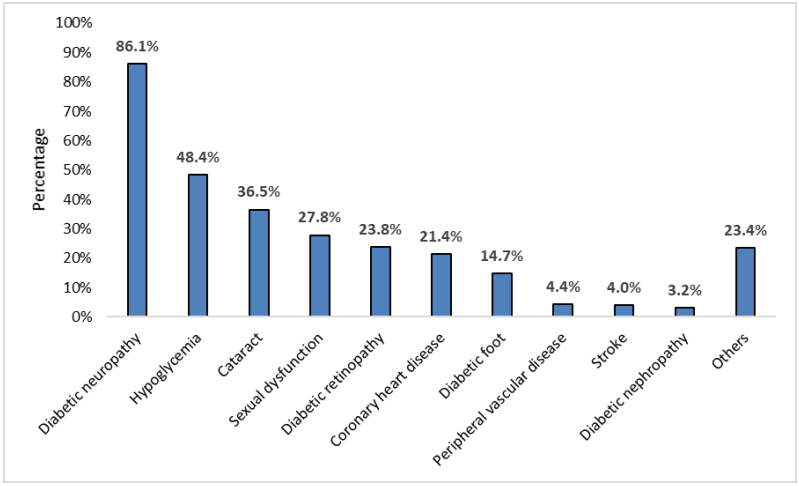

Figure 1: Percentage of Complications Among Type 2 Diabetic Patients

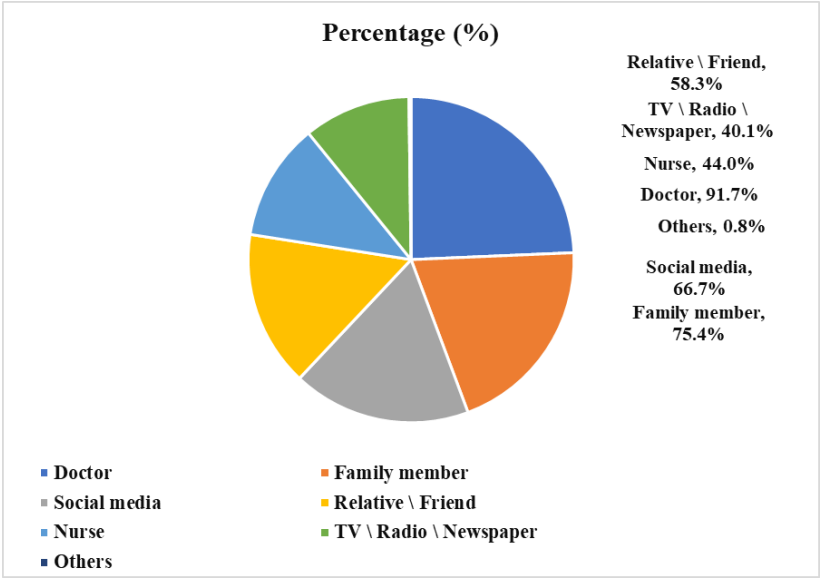

Figure 2: Sources of Information About Diabetes

3.3. Attitude Assessment

The mean score, mean score percentage, and its 95% Confidence Interval of the attitudes of the patients are shown in (Table 4). About three-quarters of the patients (72.6%) had a moderate attitude level. The attitude levels are shown in (Fig.3). The number and percentage of patients who expressed positive or negative attitudes toward each item in the attitude questionnaire are shown in (Table 5). The choices strongly disagree (SD) and disagree (DA) for items 1, 2, 3 and 9 and the choices agree (A) and strongly agree (SA) for items 4, 5, 6, 7, 8 and 10 both corresponded to a positive attitude. The neutral choices (NE) were included in the negative attitudes.

221 (87.7%) of the patients found it hard to believe that they really had diabetes. 190 (75.4%) felt unhappy and depressed because of their diabetes. 191 (75.9%) felt they were not as good as others because of their diabetes. 103 (40.8%) could do just about anything they set out to do. 244 (96.8%) said they should control their weight to control diabetes. Only 74 (29.3%) thought that things were going very well for them right now. 247 (98.0%) said they should monitor their blood glucose at home. 39 (15.4%) thought that diabetes did not affect their life at all. Only 22 (8.8%) said that eating restrictions were no longer required once diabetes was controlled. 250 (99.2%) thought regular exercise helped keep diabetes under control.

Table 4: The Patients' Mean Scores, Mean Score Percentages, And Their 95% Confidence Intervals of Attitude (N = 252)

Scores	Minimum	Maximum	Mean	Mean percentage %	95% Confidence Interval of Mean percentage %
Attitude score	19	48	31.55	63.10	61.64 - 64.69

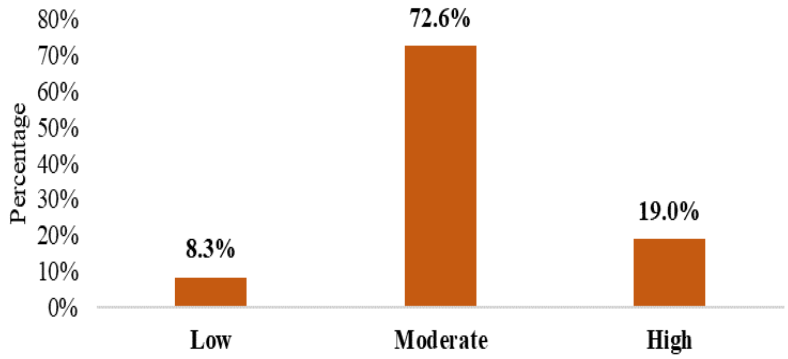

Figure 3: Distribution of Attitude Levels of The Patients (N = 252)

Table 5: The Responses of the Patients Regarding Their Attitudes Towards Diabetes

Attitudes	SD *N*(%)	DA *N*(%)	NE *N*(%)	A *N*(%)	SA *N*(%)	Positive *N*(%)	Negative *N*(%)
I find it hard to believe that I really have diabetes	9(3.6)	22(8.7)	4(1.6)	63(25.0)	154(61.1)	31(12.3)	221(87.7)
I feel unhappy and depressed because of my diabetes	15(6.0)	47(18.7)	8(3.2)	39(15.5)	143(56.7)	62(24.7)	190(75.4)
I feel I am not as good as others are because of my diabetes	15(6.0)	46(18.3)	10(4.0)	77(30.6)	104(41.3)	61(24.3)	191(75.9)
I can do just about anything I set out to do	68(27.0)	78(31.0)	3(1.2)	54(21.4)	49(19.4)	103(40.8)	149(59.2)
People with diabetes should control their weight	1(0.4)	1(0.4)	6(2.4)	53(21.0)	191(75.8)	244(96.8)	8(3.2)
Things are going very well for me right now	57(22.6)	80(31.7)	41(16.3)	57(22.6)	17(6.7)	74(29.3)	178(70.6)
People with diabetes should monitor their own blood glucose at home	0(0)	2(0.8)	3(1.2)	17(6.7)	230(91.3)	247(98.0)	5(2.0)
Diabetes does not affect my life at all	153(60.7)	53(21.0)	7(2.8)	23(9.1)	16(6.3)	39(15.4)	213(84.5)
Once diabetes is controlled, eating restrictions are no longer required	66(26.2)	164 (65.1)	3(1.2)	13(5.2)	6(2.4)	230(91.3)	22(8.8)
Regular exercise helps in keeping diabetes under control	0(0)	0(0)	2(0.8)	30(11.9)	220(87.3)	250(99.2)	2(0.8)

3.4. Attitudes and Sociodemographic Characteristics

The relationship between the attitude scores and the sociodemographic characteristics of the patients is displayed in (Table 6). There was a significant relationship between the attitude scores and the gender of the patients (P = 0.01), where the male patients had higher attitude scores than the female patients (32.89 ± 6.668 vs. 30.81 ± 5.790).

There was a highly significant relationship between attitude scores and the educational level of the patients (P <0.001), where the attitude score increased as the educational level increased, where the mean attitude score for neither reads nor writes versus preparatory, university \ institute and postgraduate educational levels was (29.55 ± 6.535 vs. 35.55 ± 5.176, 34.56 ± 6.609 and 37.4 ± 7.301) respectively. There was a highly significant relationship between attitude scores and job type (P < 0.001), where the government employee had higher attitude scores than the housewives (34.43

± 6.372 vs. 30.39 ± 5.684) and the retired patients had higher attitude scores than the housewives (34.1 ± 6.629 vs. 30.39 ± 5.684). There was a highly significant relationship between attitude scores and economic status (according to the patients' assessments) ($P < 0.001$), where the attitude scores increased as the economic status increased, where the mean attitude score for good economic status versus average and weak economic status was (37.64 ± 5.969 vs. 32.58 ± 6.036 and 29.98 ± 5.764) respectively.

Table 6: Sociodemographic Characteristics of the Patients with Differences in Attitude Scores

Variables	Categories	No.	Attitude score			
			Mean	±	St.D	P
Gender	Male	90	32.89	±	6.668	0.010*
	Female	162	30.81	±	5.79	
Age Groups / years	Under 45	30	32.27	±	7.46	0.605
	45-54	87	31.14	±	5.587	
	55-64	80	31.7	±	6.327	
	65-74	46	32.11	±	6.287	
	75 and older	9	29	±	5.701	
Marital Status	Single	3	27.33	±	4.933	0.435
	Married	199	31.78	±	6.23	
	Widower / Widow	46	31.09	±	6.099	
	divorced	4	28.75	±	5.56	
Educational level	Neither reads nor writes	62	29.55	±	6.535	<0.001*
	Primary	106	30.52	±	4.925	
	Intermediate	34	33	±	6.814	
	Preparatory	20	35.55	±	5.176	
	University \ Institute	25	34.56	±	6.609	
	Postgraduate	5	37.4	±	7.301	
Job type	Government employee	30	34.43	±	6.372	<0.001*
	Retired	41	34.1	±	6.629	
	Free Business	32	31.47	±	6.278	
	Not working	6	27.83	±	3.71	
	Housewife	143	30.39	±	5.684	
Residence	Urban	230	31.67	±	6.324	0.311
	Rural	22	30.27	±	4.399	
House type	Owned	139	32.15	±	6.138	0.173
	Rented	43	31.51	±	6.84	
	Slum	27	29.37	±	6.488	
	Agricultural	43	31.02	±	5.244	
Economic Status according to the patients' assessments	Weak	127	29.98	±	5.764	<0.001*
	Average	111	32.58	±	6.036	
	Good	14	37.64	±	5.969	

Economic status according to Crowding Index Level	Weak	64	30.11 ± 5.538	0.096
	Average	167	32.02 ± 6.373	
	Good	21	32.19 ± 6.129	
Smoking	Yes	31	30.97 ± 5.32	0.531
	No	187	31.46 ± 6.137	
	Ex-smoker	34	32.59 ± 7.182	
St.D = Standard Deviation				

3.5. Attitudes and Clinical Data About Diabetes Mellitus

The relationship between the attitude scores and the clinical data about diabetes mellitus is displayed in (Table 7). There was a significant relationship between the attitude scores and diabetes duration groups (P = 0.011), where the attitude scores increased as the diabetes duration decreased, where the mean attitude score for those patients who had diabetes for 5 years or less was higher than that for those patients who had diabetes for 6-10 years (33.58 ± 7.185 vs. 30.85 ± 5.804) and also was higher than that for those patients who had diabetes for 11-15 years (33.58 ± 7.185 vs. 30.52 ± 4.892). There was a significant relationship between the attitude scores and the type of treatment (P = 0.005), where the patients who took oral pills-only had higher attitude scores than those who took insulin-only (32.25 ± 6.47 vs. 28.84 ± 5.86). There was a highly significant relationship between attitude scores and the patients' perceptions that their disease was under control (P < 0.001), where the patients who responded yes had higher attitude scores than both those who responded somewhat and no (35.65 ± 7.70 vs. 31.75 ± 5.43 and 28.92 ± 4.09) respectively.

There was a highly significant relationship between attitude scores and the presence of complications (P < 0.001), where the patients who didn't have complications had higher attitude scores than those who had complications (39.40 ± 4.88 vs. 31.23 ± 6.02).

Table 7: Clinical Data About Diabetes Mellitus with Differences in Attitude Scores

Variables	Categories	No.	Attitude score			
			Mean	±	St.D	P
Diabetes duration groups	5 years or less	79	33.58	±	7.185	0.011*
	6-10 years	74	30.85	±	5.804	
	11-15 years	50	30.52	±	4.892	
	16-20 years	32	30.69	±	5.337	
	More than 20 years	17	29.82	±	5.982	
Take treatment	Yes	250	31.58	±	6.204	0.485
	No	2	28.5	±	0.707	
Type of treatment	Oral pills only	179	32.25	±	6.466	0.005*
	Insulin only	25	28.84	±	5.857	
	Oral pills and insulin	38	29.47	±	4.065	
Disease under control	Yes	62	35.65	±	7.691	<0.001*
	Somewhat	87	31.75	±	5.429	
	No	103	28.92	±	4.091	
Complication	Yes	242	31.23	±	6.025	<0.001*
	No	10	39.4	±	4.881	
Family history	Yes	194	31.41	±	5.916	0.499
	No	58	32.03	±	7.049	
Other chronic diseases	Yes	229	31.41	±	6.044	0.269
	No	23	32.91	±	7.471	
St.D = Standard Deviation						

3.6. Correlations of Attitude Scores

Attitude score had a moderate significant negative correlation with both HbA1c and FBS at the 0.01 level with a P value of high significance (<0.001) as shown in Table 8.

Table 8: Correlations of the Patients' Hemoglobin Hba1c, Fasting Blood Sugar, and Random Blood Sugar with Their Attitude Scores

Variables		Attitude score
Hemoglobin HbA1c	Pearson Correlation	**-.410****
	Sig. (2-tailed)	**<0.001**
	n	**147**
Fasting Blood Sugar	Pearson Correlation	**-.375****
	Sig. (2-tailed)	**<0.001**
	n	**120**
Random Blood Sugar	Pearson Correlation	-0.017
	Sig. (2-tailed)	0.866
	n	97
**. Correlation is significant at the 0.01 level (2-tailed).		

4. Discussion

4.1. Attitude Assessment

In this study, the patients' level of attitude was found to be moderate in the majority (72.6%). This finding is similar to a study carried out in Iran (Mohammadi et al., 2015) which reported that most participants have a moderate attitude level, and differs from the findings of a study conducted in Baghdad, Iraq (Abbas et al., 2016) which stated that a large number of patients had a poor attitude level and from a study conducted in Iran (Niroomand et al., 2016) in which majority had good attitude level. The variations in the outcomes of these studies can be attributed to numerous factors that include the level of education, socioeconomic level, duration of diabetes, and glycemic control. The majority of patients (87.7%) reported a negative attitude of finding it hard to believe that they have diabetes, which is similar to the finding from a study conducted in the United Arab Emirates (Al-Maskari et al., 2013) and differs from a study conducted in Saudi Arabia (Al-Aboudi et al., 2016). The majority of patients reported a positive attitude toward the importance of DM care through controlling their weight (96.8%), monitoring their blood glucose at home (98.0%), and keeping diabetes under control through regular exercise (99.2%). This finding is similar to (Al-Maskari et al., 2013) and differs from (Mohammadi et al., 2015). 75.4% of the patients reported that they feel unhappy and depressed because of their diabetes and 75.9% of them reported that they feel they are not as good as others are because of their diabetes. The previous two statements are negative attitudes and their percentages are higher than those of (Al-Aboudi et al., 2016). 8.8% of the patients agreed with the statement that once diabetes is controlled, eating restrictions are no longer required which is a negative attitude. This finding is similar to a study conducted in India (Mukhopadhyay et al., 2010) and differs from (Mohammadi et al., 2015). 40.8% of the patients reported that they can do just about anything they set out to do; 29.3% reported that things are going very well for them right now and 15.4% reported that diabetes does not affect their lives at all. The previous statements are positive attitudes and their percentages are lower than those of (Al-Aboudi et al., 2016). This study's mean attitude score is 31.55 out of 50, which is positive. This finding is similar to the finding from (Al-Aboudi et al., 2016) and differs from the finding from (Al-Maskari et al., 2013) which showed a negative average attitude. This indicates that the patients are willing to change their lifestyles to manage their disease.

4.2. Attitudes and Sociodemographic Characteristics

The male patients had higher attitude scores than the female patients which is similar to the findings from Iraq (Abbas et al., 2016) and Tanzania (Joho et al., 2023), and differs from a study conducted in Malaysia (Abbasi et al., 2018). It is noted that males with diabetes manage their condition better, experiencing less anxiety and depression. They feel less anxious about society and are pleased with how they are handling their health (Siddiqui et al., 2013). This may explain this finding. The attitude score increased as the educational level increased. This finding is similar to the findings of studies from Iraq (Abbas et al., 2016), Egypt (Lotfy et al., 2022), Saudi Arabia (Mahzari et al., 2022), and Malaysia (Abbasi et al., 2018) and differs from that conducted in the United Arab Emirates (Al-Maskari et al., 2013) which didn't show a significant association. Higher-educated people might have a more positive attitude toward their

disease because they have a greater understanding of it and how to treat it, which could account for this finding. Also, Education is seen to provide people with the information and the skills needed to manage their diabetes properly. This in turn provides the person with a more positive outlook about life. Furthermore, higher education can increase a person's ability to obtain healthcare resources and knowledge, giving the person the capability to participate in the management of their diabetes and be the one making informed decisions about its treatment.

The government employees and the retired patients had higher attitude scores than the housewives. This result is similar to the other studies conducted in Iraq (Abbas et al., 2016), and Malaysia (Abbasi et al., 2018) but in contrast with the finding from Iran (Karbalaeifar et al., 2016) which didn't show a significant association. This evidence may be explained by the fact that employment is known to be linked with better educational achievement, which has a positive effect on attitude. Hence, the patients who are employed or retired, who are more likely to be educated, have higher attitude scores than housewives, who may be less educated. The attitude scores increased as the economic status (according to the patients' assessments) increased. This finding is similar to (Abbasi et al., 2018), but varies from (Al-Maskari et al., 2013) which reported no relationship between attitude scores and economic status. This might be due to the fact that those with higher socioeconomic status have better access to healthcare resources, education, and information, which contribute to a more positive attitude adopted in diabetes management.

4.3. Attitudes and Clinical Data About Diabetes Mellitus

The attitude scores increased as the diabetes duration decreased. This finding is in line with studies from Palestine (Thultheen et al., 2021), and Egypt (El-Khawaga and Abdel-Wahab, 2015), while (Abbasi et al., 2018) reflected an upgrade in the attitude scores as the diabetes duration increased, and (Al-Maskari et al., 2013) didn't show a significant association. Such findings may result from the idea that individuals with a short duration of diabetes are less likely to have complications from their disease, thereby having a more positive attitude toward it, while those with a longer duration are more likely to have complications as a result of it, hence having a more negative attitude.

The patients who took solely oral pills got higher attitude scores than those who used only insulin. This finding correlates well with what was observed in (Al-Maskari et al., 2013). It is different from (Niroomand et al., 2016), where the patients who took oral pills-only had lower attitude scores than those who took insulin-only, and from (Al-Aboudi et al., 2016) which did not show a substantial relationship. This result could be due to the fact that people who are on oral diabetes medication only believe their condition is not as serious as compared to patients taking insulin, which may result in a more positive attitude. Moreover, individuals taking oral medication only may be favorable in their attitude regarding the disease because of their better understanding of the disease and how to manage it. Besides, those taking only oral medicine may have fewer complications and shorter-term diabetes than those relying on insulin treatment, which might enhance their optimistic outlook. Switching from oral antihyperglycemic medications to insulin therapy may elicit negative attitudes among these patients due to fears of hypoglycemia, uncomfortable injections, and storage, which could be associated with the lower attitude scores observed among these individuals. Therefore, patients taking tablets-only treatment for type 2 diabetes may have higher attitude scores due to the mixture of lower-risk beliefs and reluctance to take insulin.

The patients who didn't have complications had higher attitude scores than the ones who had complications. The result is like the finding from the study conducted in India (Aswathi et al., 2019), while it is different from that in the study from Eritrea (Adgoy et al., 2021). This result could be due to the fact that patients with no complications are more informed about their condition and experience a better quality of life. In addition to that, their view may be more supportive, and they might perceive their ability to manage diabetes or carry out self-care more positively. Moreover, due to the obstacles and limitations imposed by their disease, patients who have complications tend to get negative about life more easily.

4.4. Correlations of Attitude Scores

Attitude scores had a moderate, highly significant negative correlation with both hemoglobin A1c and fasting blood sugar. This is contrary to the studies conducted in India (Solanki et al., 2017), and Iran (Karbalaeifar et al., 2016) where no significant correlations were reported. Outcomes might be caused by the fact that patients can have more negative emotions or behaviors in case they are thinking wrongly and the way they see their illness is distorted. Posing negative attitudes to people living with diabetes can hinder beneficial self-management behaviors, for instance, medicine compliance, food control, and doing physical activities frequently, which in turn may cause poor glycemic control, as evidenced by high HbA1c and fasting blood sugar levels.

5. Conclusions

Most patients exhibited a moderate level of attitude, with an overall positive outlook on their condition, suggesting openness to diabetes self-management education programs and a willingness to take responsibility for their care. Doctors, family members, and social media were identified as the most common sources of information for diabetics, with social media platforms playing an increasingly important role in providing guidance. Despite this, patients generally had inadequate blood glucose control, as indicated by their mean hemoglobin A1c, fasting blood sugar, and random blood sugar levels. Attitudes toward type 2 diabetes were significantly correlated with hemoglobin A1c and fasting blood sugar, underscoring the relationship between attitude and glycemic control.

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Association of FTO Gene Variants with Some Biochemical Markers of Type 2 Diabetes Mellitus Patients in Iraqi Population

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Abstract

Background: The Fat-mass and obesity associated (*FTO*) gene modulates the gene expression through methylation–demethylation modifications since it is part of Fe (II) - and 2-oxoglutarate-dependent dioxygenases superfamily. This study was carried out in the Department of Clinical Laboratories / College of Applied Medical Sciences / University of Kerbala during the period from November 2022 to April 2024. The study aimed to investigate the association between the variation in the *FTO* gene and serum level of some biochemical markers in Type 2 Diabetes Mellitus Patients within the Iraqi Population.

Patients and Methods: One hundred volunteers participated in this study, 50 individuals with Type 2 DM as a patient's group (25 females and 25 males), and 50 apparently healthy individuals as a control group (25 females and 25 males). The ages of all participants were ranged between 25 to 75 years at the time of the investigation. We investigate three sites in the *FTO* gene (*FTO* 1, *FTO* 2, and *FTO* 3). The variation of the *FTO* gene was investigated by the Sanger sequencing method. The levels of biochemical markers were measured in blood serum.

Results: The results of the present study identified the presence of four previously registered variants in *FTO* gene. These variants might be of interest to *FTO* gene studies due to their presence in the coding regions that included in the gene expression**.**

Conclusion: The two variants, 53769662 T/A and 53782363 C/A, may be the most important variables because there are statistical associations with some biochemical markers.

ارتباط متغيرات جين *FTO* مع بعض المعلمات الكيموحيوية عند مرضى السكري من النوع الثاني في المجتمع العراقي

زيد عبد الحسين كاظم، جودت نوري غائب

الخلاصة

المقدمة: يُعد جين FTO (مرتبط بالكتلة الدهنية والسمنة) أحد الأعضاء في عائلة الإنزيمات ثنائية الأكسجين التي تعتمد على الحديد (Fe II) والـ 2-أوكسوجلوتارات، حيث يُعدّل تعبير الجين من خلال تعديلات الميثيل والدي-ميثيل. تم إجراء هذه الدراسة في قسم المختبرات السريرية / كلية العلوم الطبية التطبيقية / جامعة كربلاء خلال الفترة من نوفمبر 2022 إلى أبريل 2024. هدفت الدراسة إلى التحقيق في العلاقة بين التباين في جين FTO ومستوى بعض العلامات البيوكيميائية في مصل الدم لدى مرضى السكري من النوع الثاني داخل المجتمع العراقي.

المرضى وطرق العمل: شارك في هذه الدراسة مئة متطوع، 50 منهم يعانون من مرض السكري من النوع الثاني كمجموعة مرضى (25 أنثى و25 ذكر)، و50 فردًا يتمتعون بصحة جيدة كمجموعة ضابطة (25 أنثى و25 ذكر). تراوحت أعمار جميع المشاركين بين 25 إلى 75 عامًا في وقت إجراء الدراسة. تم التحقيق في ثلاثة مواقع في جين FTO (FTO 1، FTO 2، وFTO 3). تم تحليل التباين في جين FTO باستخدام طريقة تسلسل سانجر. كما تم قياس مستويات العلامات البيوكيميائية في مصل الدم.

النتائج: حددت نتائج هذه الدراسة وجود أربع متغيرات مسجلة سابقًا في جين FTO. قد تكون هذه المتغيرات ذات أهمية في دراسات جين FTO نظرًا لوجودها في المناطق المرمزة التي تشارك في تعبير الجين.

الاستنتاج: قد يكون المتغيران T/A 53769662 وC/A 53782363 هما الأكثر أهمية، نظرًا لوجود ارتباطات إحصائية مع بعض العلامات البيوكيميائية.

1. Introduction

The FTO also known as alpha-ketoglutarate-dependent dioxygenase FTO is an enzyme that in humans is encoded by the *FTO* gene that is located on chromosome 16. As one homolog in the Alkylatin B family proteins, it is the first mRNA demethylase that has been identified(Jawiarczyk-Przybyłowska et al., 2023; Jia et al., 2012). Human obesity appears to be associated with specific *FTO* gene variations(Popović et al., 2023; R.J.F. and G.S.H., 2014). The transcribed FTO protein's amino acid sequence bears a strong resemblance to that of the oxidatively demethylating enzyme AlkB. *FTO* belongs to the superfamily of non-heme iron-containing proteins called alpha-ketoglutarate-dependent hydroxylases. It was initially found that recombinant FTO protein could, albeit inefficiently, catalyze the demethylation of 3-methylthymine in single-stranded DNA and 3-methyluridine in single-stranded RNA(Gerken et al., 2007; Xu et al., 2023). N6-methyladenosine (m6A), a nucleoside that is often modified in RNA, was later discovered to be a significant substrate of *FTO*. Obesity raises the risk of several common diseases, making it an important global health concern. It's unclear whether hereditary factors contribute to obesity. A common mutation in the *FTO* gene, which predisposes to diabetes through an influence on body mass index, was found during a genome-wide search for genes linked to type 2 diabetes susceptibility(Frayling et al., 2007; Tian et al., 2023). A typically elevated triglyceride deposition and the generation of hepatic glucose can result from enhanced *FTO* expression, which can also promote de novo lipogenesis, decrease lipolysis and fatty acid oxidation, and boost gluconeogenesis(Witka et al., 2019).

Diabetes Mellitus term describes a metabolic disorder of multiple an etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both(Ahmed, 2024; AL-Sahi et al., 2024) . DM is characterized by immune-mediated (Type 1 diabetes), insulin resistance (Type 2 diabetes), gestational hyperglycemia, or other chronic hyperglycemia; genetic, environmental, infectious, or medication-induced problems; or affects the beta cells of the islets of Langerhans(Abdulhakeem et al., 2023; Azeez et al., 2024). Type 2 diabetes mellitus (T2DM) is characterized by hyperglycemia, insulin resistance and relative insulin deficiency. Over 23 million Americans live with diabetes mellitus, out of the 366 million individuals who have the condition worldwide. By 2030, this figure will increase to 552 million.(Damanik and Yunir, 2021; Qalaf et al., 2024) The causes of T2DM are not completely understood but there is a strong association between overweight, obesity, family history, and ethnicity(Aschner, 2017; Basu et al., 2013). The aim of the study: Investigate the Association of FTO gene variants with some biochemical markers in Type 2 Diabetes Mellitus patients of Iraqi Population.

2. Materials and Methods

2.1. Blood Sample Collection

One hundred volunteers participated in this study, 50 individuals with Type 2 Diabetes Mellitus as a patient's group (25 females and 25 males) and 50 apparently healthy individuals as a control group (25 females and 25 males). The ages of all participants were ranged between 25 to 75 years at the time of the investigation. The blood samples were collected from the individuals in Al-Imam Al-Hassan Center for Endocrinology and Diabetes in Karbala city / Iraq. An ethical consent form was signed by each volunteer. Six milliliters of the venous blood sample were obtained from each participant using gel tubes, and the blood was drawn using disposable syringes under sterile condition. The collected blood was centrifuged to separate serum to be used later. The levels of biochemical markers HbA1c, FBS, Cholesterol, TG, LDL, HDL, VLDL, FIB and CRP were measured in blood serum using the ARCHITECT c4000 clinical chemistry instrument from Abbott Diagnostics

2.2. Molecular Detection

A total volume of 25 µl was used in the PCR reaction (5 µl DNA, 2 µl from each primer Table 1, 8 µl master mix, and 8 µl nuclease free water). The PCR program that was used to amplify the target sequence of *FTO* 1, *FTO* 2, and *FTO* 3 region consisted of 35 cycles, each cycle included denaturation for 30 seconds at 94ºC, annealing for 45 seconds at 57ºC and extension for 45 seconds at 72ºC. Agarose gel electrophoresis was used to separate PCR product bands on 1% agarose gel stained by fluorescent Redsafe dye. The gel electrophoresis system was set at 70 volts for 60 minutes, and then the gel was displayed under UV transilluminator to check the PCR products(see Fig.1, Fig.2, and Fig.3).

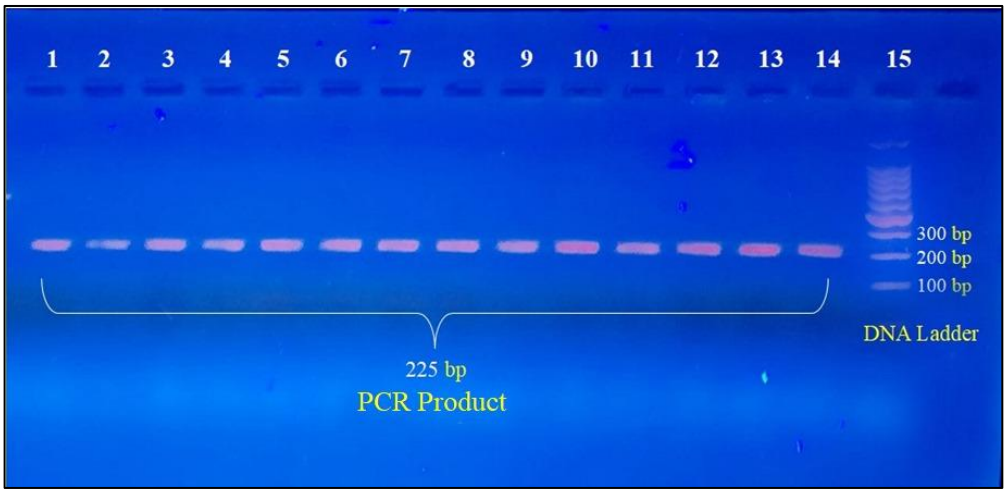

Figure 1: PCR Products (225 Bp) Found in Study Samples That Demonstrates the FTO Gene's FTO 1 Target Region's Presence. Lanes 1-14, PCR Products. Lane 15, DNA Ladder.

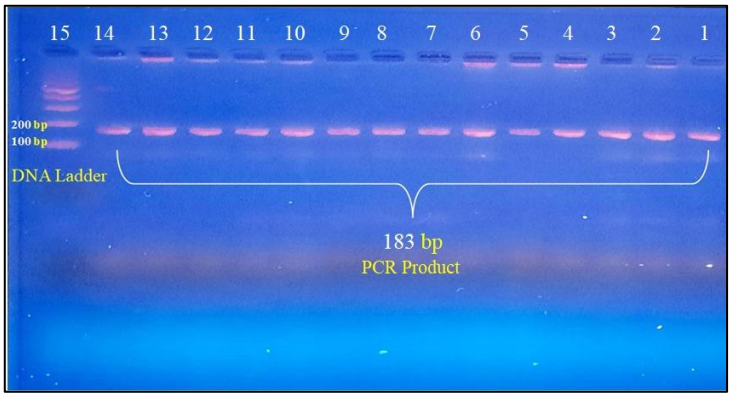

Figure 2: PCR Products (183 Bp) Found in Study Samples That Demonstrates the *FTO* Gene's *FTO* 2 Target Region's Presence. Lanes 1-14, PCR Products. Lane 15, DNA Ladder.

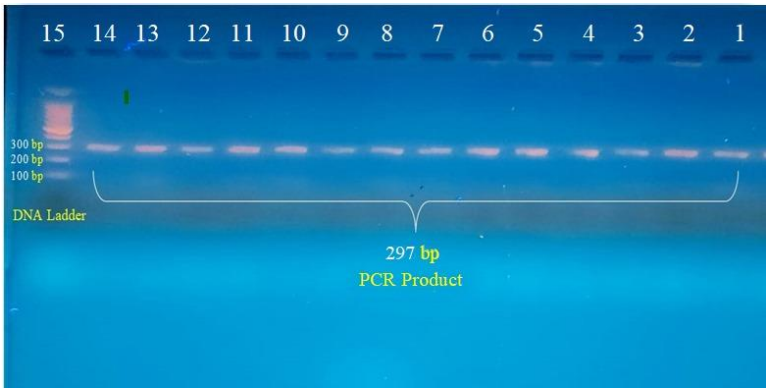

Figure 3: PCR Products (297 Bp) Found in Study Samples That Demonstrates the *FTO* Gene's *FTO* 3 Target Region's Presence. Lanes 1-14, PCR Products. Lane 15, DNA Ladder.

Table 1: Primers Designed for Amplification of *FTO* Gene.

Primer name		**Primer sequence**	**PCR product size**
***FTO* 1**	*FTO* -Forward	5'- TCTAAATTATTAATCAGGGCCATTT-3'	225 base pair
	FTO -Reverse	5'- TGTCCTACCACCCTGTTTACC-3'	
***FTO* 2**	*FTO* -Forward	5'- ACAGTGCCAGCTTCATAGCC -3'	183 base pair
	FTO -Reverse	5'- TTGAGGTGCCATTCCTCAAT -3'	
***FTO* 3**	*FTO* -Forward	5'- TTGAATGAAATAGGATTCAGAAGAGA -3'	297 base pair
	FTO -Reverse	5'- TGTCCAAACAGTAGGTCAGGAA -3'	

2.3. Nucleotides Sequencing and Analysis

In the present study, three regions of the *FTO* gene were selected, and we named them *FTO* 1, *FTO* 2, and *FTO* 3. (see Fig.4, Fig.5, and Fig.6). PCR products from 24 patients, along with 12 PCR products from control group (total= 36) for each target region of the *FTO* gene were sent to the Alpha DNA (S.E.N.C.) Corporation in (Montreal, Quebec, Canada) to perform nucleotide sequencing. Sanger sequencing method was applied using an automated DNA sequencer (see Fig.7). The results of sequencing were manually examined using bioinformatics tools, and they were compared to human reference gene sequences that already uploaded to the National Center for Biotechnology Information (NCBI). The NCBI's Basic Local Alignment Search Tool (BLAST) was used to complete the alignments. The target gene's sequenced area was examined by using Molecular Evolutionary Genetics Analysis X (MEGAX), where the CLUSTALW program carried out several sequence alignments to validate the existence of variants found by the BLAST tool. The locations of every variant found in the current study were reported and examined using Ensembl Genome Browser's tools to determine the type of variant and forecast its functional implications (see Fig.8, Fig.9, Fig.10 and Fig.11).

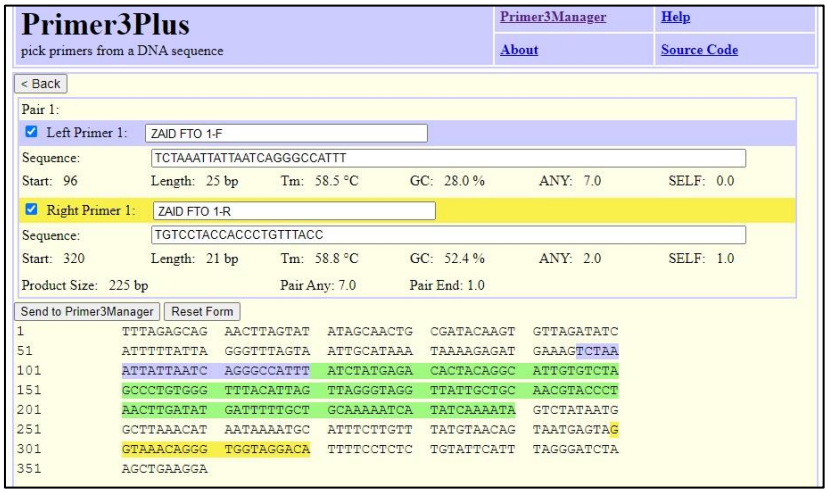

Figure 4: Forward and Reverse Primer Design for *FTO* 1 Gene in Primer 3 Plus Program.

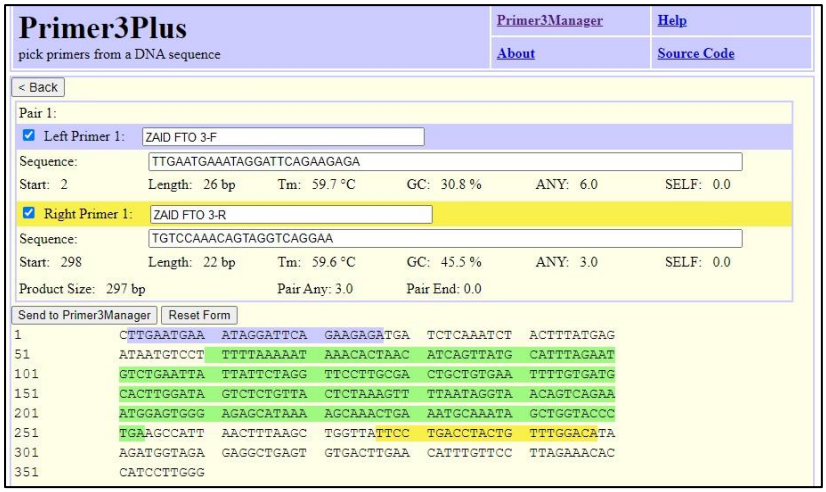

Figure 5: Forward and Reverse Primer Design for *FTO* 2 Gene in Primer 3 Plus Program.

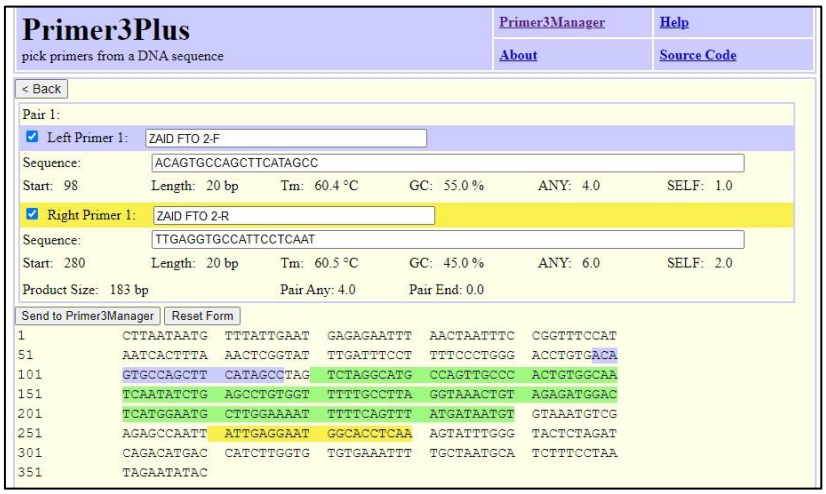

Figure 6: Forward and Reverse Primer Design for FTO 3 Gene in Primer 3 Plus Program.

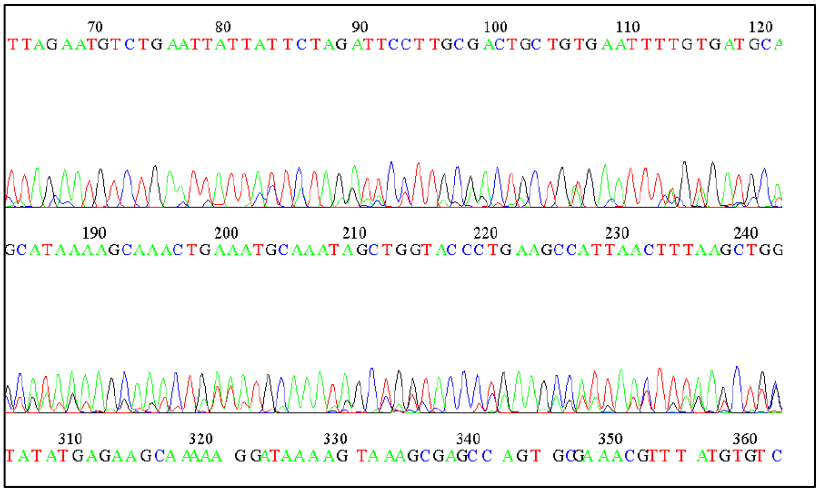

Figure 7: An Automated Sanger DNA Sequencing Method Shows the Electropherogram with Peaks of The Forward Strand of the Sample Sequence

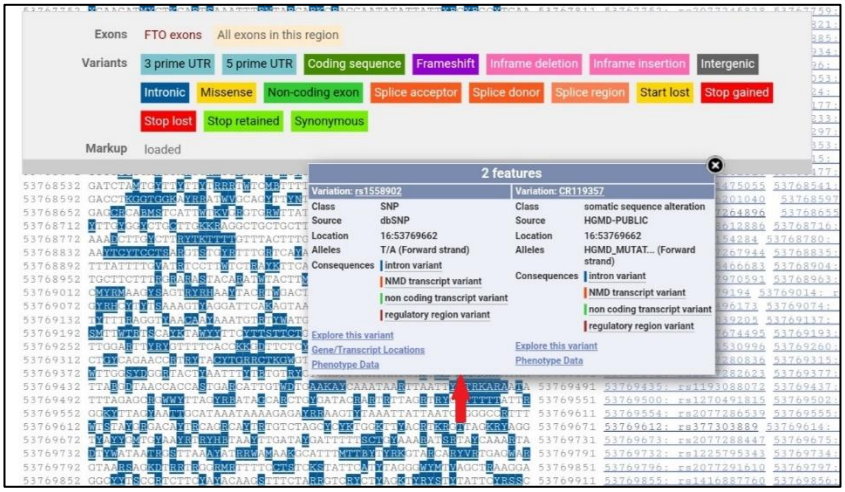

Figure 8: Detect (53769662 T/A) Variant in Study Sample

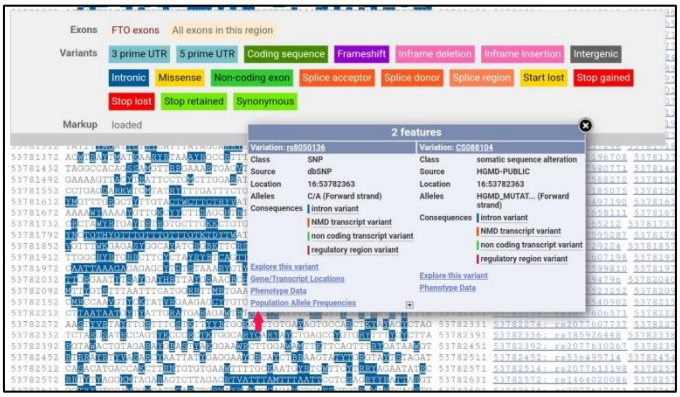

Figure 9: Detect (53782363 C/A) Variant in Study Sample

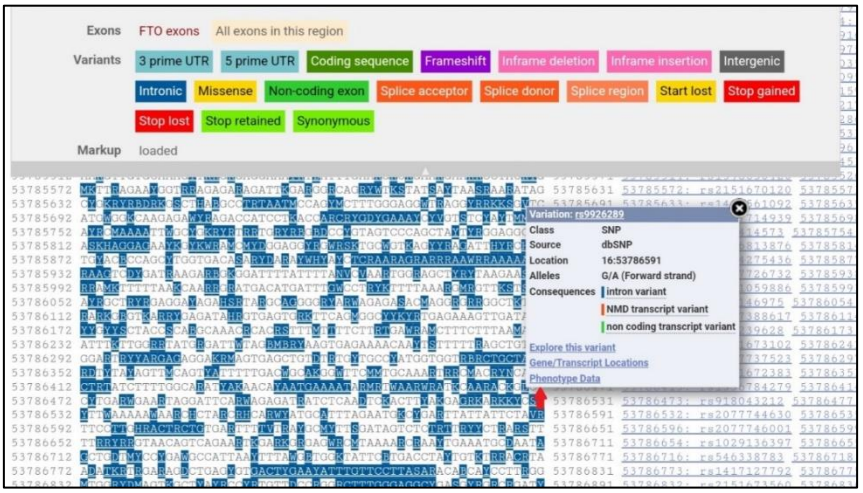

Figure 10: Detect (53786591 G/A) Variant in Study Sample

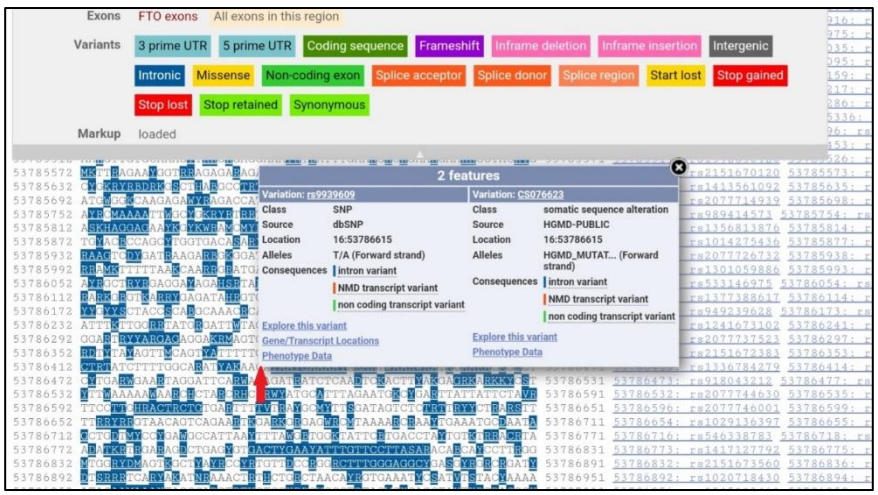

Figure 11: Detect (53786615 T/A) Variant in Study Sample

2.4. Statistical Analysis

Statistical analysis was carried out using SPSS version 22.0 (SPSS, IBM Company, Chicago, IL 60606, USA). Data were expressed as means ± standard deviation if the data were normally distributed. Data were expressed as median±IQR if the data were non-normal distributed. $P \leq 0.05$ was statistically significant.

3. Results

The results of the statistical analysis showed the mean value, standard deviation, standard error, minimum and maximum value for the parameters included in the study (BMI, HbA1c, FBS, Cholesterol, TG, LDL, HDL, VLDL, FIB and CRP) for the patient's group and the control group. This information is presented in Table 2 and Table 3.

Table 2: Mean Values of the Biochemical Markers in The Patient Group.

Variable	Description				
	Normal Value	**Mean ±SD**	**Min.**	**Max.**	**SE**
BMI	18.5-24.9	31.36±4.67	25.7	43.0	0.66
HbA1c	Below 5.7 %	8.42±1.72	6.3	11.4	0.24
FBS	Below 100 mg/dl	194.38±49.14	134	280	6.95
Cho	120-200 mg/dl	185.16±42.63	122	242	6.03
TG	35-160 mg/dl	148.82±56.16	68	271	7.94
HDL	30-70 mg/dl	43.0±14.88	25	65	2.1
LDL	30-130 mg/dl	112.36±33.81	54	154	4.78
VLDL	13-60 mg/dl	30.61±11.02	14	54	1.55
FIB	200-400 mg/dl	307.91±52.27	182	392	7.39
CRP	0-6 mg/l	4.16±3.09	0.7	11.1	0.43

The data presented in the table as means: ±SD standard deviation of mean, Min minimum, Max maximum, SE standard error. BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar, Cho cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density, lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen, CRP c-reactive protein

Table 3: Mean Values of The Biochemical Markers in The Control Group.

Variable	Description				
	Normal Value	**Mean ±SD**	**Min.**	**Max.**	**SE**
BMI	18.5-24.9	32.16±4.58	26.4	46.5	0.64
HbA1c	Below 5.7 %	5.59±0.41	5.0	6.0	0.55
FBS	Below 100 mg/dl	113.73±11.96	97.0	126.0	1.69
Cho	120-200 mg/dl	195.24±34.04	135.0	242.0	4.81
TG	35-160 mg/dl	124.06±48.2	57.0	158.0	6.81
HDL	30-70 mg/dl	44.44±10.85	21.0	69.0	1.53
LDL	30-130 mg/dl	132.04±37.87	81.0	192.0	5.35
VLDL	13-60 mg/dl	24.71±9.63	11.0	32.0	1.36
FIB	200-400 mg/dl	274.62±25.83	203.0	297.0	3.65
CRP	0-6 mg/l	3.03±1.92	1.2	4.6	0.27

The data presented in the table as means: ±SD standard deviation of the mean, Min minimum, Max maximum, SE standard error. BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar, Cho cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen, CRP c-reactive protein.

The results of this work indicated that there was no significant difference when the mean height, weight, and BMI of the patients and controls were compared ($p = 0.33$, $p = 0.09$, $p = 0.19$, respectively). The comparisons of the mean FBS and HbA1C between the patients and control groups revealed a very highly statistically significant difference ($p<0.001$) with effect sizes of 2.23 and 2.26, respectively. The lipid profile analysis showed inconsistent findings, with statistically insignificant differences observed when comparing the mean serum cholesterol and HDL levels of the patients and controls ($p=0.09$, $p=0.29$). However, TG, LDL, and VLDL levels exhibited statistically significant differences, with highly significant disparities noted ($p=0.01$, 0.004, 0.003) when comparing the same two groups. The effect sizes of the comparisons mentioned later were as follows: 0.47, -0.55, and 0.57. Finally, the comparison of FIB revealed a highly statistically significant difference between the mean concentration levels of the patients and controls ($p<0.001$), with an effect size of 0.8. Additionally, CRP levels showed a statistically significant difference ($p=0.016$) between the two groups, with an effect size of 0.44 see Table 4.

Table 4: Description of Biochemical Marker P-Values (n=100)

Variable	Category	Mean	Standard Deviation	Standard Error	P-value	Cohen sd
Weight	Patients	85.344	11.5001	1.6264	0.09	-0.27
	Controls	88.920	14.9933	2.1204		
Height	Patients	165.320	9.4484	1.3362	0.33	-0.09
	Controls	166.180	9.8077	1.3870		
BMI	Patients	31.362	4.6729	0.6608	0.19	-0.17
	Controls	32.166	4.5818	0.6480		
HbA1c	Patients	8.428	1.7272	0.2443	<0.001	2.26
	Controls	5.590	0.4171	0.0590		
FBS	Patients	194.38	49.148	6.951	<0.001	2.23
	Controls	113.73	11.967	1.692		
Cho	Patients	185.16	42.638	6.030	0.09	-0.26
	Controls	195.24	34.043	4.814		
TG	Patients	148.82	56.165	7.943	0.01	0.47
	Controls	124.06	48.206	6.817		
HDL	Patients	43.00	14.885	2.105	0.29	-0.11
	Controls	44.44	10.857	1.535		
LDL	Patients	112.36	33.819	4.783	0.04	-0.55
	Controls	132.04	37.871	5.356		
VLDL	Patients	30.61	11.023	1.559	0.003	0.57
	Controls	24.71	9.630	1.362		
FIB	Patients	307.92	52.272	7.392	<0.001	0.8
	Controls	274.62	25.839	3.654		
CRP	Patients	4.162	3.0936	0.4375	0.016	0.44
	Controls	3.038	1.9232	0.2720		

The data presented in the table as means: $p\leq0.05$ is considered statistically significant, BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar, Cho cholesterol, TG triglycerides, HDL high density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen, CRP c-reactive protein

Conventional PCR was employed to amplify the DNA target regions within the *FTO* gene. The PCR products (225 base pairs) of the *FTO* 1 region, (183 base pairs) of the *FTO* 2 region and (297 base pairs) of the *FTO* 3 region were detected in all of the study samples. This indicates the presence of the target regions in the *FTO* gene. Genetic analysis of the results detected four registered variants: [5376966 T/A; rs1558902] were detected in the *FTO* 1 region [53782363 C/A; rs8050136] were detected in the *FTO* 2 region and [53786591 G/A; rs996289, 53786615 T/A; rs9939609] were detected in the *FTO* 3 region see Table 5.

Table 5: Previously Registered Variants that are Detected in Study Samples.

No.	Region	Variant Location	Allele	Consequence	Sample No.	Total samples
1	Intron	53769662	T/A	Intron Variant	1,3,4,5,6,7,9,11,12,13,14 15,16,17,19,21,22,23,24,25 27,28,29,31,33,34,35	28
2	Intron	53782363	C/A	Intron Variant	3,6,7,11,15	5
3	Intron	53786591	G/A	Intron Variant	1,3,4,5,6,9,10,11,12,14,15 16,17,19,22,23,24,25,27,28 29,32,33,34,36	25
4	Intron	53786615	T/A	Intron Variant	3,6,11,15,22	5

The effects of *FTO* gene variations on the study parameters (BMI, HbA1c, FBS, Cho, TG, HDL, LDL, VLDL, FIB, and CRP) were investigated by comparing the level of each parameter in the samples sharing the same variation. It was found that the variant 53769662 T/A of the *FTO* 1 region is statistically significantly associations with cholesterol serum levels (p=0.03). The results revealed that there were no other significant associations with the rest of the parameters see Table 6 and Table 7.

Table 6: Effects Of 53769662 T/A Variants on Study Parameters (Normally Distributed)

Parameters	53769662 T/A mutation	Mean±SD	Frequency	P value
Cho	Mutant	178.5±27.5	28	0.03*
	Non-mutant	205.3±36.1	8	
TG	Mutant	143.6±58.4	28	0.16
	Non-mutant	122.8±25.9	8	
HDL	Mutant	41.3±10.7	28	0.92
	Non-mutant	40.9±19.0	8	
LDL	Mutant	109.8±25.3	28	0.48
	Non-mutant	118.1±42.5	8	
VLDL	Mutant	28.7±11.7	28	0.17
	Non-mutant	24.6±5.4	8	
FIB	Mutant	288.9±54.2	28	0.58
	Non-mutant	277.4±35.7	8	

The data presented in the table as means: ±SD standard deviation of the mean $p \leq 0.05$ is considered statistically significant Cho refers to cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen

Table 7: Effects of 53769662 T/A Variants on Study Parameters (Non-normally Distributed)

Parameters	53769662 T/A mutation	Median±IQR	Frequency	P value
BMI	Mutant	31.6±5.8	28	0.27
	Non-mutant	34.1±6.4	8	
HbA1c	Mutant	7.1±3.6	28	0.99
	Non-mutant	7.1±5.5	8	
FBS	Mutant	155.6±103.0	28	0.99
	Non-mutant	155.6±158.0	8	
CRP	Mutant	2.7±2.9	28	0.72
	Non-mutant	3.5±2.6	8	
The data presented in the table as means: ±IQR the interquartile, p≤0.05 is considered statistically significant**,** BMI, refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar**,** CRP c-reactive protein				

It was found that the variant 53782363 C/A of the *FTO* 2 region is statistically significantly associations with the FIB and CRP serum levels (p=0.04 respectively). The results revealed that there were no other significant associations with the rest of the parameters (see Table 8 and Table 9).

Table 8: Effects Of 53782363 C/A Variants on the Study Parameters (Normally Distributed)

Parameters	53782363 C/A mutation	Mean±SD	Frequency	P value
Cho	Mutant	181.0±35.4	5	0.79
	Non-mutant	185.0±31.0	31	
TG	Mutant	156.0±48.1	5	0.45
	Non-mutant	136.3±54.3	31	
HDL	Mutant	39.2±9.3	5	0.66
	Non-mutant	41.5±10.7	31	
LDL	Mutant	104.4±31.5	5	0.56
	Non-mutant	112.8±29.5	31	
VLDL	Mutant	31.3±9.6	5	0.44
	Non-mutant	27.3±10.9	31	
FIB	Mutant	328.8±57.4	5	0.04*
	Non-mutant	279.5±46.7	31	
The data presented in the table as means: ±SD standard deviation of the mean**,** p≤0.05 is considered statistically significant**,** Cho refers to cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen				

Table 9: Effects of 53782363 C/A Variants on the Study Parameters (Non-normally Distributed).

Parameters	53782363 C/A mutation	Median±IQR	Frequency	P value
BMI	Mutant	31.0±10.5	5	0.86
	Non-mutant	32.7±6.9	31	
HbA1c	Mutant	7.1±4.0	5	0.89
	Non-mutant	6.7±2.3	31	
FBS	Mutant	157.1±115.0	5	0.89
	Non-mutant	145.6±66.0	31	
CRP	Mutant	3.6±2.9	5	0.04*
	Non-mutant	1.4±1.9	31	
The data presented in the table as means: ±IQR the interquartile, p≤0.05 is considered statistically significant**,** BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar**,** CRP c-reactive protein				

There were no statistically significantly associations between the 53786591 G/A of the *FTO* 3 region and all the parameters see Table 10 and Table 11.

Table 10: Effects Of 53786591 G/A Variants on Study Parameters (Normally Distributed).

Parameters	53786591 G/A mutation	Mean±SD	Frequency	P value
BMI	Mutant	32.9±5.7	25	0.49
	Non-mutant	31.6±2.8	11	
Chol	Mutant	182.1±31.4	25	0.51
	Non-mutant	189.7±31.4	11	
TG	Mutant	142.6±56.2	25	0.55
	Non-mutant	130.8±47.6	11	
HDL	Mutant	42.9±11.4	25	0.14
	Non-mutant	37.3±7.4	11	
LDL	Mutant	112.6±30.5	25	0.76
	Non-mutant	109.3±28.2	11	
VLDL	Mutant	28.5±11.2	25	0.55
	Non-mutant	26.2±9.6	11	
FIB	Mutant	286.7±51.5	25	0.95
	Non-mutant	285.5±50.4	11	
The data presented in the table as means: ±SD standard deviation of the mean, p≤0.05 is considered statistically significant, BMI refers to body mass index, Cho cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen				

Table 11: Effects Of53786591 G/A Variants on Study Parameters (Non-Normally Distributed).

Parameters	53786591 G/A mutation	Median±IQR	Frequency	P value
HbA1c	Mutant	7.3±3.4	25	**0.54**
	Non-mutant	8.1±3.6	11	
FBS	Mutant	145.6±96.0	25	**0.54**
	Non-mutant	185.7±103	11	
CRP	Mutant	2.4±2.8	25	**0.17**
	Non-mutant	4.0±2.3	11	
The data presented in the table as means: ±IQR the interquartile range, p≤0.05 is considered statistically significant, HbA1c refers to hemoglobin A1c, FBS fasting blood sugar, CRP c-reactive protein				

There were no statistically significantly associations between the 53786615 T/A of the *FTO* 3 region and all the parameters see Table 12 and Table 13.

Table 12: Effects Of 53786615 T/A Variants on Study Parameters (Normally Distributed).

Parameters	**53786615 T/A mutation**	**Mean±SD**	**Frequency**	**P value**
Cho	Mutant	189.6±35.2	5	**0.70**
	Non-mutant	183.6±31.0	31	
TG	Mutant	164.6±42.0	5	**0.25**
	Non-mutant	134.9±54.3	31	
HDL	Mutant	43.2±6.7	5	**0.65**
	Non-mutant	40.9±11.1	31	
LDL	Mutant	118.4±32.9	5	**0.59**
	Non-mutant	110.5±29.3	31	
VLDL	Mutant	33.0±8.4	5	**0.25**
	Non-mutant	27.0±10.9	31	
FIB	Mutant	323.2±50.7	5	**0.08**
	Non-mutant	280.4±48.6	31	
The data presented in the table as means: ±SD standard deviation of the mean, p≤0.05 is considered statistically significant, Cho cholesterol, TG triglycerides, HDL high-density lipoprotein, LDL low-density lipoproteins, VLDL very-low-density lipoprotein, FIB fibrinogen				

Table 13: Effects of 53786615 T/A Variants on Study Parameters (Non-Normally Distributed).

Parameters	**53786615 T/A mutation**	**Median±IQR**	**Frequency**	**P value**
BMI	Mutant	29.6±10.1	5	**0.30**
	Non-mutant	32.7±5.7	31	
HbA1c	Mutant	6.7±2.6	5	**0.79**
	Non-mutant	7.1±3.9	31	
FBS	Mutant	145.6±76.0	5	**0.79**
	Non-mutant	157.1±112.0	31	
CRP	Mutant	1.4±4.5	5	**0.16**
	Non-mutant	3.0±2.7	31	
The data presented in the table as means: ±IQR the interquartile range, p≤0.05 is considered statistically significant, BMI refers to body mass index, HbA1c hemoglobin A1c, FBS fasting blood sugar, CRP c-reactive protein				

Discussion

Obesity raises the risk of several common diseases, making it an important global health concern. It's unclear whether hereditary factors contribute to obesity. A common mutation in the *FTO* (fat mass and obesity associated) gene, which predisposes to diabetes through an influence on body mass index, was found during a genome-wide search for genes linked to type 2 diabetes susceptibility(Frayling et al., 2007). A typically elevated triglyceride deposition and the generation of hepatic glucose can result from enhanced *FTO* expression, which can also promote de novo lipogenesis, decrease lipolysis and fatty acid oxidation, and boost gluconeogenesis(Witka et al., 2019). These results imply that *FTO* is connected to the regulation of both body weight and glucose metabolism. While there is no doubt that variations in the *FTO* gene are linked to type 2 diabetes and obesity, the biological role of *FTO* remains unclear(Gerken et al., 2007; Han et al., 2010). Finally, we believe that the results of this study, particularly the four variants in the *FTO* gene's *FTO* 1 regions (53769662 T/A), *FTO* 2 regions (53782363 C/A) and *FTO* 3 regions (53786591 G/A and 53786615 T/A) could be significant in the field of *FTO* gene studies. The presence of these variants in important coding regions suggests their potential importance.

Furthermore, among four variants, two variants (53769662 T/A and 53782363 C/A) might be the most significant, as they exhibited a significant effect on certain study parameters. Where the variant 53769662 T/A showed a significant effect on the level of cholesterol in the blood, the variant 53782363 C/A showed a significant effect on the serum levels of FIB and CRP. To precisely identify their role in Type 2 Diabetes Mellitus patients, further studies are needed in future research. It must be noted that the study results were limited by the relatively small sample size of the patients and controls, suggesting the need for large-scale studies to corroborate the results and validate the findings.

Ethics approval and consent to participate

The Institutional Ethics Committee in the Department of Clinical Laboratories / College of Applied Medical Sciences/ University of Kerbala approved this study (IQ.UOK.CAMS.DCL.REC.2). Informed consent was taken from every patient in their language regarding willingness to participate in the study. Patient confidentiality was maintained during all research procedures.

Author contributions

Both authors have contributed to the writing and approved the manuscript before submission.

Conflicts of interest

There are no conflicts of interest.

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The Association of Five Novel Variants of *TLR7* Gene with Some Biochemical Markers in Breast Cancer Patients from Iraqi Women

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Abstract

Background: The TLR7 gene carries multiple polymorphisms that are likely associated with human diseases, including cancer. The study aimed to shed light on the relationship between the variation of the TLR7 gene and serum level of biochemical markers (CA15-3, CEA, CA125, and CA27-29) in Iraqi women with breast cancer.

Methods: a case-control study involving 100 women volunteers: 50 with breast cancer as a patient group and 50 who appeared to be healthy as a control group. The ages of all participants were ranged between 29 to 75 years. This study was conducted from November 2022 to April 2024 at the Department of Clinical Laboratories, College of Applied Medical Sciences, University of Kerbala . Sanger sequencing was used to investigate variants of the TLR7 gene. The enzyme-linked immunosorbent assay (ELISA) method was used to evaluate the levels of CA15-3, CEA, CA125, and CA27-29 in serum.

Results: Presence of 5 novel unregistered variants in the Intron 2 region. It was found that a significant effect of TLR7 - 12871749 G\C variant (SNP) of the Intron region on serum level of CEA (point biserial correlation coefficient =-0.396, p-value=0.03). A significant effect of TLR7 - 12871764 A\G variant of the Intron region on serum level of CA 15-3 (point biserial correlation coefficient =-0.385, p-value=0.03). The results showed no significant, weak, moderate, or strong associations between all other types of variants when tested individually and the four biochemical markers under study.

Conclusion: Among the five new variations, 12871749 G\C and 12871764 A\G may be the most significant variants because there is a statistical association with some biochemical markers, therefore, Future research should delve deeper into the role of TLR7 polymorphisms in the fields of tumor immunology, which may open new perspective in early diagnosis and prevention of cancer.

ارتباط خمسة متغيرات جديدة في جين *TLR7* مع بعض المعلمات الكيموحيوية عند مرضى سرطان الثدي من النساء العراقيات

زهراء ماجد جاسم , جودت نوري غائب الجشعمي

الخلاصة

المقدمة: يحتوي جين TLR7 أشكالًا متعددة من المحتمل أن تكون مرتبطة بالأمراض التي تصيب الإنسان مثل السرطان. هدفت الدراسة إلى إلقاء الضوء على العلاقة بين تباين جين TLR7 و مستوى بعض المؤشرات الكيموحيوية (CA15-3، CEA، CA125، وCA27-29) لدى مرضى سرطان الثدي من النساء العراقيات.

المواد وطرق العمل: دراسة الحالات والشواهد التي تشمل 100 امرأة متطوعة: 50 امرأة مصابة بسرطان الثدي كمجموعة مرضى و50 امرأة تبدو بصحة جيدة كمجموعة ضابطة، تتراوح أعمار جميع المشاركين بين 29 الى 75 عاماً. أجريت الدراسة في الفترة من تشرين الثاني 2022 إلى نيسان 2024 في قسم المختبرات السريرية، كلية العلوم الطبية التطبيقية، جامعة كربلاء. تم التحقيق في تباين جين TLR7 بواسطة طريقة التسلسل Sanger. تم قياس مستويات المعلمات الكيموحيوية في مصل الدم بواسطة طريقة (ELISA).

النتائج: حددت نتائج الدراسة الحالية وجود 5 متغيرات جديدة غير مسجلة في منطقة Intron 2 لجين TLR7. من بين هذه المتغيرات كان هناك فروق ذات دلالة احصائية ($P\leq0.05$) بين (TLR7 - 12871749 G\C SNP) ومستوى CEA في المصل , وبين (TLR7 - 12871764 A\ SNP) ومستوى CA 15-3 , ولم تكن هناك ارتباطات احصائية ضعيفة أو متوسطة أو قوية بين جميع أنواع المتغيرات الأخرى عند اختبارها بشكل فردي مع المعلمات الكيموحيوية الأربعة قيد الدراسة.

الاستنتاج: قد يكون المتغيران 12871749 G\C و 12871764 A\G من اهم المتغيرات لوجود ارتباط احصائي مع بعض المعلمات الكيموحيوية , لذلك ينبغي أن تتعمق الأبحاث المستقبلية في دور تعدد أشكال TLR7 في مجالات علم المناعة المتعلق بالأورام ، والتي قد تفتح آفاقا جديدة في التشخيص المبكر والوقاية من السرطان.

1. Introduction

Toll-like receptors (TLRs) are a type of pattern recognition receptor (PRR), proteins that enhance immunity by identifying damage-associated molecular patterns (DAMP) and pathogen-associated molecular patterns (PAMP) (Amarante-Mendes et al., 2018). TLR agonists, including TLR7, stimulate cytokine responses that promote antitumor immunity by enhancing CD8þ T-cell activation, increasing myeloid-derived suppressor cells (MDSC) maturation, and T-regulatory cells (Treg) inhibition (Tang et al., 2013; Spinetti et al., 2016). In the human body, there are ten TLR members, some of which are located on the cell membrane (TLR1, TLR2, TLR4, TLR5, and TLR6), while others in endosomes (TLR3, TLR7, TLR8, and TLR9) (Chi et al., 2017). One member of the TLRs family is TLR7, encoded by the TLR7 gene located on the short arm of the X chromosome (Kemball et al., 2010; Kutikhin, 2011). The TLR signaling pathway is expressed in both the tumor and immune cell types, which significant for stimulating immune system responses. so, may be associated with developing forms of malignancies (Singh et al., 2023). Normal cells can turn into cancer cells by mutation or other genetic reasons resulting in loss the normal cell cycle (Al-mosawy et al., 2020). Breast cancer is an extremely significant public health problem that affects many women worldwide (Abdulridha Al-Ganimi & Abd Al-Salam, 2023). Breast cancer (BC) is a complex disease due to its diverse morphological features, variable clinical outcomes, and response to different therapeutic options (*KHALID*, 2017). The risk factors of this cancer include aging, reproductive patterns, history of menarche, breast features, hormone use, tobacco and alcohol consumption, body routines, and diet. In the early stages, there are few indications or symptoms, so early detection is an essential method to improve outcomes (Fillatreau et al., 2021). Early detection of breast cancer leads to longer-term reductions in mortality rates, so detecting cancer cells in their early stages is essential for a better prognosis. Increased data suggests a significant relationship between TLRs and breast cancer progression (Singh et al., 2023). Breast cancer genesis and progression is a complicated, multi-step process involving many genetic and epigenetic changes. External environmental variables and internal cellular microenvironmental cues impact the development of the alterations that cause tumorigenesis (Thakur et al., 2022). The study aimed to shed light on the relationship between TLR7 gene polymorphisms and serum levels of biochemical markers [Cancer Antigen 15-3) CA 15-3), Carcinoembryonic Antigen (CEA), Cancer Antigen 125 (CA125), and Cancer Antigen 27- 29 (CA 27-29)] in Iraqi women with breast cancer.

2. Material, Patients and Method

2.1. Patients

This study included 100 women volunteers: 50 with breast cancer as a patient group and 50 who appeared to be healthy as a control group. The ages of all participants were ranged between 29 to 75 years.

2.2. Study Design

The present study was designed as a Case-Control study. The study design has two parts: the first part is related to the biochemical study and includes: biochemical assays [Cancer Antigen 15-3 (CA15-3), Carcinoembryonic Antigen (CEA), Cancer Antigen 125 (CA125), and Cancer Antigen 27- 29 (CA 27-29)] while the second part is related to the genetic study and includes: DNA Extraction, agarose gel electrophoresis, primer desion, PCR, and sequencing. This study was conducted from November 2022 to April 2024.

2.3. Ethics Approval and Consent to Participate

This study was authorized by the Institutional Ethics Committee of Clinical Laboratories, College of Applied Medical Sciences, University of Kerbala, (IQ.UOK.CAMS.DCL.REC.1). Each patient provided informed permission in their

original language, indicating their desire to participate in the study. Patient confidentiality was preserved throughout the research process.

2.4. Blood Collection

The samples of blood were collected from the volunteers at the Al-Imam Al-Hussein Center for Oncology and Hematology in Karbala, Iraq. Five milliliters (5ml) of the venous blood sample from each participant was obtained and separated into two parts: two milliliters (2ml) in EDTA tubes for DNA extraction and three milliliters (3ml) in gel tubes for biochemical tests. Disposable syringes were used to draw the blood in sterile conditions. The blood collected in gel tubes was centrifuged to extract serum, which was subsequently used in biochemical assays [Cancer Antigen 15-3 (CA 15-3), Carcinoembryonic Antigen (CEA), Cancer Antigen 125 (CA125) and Cancer Antigen 27- 29 (CA 27-29)] using enzyme-linked immunosorbent assay (ELISA) kits. Special research kits (sandwich ELISA kits) for these four biochemical markers from BT LAB Company, China were used.

2.5. DNA Extraction Kit

In order to extract DNA from the obtained blood, the EDTA tube was frozen. The ReliaPrep™ Blood gDNA Miniprep System (Promega Company, USA) used to extract DNA from blood samples. The DNA extraction kit consists of Binding Buffer (BBA), Cell Lysis Buffer (CLD), Collection Tubes (2ml), Column Wash Solution (CWD), Nuclease-Free Water, Proteinase K Solution (PK), and Binding Columns. It was used to isolate 100 genomic DNA isolates from 300 µl of the whole blood for each sample.

2.6. Molecular Detection

The total volume used in PCR reaction in this study was 25 µl and included 5 µl DNA, 2 µl from each primer, 8 µl nuclease-free water, and 8 µl master mix (a ready-to-use mixture of Easy Taq DNA Polymerase, dNTPs, and optimized buffer) from Promega Company, USA as described in Table 1. The target sequence of the Intron 2 region in the TLR7 gene was amplified using a specific PCR program as shown in Table 2.

Table 1: Primers Designed for TLR7 Gene Amplification in the Intron 2 Region of this Study

Primer name		Primer sequence	PCR product size
Intron 2	*TLR7*-Forward	5'-CATGGTGATGATGACAGCAA-3'	580 base pair
	TLR7-Reverse	5'-GGCCACTCAAGGACAGAACT-3'	

Table 2: The PCR Program.

Steps	Stage	Cycle	Step	Temp.	Time
Initial denaturation	1	1	1	94.0	4:00
Denaturation	2	35	1	94.0	0:30
Annealing			2	57.0	0:45
Extension			3	72.0	0:30
Final extension	3	1	1	72.0	5:00
Hold			2	4.0	HOLD

The PCR product bands were separated using agarose gel electrophoresis on a 1% agarose gel dyed with fluorescent Red Safe dye (Promega Company, USA). The gel electrophoresis system was set for 60 minutes at 70 volts, an UV transilluminator was used to see the gel Fig.1. The Primer3Plus bioinformatics program was utilized to design the primers

(Forward and Reverse Primer for the Intron 2 region of TLR7 Gene) for this investigation (Figure 2; Figure 3). The primers were ordered from Alpha DAN, S.E.N.C. in (Montreal, Quebec, Canada), and synthesized on automated computer-controlled synthesizers using standard phosphonamidite chemistry.

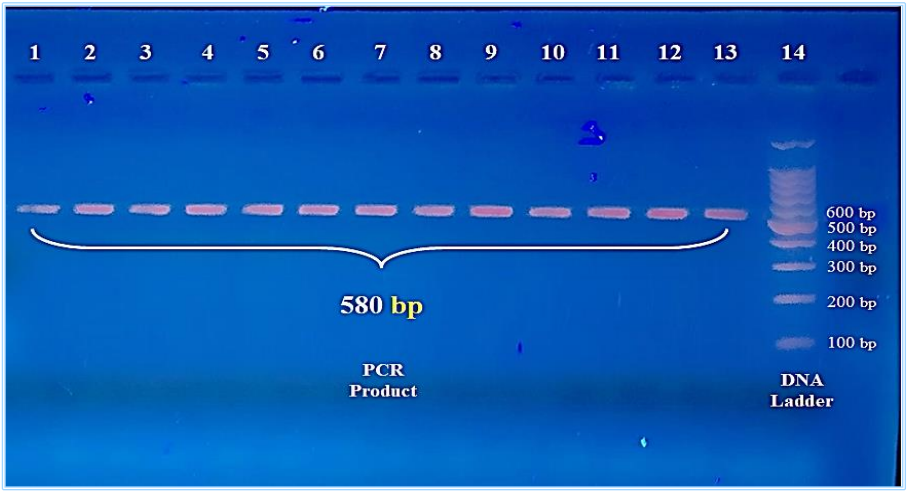

Figure 1: PCR Products (580 Base Pair) Detected in Study Samples, Indicating That the Target Region (Intron 2) Is Present In The TLR7 Gene (The Mentioned Figure Explain And Shows Our Study PCR Products Results)

2.7. Nucleotides Sequencing and Analysis:

PCR products for 20 samples of the patient group were sent to Alpha DNA (S.E.N.C.) Corporation in Montreal, Quebec, Canada, for nucleotide sequencing. A sequencing data were manually analyzed using bioinformatics tools, compared to human reference gene sequences that had already been uploaded to the National Center for Biotechnology Information (NCBI). A basic Local Alignment Search Tool (BLAST), one tool of NCBI, is used to perform alignments Fig.2. Molecular Evolutionary Genetics Analysis X (MEGAX) was employed to examine the sequenced region of the target gene. Multiple sequence alignments were carried out using the CLUSTALW program to verify existence variants identified by the BLAST tool. The variants' location in sequenced DNA samples found in the current study were reported and examined using tools from the Ensemble Genome Browser tools to identify the type of variant and forecast the functional implications Fig.3. The previously described browser was also used to identify new (novel) variations found in this study.

2.8. Statistical Analysis

Statistical analysis was carried out using SPSS version 22.0 (SPSS, IBM Company, Chicago, IL, USA). Data was expressed as means ± standard error (SE) normally distributed and were compared using the independent samples T-test. Data was expressed as a median ± Interquartile range (IQR) if non-normal distributed and were compared using the Mann-Whitney U test. The P-values ≤ 0.05 were considered statistically significant differences. The Kruskal-Wallis test and one-way analysis of variance were also used. Cohen's D describe the effect size used to indicate the standardized difference between the two means. Also, use the point biserial correlation coefficient (r_{pb}).

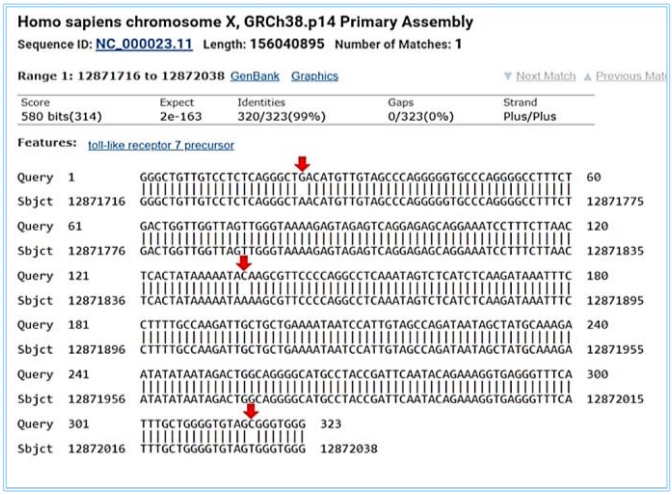

Figure 2: Alignment of Sequence by The BLAST Tool
(The Mentioned Figure Explain and Shows Our Study PCR Products Results)

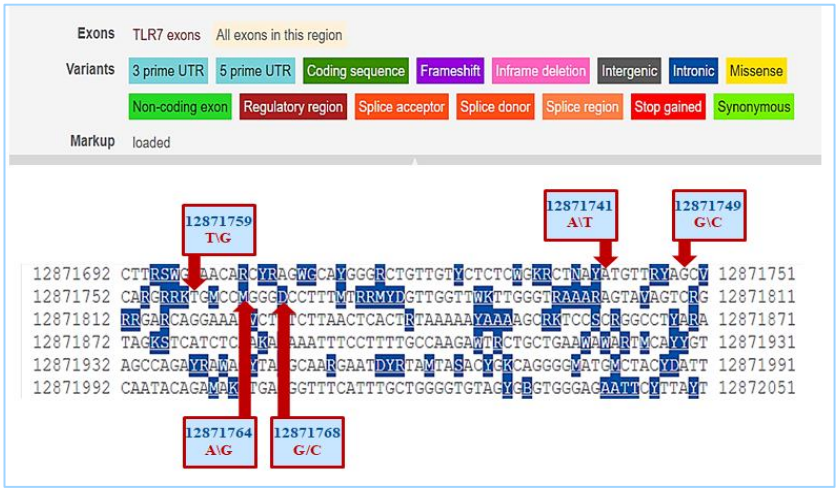

Figure 3: The Molecular Location of Newly Detected, Non-Registered Variations in Study Samples

3. Results

The results of this study showed that there were statistically significant differences when the serum levels of the four tumor markers namely, CEA, CA 125, CA 15-3, and CA 27-29 were compared between control and patient groups ($p<0.001$). The calculated Cohen's D for the mentioned four comparisons of -0.861, -0.753, -0.705, and -1.021 suggests a large-sized effect, indicating that there was a practical and meaningful difference in the mean serum levels of the four tumor markers under study. The advantage was in favor of the patient group, highlighting it was potential significance for the differentiation between controls and patients Table 3.

Table 3: The Description of Mean Values for Biochemical Markers by Using Cohen's D (N=100).

Biochemical marker	Category	Frequency	Mean	Std. Error Mean	p-value	Cohen's D
CEA Pg/ml	Control	50	237.23	12.19	<0.001	-.861
	Patient	50	317.07	13.95		
CA 125 Ku/ml	Control	50	13.04	0.75	<0.001	-.753
	Patient	50	21.84	2.21		
CA 15-3 U/ml	Control	50	77.17	5.12	<0.001	-.705
	Patient	50	164.77	24.3		
CA 27-29 U/ml	Control	50	28.715	1.15	<0.001	-1.021
	Patient	50	46.42	3.27		

Conventional PCR used to amplify the target region of DNA (the Intron 2 region of the TLR7 gene), which includes (580 base pair). PCR products (580 bp) of the Intron 2 region were detected in all study samples, indicating the presence of the target regions in the TLR7 gene. The genetic analysis of the 20 samples showed that the 6 out of the 20 samples demonstrated double variations Table 4, and the rest of the 14 samples displayed multiple variations Table 5 in their intron 2 region sequences Fig.3 in the methods section.

Table 4: Double Variants on the Intron2 Region Appeared in the Study Samples

Sample#	Number of Variations	Variants Location			
5	2	A\G	12871738	A\T	12871741
9		A\T	12871741	G\C	12871749
14		A\G	12871738	A\C	12871850
17		T\G	12871759	T\A	12871888
19		A\G	12871738	A\C	12871850
20		A\G	12871738	A\C	12871850

Table 5: Multiple Variants on Intron2 Region Appeared in the Study Samples

Sample#	Number of Variations	Variants Location					
1	3	A\G	12871738	G\C	12871749	A\T	12871741
2	5	A\G	12871738	G\C	12871768	G\C	12871749
		A\T	12871741	A\C	12871850		
3	7	A\C	12871850	T\G	12871759	G\C	12871749
		A\G	12871738	A\G	12871764	G\C	12871768
		A\T	12871741				
4	3	A\G	12871738	G\C	12871749	A\T	12871741
6	5	A\G	12871738	A\G	12871764	T\G	12871759
		A\T	12871741	T\A	12871888		
7	6	A\G	12871738	A\G	12871764	G\C	12871749
		A\T	12871741	G\C	12871768	A\C	12871850
8	3	G\C	12871749	T\A	12871888	T\G	12871759
10	4	A\T	12871741	G\C	12871749	T\G	12871759
		G\C	12871768				
11	5	A\G	12871738	A\G	12871764	T\G	12871759
		G\C	12871749	A\C	12871850		
12	7	T\G	12871759	G\C	12871749	A\T	12871741
		A\G	12871764	A\C	12871850	T\A	12871888
		A\G	12871738				
13	4	A\G	12871738	A\G	12871764	A\C	12871850
		G\C	12871749				
15	4	A\G	12871738	T\G	12871759	A\C	12871850
		G\C	12871749				
16	5	A\G	12871738	A\C	12871850	G\C	12871768
		T\G	12871759	T\A	12871888		
18	6	G\C	12871768	A\G	12871764	T\G	12871759
		A\G	12871738	A\C	12871850	T\A	12871888

The genetic analysis detected Five novel non-registered variants in the Intron 2 region: [12871741 A\T, 12871749 G\C, 12871759 T\G, 12871764 A\G, 12871768 G\C] Table 6; Fig.3 in the. methods section

Table 6: Novel Non-Registered Variations Detected in the Study Samples.

Region	Variants Location	Allele	Sample#	Total
Intron2	12871759	T\G	3,6,8,10,11,12,15,16,17,18	10
	12871749	G\C	1,2,3,7,8,9,10,11,12,13,15	11
	12871741	A\T	1,2,3,5,6,7,9,10,12	9
	12871764	A\G	3,6,7,11,12,13,18	7
	12871768	G\C	2,3,7,10,16,18	6

The effect of TLR7 gene variants on biochemical markers CEA, CA125, CA15-3, and CA27-29 was studied. This was done by comparing the levels of each biochemical marker in the samples pooled with a number of mutations. The results of the samples were divided into three categories according to the number of mutations in each sample. The first category includes the samples that contain fewer than 3 mutations, the second category includes the samples that contain 3 to 5 mutations, and the third category includes the samples that contain more than 5 mutations. The results showed the effect of TLR7 gene variations on serum levels of CEA, CA125, CA15-3, and CA27-29 in the Intron 2 region. Non-significant differences between the three categories (<3, 3-5, and >5 mutations) in the level of CEA, CA 125, CA 15-3, and CA 27-29 with p-value 0.56, 0.89, 0.14, and 0.69, respectively Table 7.

Table 7: Comparison of Biochemical Markers Between Different Groups for The Intron 2 Region (Three Groups Divided Based on The Number of Mutations In Each Sample).

No. mutations	Total Samples	CEA (Median±IQR)	CA 125 (Median±IQR)	CA 15-3 (Median±IQR)	CA 27-29 (Mean±SE)
<3	9	271.26±162.4	11.2±14.8	65.3±24.4	45.59±6.67
3-5	7	276.2±97.2	11.1±2.6	112.5±218.7	38.44±8.85
>5	4	339.7±170.0	21.3±24.0	93.5±187.2	49.0±9.35
P-value		0.56	0.89	0.14	0.69
The results indicated a non-significant difference by using the Kruskal-Wallis test, and one-way ANOVA					

The result of samples was divided into three groups based on the status of variations present (either previously registered variations or new, non-registered variations). The first group (New) included samples that contained new, non-registered (novel) variations only, the second group (Mixed) included samples that contained previously registered variations and new, non-registered variations, while the third group includes previously registered variations only. The result showed non-significant differences between the new, mixed, and old variations in the level of CEA, CA125, CA15-3, and CA27-29, with a p-value 0.64, 0.87, 0.35, and 0.92, respectively Table 8.

Table 8: Differences in Mean Values of Biochemical Markers According to Different Variations in Intron (N=20)

Variations status	N.	CEA (Mean±SE)	CA 125 (Median±IQR)	CA 15-3 (Median±IQR)	CA 27-29 (Mean±SE)
New	2	271.0±17.81	11.12±0	81.94±0	43.29±18.19
Mixed	15	293.95±27.81	11.62±20.5	81.77±52.5	44.76±5.9
Old	3	356.5±96.45	11.15±0	58.82±0	39.15±4.61
P-value		0.64	0.87	0.35	0.92
The results indicated no significant difference by Kruskal-Wallis test and one-way ANOVA					

A point biserial correlation coefficient (r_{pb}) was conducted to examine the relationship between each SNP (Single Nucleotide Polymorphism) with the four biochemical markers (CEA, CA125, CA15-3, CA27-29). SNP A/G (12871764) of the Intron 2 region was moderately associated with the CA 15-3 biochemical marker levels (point biserial correlation coefficient = -0.385), and the two variables were statistically significantly associated (p = 0.036). SNP G/C (12871749) of the Intron 2 region was moderately negatively and statistically significantly correlated with the CEA biochemical marker serum levels (point biserial correlation coefficient = -0.396, p = 0.03).

4. Discussion

TLR7 gene polymorphism significance for oncogenomic (a sub-field of genomics that characterizes cancer-associated genes) remains unclear. The TLR7 gene contains significant functional polymorphisms that may impact not only the malignant susceptibility but may also affect responsiveness to therapy. When TLR7 is activated, it promotes B-cell differentiation, which increases the production of autoantibodies and stimulates the production of type I interferon and cytokines, enhancing the immune response (Al-Humairi et al., 2019; Fillatreau et al., 2021).

TLR7 stimulation in tumor-bearing hosts activates antitumoral immunity that can improve disease outcomes in numerous malignancies (Spinetti et al., 2016). Other studies have revealed that TLR7 can induce apoptosis in certain cancer cells, playing an antitumor role. TLR 7 agonists activate innate immune cells, humoral and cellular immunity, and thus produce anti-tumor activities (Chi et al., 2017). TLR7 can be used as novel diagnostic biomarkers, progression and prognostic indicators, and immunotherapeutic targets for cancer. TLR7 agonists have been investigated as possible treatments for immune therapy that targets tumors (Sun et al., 2022).

On the other hand, multiple studies support the notion that TLRs are cancer activators. The expression of TLRs in cancer cells indicates that TLR-mediated signaling is important in cancer tumor progression. TLRs are extensively expressed in breast cancer cells, and activation of these receptors can result in cancer cell proliferation, invasion, migration, and metastasis. Furthermore, When TLR binds to cancer cells, it leads to increased production of immune-suppressive cytokines (Singh et al., 2023), suggesting that tumor cells may use TLR activation to escape from tumor immune surveillance (Kidd et al., 2013; Semlali et al., 2017). The significance of multiple TLR expression in tumor cells is not completely unknown, so more research is needed into the gene variety of TLRs in cancer. Few previous studies were done about the relationship and effect of TLR7 gene polymorphisms on breast cancer. Among the previously registered TLR7 gene variants that were discovered in this study is: rs179019 (X:12871850 A\C), which no previous studies revealed an association between this variant and breast cancer, But it has been significantly associated with other diseases, such as the study by (Ranjan & Panda, 2023) that showed a significant association between the rs179019 variant and susceptibility to SLE development. This association might be explained by the overproduction of pro-inflammatory cytokines in addition to type I Interferon. Also, the rs179020 (X:12871738 A\G), associated with some diseases has been discovered, but its relationship to breast cancer has not been proven, such as the study by (Traks et al., 2015) that showed a significant association between the rs179020 variant and a vitiligo disease (Galimova et al., 2017) in a study on psoriasis disease, revealed a significant association between the risk of developing this disease with the rs179020 variant (Al-Humairi et al., 2019) showed a significant association between this variant with a potential risk for urinary bladder cancer (UBC).The results presented from this study highlighted the importance of the Intron 2 region in the TLR7 gene by detecting novel variants that may affect on the breast cancer prognosis and response to treatment. Notably, five novel variants found in the *TLR7* gene's Intron 2 region (12871741 A\T, 12871749 G\C, 12871759 T\G, 12871764 A\G, 12871768 G\C), may become more significant in the field of *TLR7* gene studies because these mutations exist in a crucial coding region. To precisely identify their role in breast cancer patients, further studies are needed. It must be noted that the results of this study were limited by the relatively small sample size of patients and controls, suggesting the need for large-scale studies to corroborate the results and validate the findings.

Conclusions

Based on the results of the present study, the study found a significant effect of *TLR7*- 12871749 G\C variant of the Intron region on serum level of CEA, a significant effect of *TLR7*- 12871764 A\G variant of the Intron region on serum level of CA 15-3 and the two variants, 12871749 and 12871764, might be the most important variants among the five novel variants.

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Evaluation Of E-Selectin Levels in Iraqi Patients with Acute Coronary Artery Syndrome

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Abstract

Background: There is a great deal of mortality and morbidity associated with various cardiovascular diseases that comprise acute coronary syndrome (ACS). One crucial factor in the development of ACS is inflammation of the coronary plaque. Cell adhesion molecules play a key role in the inflammatory cascade. The vascular endothelium is directly affected by elevated levels of pro-inflammatory cytokines and other systemic inflammatory markers in ACS related to atherogenesis. This causes an increase in the expression of adhesion molecules, such as selectins.

Objective: This study aims to document the inflammatory response after acute coronary syndrome by evaluating the association between serum E-selectin levels and the risk and severity of acute coronary syndrome.

Materials and Methods: A case-control study involving 120 male subjects aged 41–70 years, who were divided into two groups: 60 ACS patients and 60 healthy individuals as a control. Serum E-selectin levels were measured using an ELISA technique.

Results: The study revealed a significant increase in serum E-selectin levels when comparing patients to the healthy control group (216.07±20.26 pg/ml Vs 179.74±53 pg/ml, $P \leq 0.0001$) respectively. The analysis of the receiver operating curve (ROC) for E-selectin showed a sensitivity of 85%, a specificity of 70%, a 95% confidence interval (CI) of 0.673-0.863, and the area under the curve (AUC) was 0.768. The cut-off point was set at 197.37 pg/ml or higher.

Conclusion: Elevated serum E-selectin levels in ACS patients suggest a potential role for adhesion molecules in the pathogenesis of ACS. Adhesion molecules could be considered as a biochemical marker for assessing ACS.

تقييم مستويات السليكتين E لدى المرضى العراقيين المصابين بمتلازمة الشريان التاجي الحادة

سيف سعيد حاتم؛ فاضل جواد ال طعه ؛ وهناء عدائي علي ؛ حنين سعيد الموسوي

الخلاصة

المقدمة :هناك نسبة كبيرة من الوفيات المرتبطة بأمراض القلب والأوعية الدموية المختلفة التي تشمل متلازمة الشريان التاجي الحادة .(ACS) أحد العوامل الحاسمة في تطور ACS هو الالتهاب في اللويحات التاجية. تلعب جزيئات الالتصاق الخلوي دورًا رئيسيًا في تسلسل الالتهاب. يتأثر بطانة الأوعية الدموية بشكل مباشر بمستويات مرتفعة من السيتوكينات المحفزة للالتهاب وغيرها من علامات الالتهاب الجهازية في ACS المتعلقة بتكون تصلب الشرايين. وهذا يسبب زيادة في تعبير جزيئات الالتصاق، مثل السليكتينات .

الهدف: تهدف هذه الدراسة إلى توثيق الاستجابة الالتهابية بعد متلازمة الشريان التاجي الحادة من خلال تقييم العلاقة بين مستويات السليكتين E في الدم وخطر وشدة متلازمة الشريان التاجي الحادة.

المرضى وطرق العمل :دراسة حالة-شاهد شملت 120 فرداً من الذكور الذين تتراوح أعمارهم بين 41-70 سنة، تم تقسيمهم إلى مجموعتين: 60 مريضًا بـ ACS و60 فردًا سليمًا كعينة تحكم. تم قياس مستويات السليكتين E في الدم باستخدام تقنية.ELISA

النتائج :كشفت الدراسة عن زيادة كبيرة في مستويات السليكتين E في الدم عند مقارنة المرضى بمجموعة التحكم السليمة 216.07±20.26) بيكوغرام/مل مقابل 179.74±53 بيكوغرام/مل، .($P \leq 0.0001$أظهر تحليل منحنى تشغيل المستقبل (ROC) للسليكتين E حساسية بنسبة 85%، وخصوصية بنسبة 70%، وفترة ثقة 95% (CI) بين 0.673-0.863، وكان مساحة تحت المنحنى .0.768 (AUC) تم تحديد نقطة القطع عند 197.37 بيكوغرام/مل أو أعلى.

الاستنتاج: تشير زيادة مستويات السليكتين E في الدم لدى مرضى ACS إلى دور محتمل لجزيئات الالتصاق في التسبب في متلازمة الشريان التاجي الحادة. يمكن اعتبار جزيئات الالتصاق كعلامة كيميائية حيوية لتقييم.ACS

1. Introduction

Acute coronary syndrome (ACS) is a condition characterized by reduced blood flow in the coronary arteries, leading to dysfunction or death of the heart muscle (Martelli et al., 2021), (Kimura et al., 2019). Common symptoms include chest pain resembling pressure, nausea, and sweating (Jasim et al., 2023). There are three subtypes of ACS: ST-elevation myocardial infarction (STEMI), unstable angina, and non-ST elevation myocardial infarction also known as NSTEMI, which can be distinguished by changes in the electrocardiogram (ECG) and blood tests (McGarry and Shenvi, 2021). STEMI occurs when a coronary artery is completely blocked, NSTEMI occurs when it is partially blocked, and unstable angina occurs when there is ischemia without cell damage or necrosis(Mihajlović et al., 2020), (Meyers et al., 2021). It is crucial to differentiate ACS from stable angina, which worsens with exertion or stress but improves with rest (Al-Tu'ma et al., 2016). Angina, whether new-onset or unstable, can strike suddenly, usually while at rest or with very little effort (Lindow et al., 2021). The adhesion molecules that selectins provide to the immune system of mammals are vital during tissue healing and the inflammatory response (Kristensen et al., 2022). Selectins are glycoproteins that serve as adhesion molecules that are vital to the immune system of mammals, particularly during inflamed response and tissue regeneration (Selvaraj et al., 2022). Cells in the cardiovascular system that bind to members of the selectin family of Ca^{2+}-dependent C-type lectins include the endothelial cell selectins (E-selectin and P-selectin) and the leukocyte selectin L-selectin., among many others (Barthel et al., 2007). Their interaction with cell surface glycans makes it easier for hematopoietic cells to adhere to vascular surfaces, which speeds up the process of circulating leukocytes rolling and delivering them to sites of inflammation (Hu et al., 2021), (Ganesh et al., 2021). Leukocytes adhere to endothelial cells in a series of steps known as the adhesion cascade, which relies on selectins (Ghazi et al., 2023). The adhesion molecule E-selectin, with a molecular weight of 115 kDa, is expressed exclusively by vascular endothelial cells (Cappenberg et al., 2022). E-selectin uses an N-terminal lectin domain of 119 residues with a 60-70% identity and approximately six cysteine-rich consensus repeats in its amino acid sequence to bind the oligosaccharide (McEver, 2015). Domains encoding epidermal growth factor and a calcium-dependent lectin at the N-terminus, the transmembrane domain, the intracellular cytoplasmic tail, and the chain of six consensus repeats make up this single-chain transmembrane glycoprotein (Tvaroška et al., 2020). Endothelial cells in the majority of tissues, such as skin microvessels and bone marrow, express E-selectin (Kappelmayer and Nagy Jr, 2017). On the other hand, lipopolysaccharide, interleukin-1b (IL-1b), and tumor necrosis factor-alpha (TNF-α) (Jubeli et al., 2012). stimulate E-selectin receptor expression. Various cancers, tumor angiogenesis, and metastasis have been linked to E-selectin expression (Noo et al., 2022).

The two important glycoprotein ligands that bind to selectin are E-selectin ligand-1 (ESL-1) for E-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) for P-selectin expressed by cytokine-activated endothelial cells (Yeini and Satchi-Fainaro, 2022). E-selectin plays a crucial role in recruiting white blood cells to the injured areas. The cytokines IL-1b and TNF-α are released by macrophages in inflamed tissue, which stimulate nearby endothelial cells to over-express E-selectin (Spertini et al., 2019). Leukocytes in the blood that carry the appropriate ligand bind to E-

selectin weakly when subjected to the shear stress of blood flow. This causes the leukocytes to "roll" along the blood vessel's interior surface, creating and breaking brief interactions(Babar et al., 2019) **.** E-selectin may be a risk factor for acute coronary syndrome, especially since it is positively associated with the density of inflammatory cells, which are a major cause of plaque formation in the intima (Simon and Goldsmith, 2002)**.** This requires evaluating the association between E-selectin and the inflammatory response in ACS patients, and this is what the current study seeks to document.

2. Material, Patients and Method

Using a case-control research approach, data was collected from 120 male subjects obtained between Dec. 2022 and Nov. 2023. The subjects were aged between 41 to 70 years, and divided into two groups: 60 males with ACS patients and 60 apparently healthy males as a control group. The Sandwich-ELISA method was used to measure the levels of serum E-selectin (UNO/HUMAN/ Germany), and a SMART-120 chemistry analyzer was used to measure the levels of lipid profiles and other compounds in human serum. (AFLO / Germany, colorimetric enzymatic method). The BMI is expressed as kg/m^2, which is the result of dividing the weight (in kg) by the square of the height (in m) (Mirzaei and Khajeh, 2018)**.** The questionnaire gathered demographic data such as sex, age, smoking status, sedentary lifestyle, and family history of ACS for both patients and healthy controls. Exclusion criteria included subjects with chronic diseases like diabetes, cirrhosis, end-stage renal disease, acute heart failure, stroke, skeletal muscle injury, malignancy, endocrine dysfunction, and other inflammatory conditions .

2.1.Statistical Analysis

Data analysis was conducted using IBM's Statistical Package for Social Sciences, version 22.0 (SPSS, Chicago, Illinois, USA). scale variables for normally distributed data were displayed as mean ± standard deviation. The Shapiro-Wilk test was used to assess data distribution for normality. T-tests and analysis of variance tables were employed to compare the means of the biomarkers among different groups. A p-value below 0.05 was deemed statistically significant. Through the use of receiver operating characteristic (ROC) analysis, the ideal sensitivity and specificity threshold for critical cases was ascertained.

3. Results

Table 1 and Fig.1 and Fig.2. demonstrate the levels of serum E-selectin and other biomarkers between the study groups. E-selectin levels were significantly higher in patients compared to the control group, with a mean ± SD of 216.07±20.26 pg/ml in CAD patients and 179.74± 53.14 pg/ml in the control group. The patients in the study were overweight and had dyslipidemia, and BMI significantly differences in between the ACS patients and control groups. Additionally, there are significant differences in the levels of TC, TG, HDL-C, LDL-C, and VLDL-C, as presented in Table 1. The analysis of the receiver operating curve (ROC) for E-selectin showed a sensitivity of 85%, a specificity

of 70%, a 95% confidence interval (CI) of 0.673-0.863, and the area under the curve (AUC) was 0.768. The cut-off point was set at 197.37 pg/ml or higher. As shown in Table 2 and Fig.3.

Table 1: Comparison of Anthropometric and Clinical Parameters Between the Two Groups of ACS Patients and Controls.

Parameter	ACS Mean ± SD N = 60	Control Mean ± SD N = 60	P-value
Age, (year)	57.82±8.00	54.08±8.71	0.039
BMI, (kg/m^2)	27.40±3.75	23.70±1.80	≤ 0.0001
TC, (mg/dl)	247.38 ±44.36	176.50 ±14.50	≤ 0.0001
TG, (mg/dl)	221.29 ±82.93	128.53 ±29.67	≤ 0.0001
HDL-C, (mg/dl)	34.44 ±2.48	53.85 ±7.52	≤ 0.0001
LDL-C, (mg/dl)	170.58 ±39.97	98.03 ±42.80	≤ 0.0001
VLDL-C, (mg/dl)	42.07 ±17.75	21.70 ±5.76	≤ 0.0001
E-selectin, (pg/ml)	216.07±20.26	179.74±53.14	≤ 0.0001

T-test was significant at p ≤ 0.05; SD: standard deviation; S: significant; TC: Total cholesterol; LDL-C: Low-density lipoprotein cholesterol; TG: Triglycerides; HDL-C: High-density lipoprotein cholesterol; VLDL-C: Very low-density lipoprotein cholesterol BMI: Body mass index

Table 2: Area Under the Curve (Auc), Optimal Threshold, Sensitivity, And Specificity Of E-Selectin Obtained by Roc Curve in Patients.

Parameter	Cut-off	Sensitivity	Specificity	AUC	P-value	95% CI	
E-Selectin (pg/ml)	197.37	0.85	0.70	0.768	0.0001	0.673	0.863

AUC: Area under the curve; CI: Confidence Interval

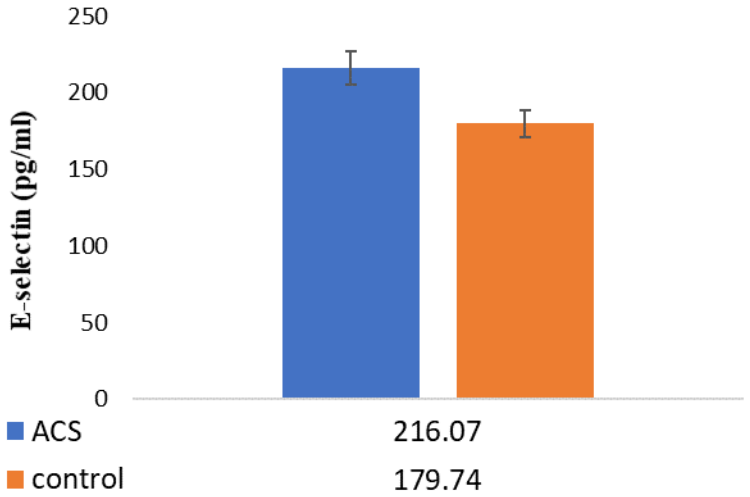

Figure 1: The Difference in The Mean Levels Of E-Selectin Between ACS And the Control Group (T-Test Was Significant at P ≤ 0.05).

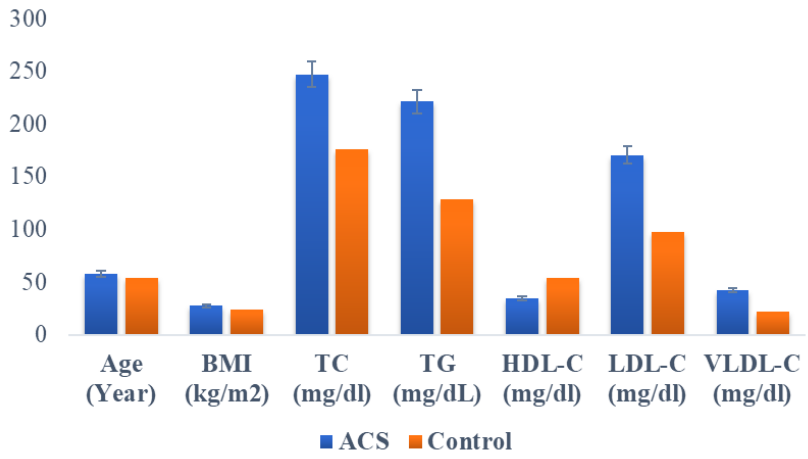

Figure 2: The Difference in Mean Levels of Age, BMI, And Lipid Profile Between the Two Groups of ACS Patients and Controls (T-Test Was Significant at $P \leq 0.05$)

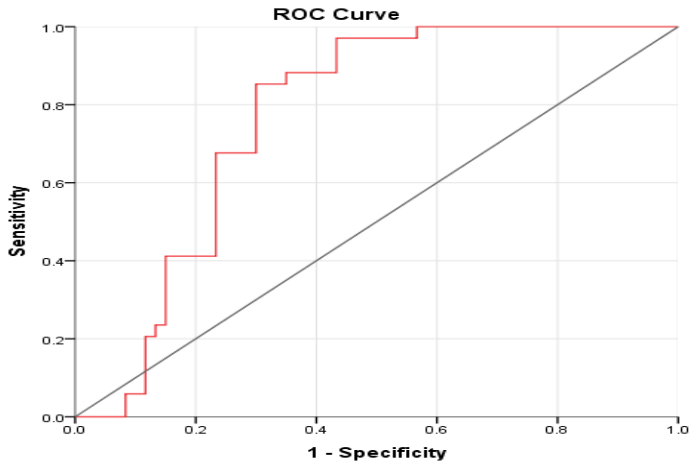

Figure 3: Receiver Operating Characteristic (ROC) Curve of Serum E-Selectin Level as Discriminators of Patients

4. Discussion

The results demonstrated that the patients' group exhibited a higher level of E-selectin. The current study demonstrated that E-selectin levels were higher in the patient group compared to the controls (ACS, 216.07 ± 20.26 pg/ml; controls, 179.74 ± 53.14 pg/ml; T-test, $P = 0.0002$). This is due to the inflammatory response and increased expression of pro-inflammatory cytokines such as IL-1b and TNF-α, which leads to the activation of endothelial cells and ultimately leads to increased E-selectin expression (Fang et al., 2011)**,** (Sutton et al., 2014)**.** Soluble E-selectin may exacerbate

inflammatory disease symptoms by activating β2 integrins and influencing leukocyte movement during the rolling process (Zhang, 2022). In this study, E-selectin was evaluated as a prognostic biomarker for patient groups in comparison to the healthy control group. De novo synthesis on endothelial cells can stimulate E-selectin production. E-selectin peaks reacting to inflammatory triggers, such as IL-1, lipopolysaccharide, or TNF-α, and then returns to baseline levels within 10 to 24 hours (Shephard, 2003). This supports the findings presented in this study, as samples are collected within a duration of 10 to 14 hours from the onset of symptoms in patients in this study. Prior human studies have been conducted on E-selectin concentrations in cardiovascular disorders. When inflammation occurs, leukocytes adhere to the walls of blood vessels through a cell adhesion molecule called E-selectin. E-selectin is necessary for effector T cells, B cells, neutrophils, monocytes, and natural killer cells to roll in the intima (Del Zoppo et al., 2000). Circulating leukocytes can cling to and cross the endothelial barrier because the vascular endothelium expresses E-selectin (González-Amaro and Sanchez-Madrid, 1999). The fact that E-selectin is exclusively expressed by endothelial cells in the intima linked to atherosclerotic lesions makes it unique and critically important. When the amount of soluble E-selectin in the bloodstream mirrors its expression on endothelial cells there is systemic inflammation and endothelial activation. An increase in E-selectin is thought to specifically indicate endothelial activation, reactive protein, and dysfunction (Calabriso et al., 2023), (Granai et al., 2023). According to previous research, many adhesion molecules are found to increase when atherosclerotic conditions, whether chronic or acute, are present, consequently, an elevation in serum E-selectin is a distinct marker of endothelial activation. Recent evidence has connected inflammation to the pathophysiology of atherosclerosis-induced plaque rupture (ACS-induced) (Manuel Gomez et al., 2007).

In this study, we found that serum E-selectin levels rise when clinically significant atherosclerosis is present. Conventional histopathology has a hard time detecting a tiny number of cells. Histological detection of even a statistically significant increase in E-selectin-positive cells would be challenging, suggesting that an increase in serum E-selectin might be more indicative of a problem (Omar, 2022).

In addition, it seems that serum E-selectin is an extremely sensitive indicator of endothelial activation. It rises in preclinical disorders like dyslipidemia and falls sharply after intensive treatment with cholesterol-lowering medications (Matera et al., 2021). Increased expression of endothelial adhesion molecules may promote vascular damage, and the expression of E-selectin and L-selectin may be stimulated by oxidized LDL (Nomura et al., 2004). Nomura, S., et al., in their studies, showed hyperlipidemic diabetics exhibited significant decreases in E-selectin and L-selectin at 6 months after pitavastatin treatment. Therefore, pitavastatin could inhibit the progression of atherosclerosis by both the decrease in LDL-C and the elevation of adiponectin (Nomura et al., 2008).

5. Conclusion

Activated endothelial is essential for the development and advancement of atherosclerosis, and our present study demonstrates that E-selectin is a diagnostic marker for this process.

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Effect of Genetic Polymorphism of CYP2C8 Enzyme on the Montelukast Therapy Responses in Iraqi Asthmatic Children

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Abstract

Background: Some reports show that the CYP2C8 enzyme plays an influential role in montelukast metabolism, and genetic polymorphism of CYP2C8 genes may influence therapeutic responses of asthmatic children to montelukast treatment.

Aim of the study: The current study aimed to detect the effects of genetic polymorphism of the CYP2C8*1B (rs7909236) gene on montelukast response in asthmatic children.

Methods and Patients: An observational cross-sectional study was carried out in the respiratory clinic center in the Kerbala Teaching Hospital of Children from early October 2022 to late September 2023.

The trial included one hundred children older than six years old with asthma who had been previously diagnosed and were taking montelukast every day for at least one month. Alle-specific PCR patients out following DNA extraction to determine each patient's genotype to determine each patient's genotype. Total serum Ig E levels, Asthma Control Test (ACT), FEV1, and PEF of Pulmonary Function Tests were also measured.

Results: The distribution of CYP2C8*1B (rs 7909236) genetics polymorphism was found to be 74% for CC wild homozygous, 22% for CA mutants heterozygous, and 4% for mutants homozygous AA, according to the results of genetic amplification. Patients with wild-type and mutant genes do not significantly differ in serum total IgE, FEV1, PEF values, or ACT scores. Therefore, this polymorphism and montelukast responsiveness is predicted to be not significantly related (p value<0.05).

Conclusions: The montelukast respond and the CYP2C8*1B g.-271 G>A (rs7909236) genetic variation did not significantly associate.

تأثير تعدد الأشكال الجيني لإنزيم CYP2C8 على استجابات العلاج مونتلوكاست لدى الأطفال العراقيين المصابين بالربو

حسناء حيدر محمد، عدي عبد الرضا، حسن محمود أبو المعالي

الخلاصة

المقدمة: خلفية البحث تبين في بعض الدراسات دور مؤثر لأنزيم CYP2C8 في ايض عقار المونتيلوكاست والتغييرات الجينية في جين هذا الانزيم من الممكن تؤثر على استجابات العلاجية لعقار المونتيلوكاست لمرضى الربو.

هدف الدراسة: هدفت الدراسة لمعرفة العالقة بين تعدد الأشكال الجيني، CYP2C8*1B (rs7909236) لدي استجابة الأطفال الذين يتناولون عقار المونتيلوكاست يوميًا.

الطرق: أجريت هذه الدراسة المقطعية من تشرين الثاني ٢٠٢٢ الى نهاية أيلول ٢٠٢٣ وقد ساهم بها ١٠٠ طفل يعانون من مرض الربو ويتناولون عقار المونتيلوكاست بشكل يومي لمدة لا تقل عن شهر قبل المشاركة في الدراسة وسحب عينة من الدم منهم. تم فحص اختبار وظائف الرئة، اختبار السيطرة على الربو وتحليل مستويات مضادات الامينوغلوبين نوع E لدي كل المشاركين في الدراسة. تم استخلاص الحامض النووي وعمل اختبار PCR خاص بنوع الاليل لتحديد الأنواع الجينية.

النتائج:

ظهرت الدراسة وجود طفرات في جين CYP2C8*1B (rs7909236) وكانت بنسبة ٧٤٪ لنمط الوراثي المتجانس (CC) وبنسبة ٢٢٪ لنمط الوراثي المتغاير (CA) وبنسبة ٤٪ للنمط الوراثي (AA).

لم يكن هنالك ارتباط كبير بين استجابة المرضى لعقار المونتيلوكاست و تعدد الجيني لانزيم CYP2C8*1B g.-271 C>A (rs7909236) .

1. Introduction

Asthma prevalence and severity remain a primary global health concern in all age groups. Asthma, which affects both adults and children, has a high morbidity rate but a low mortality rate in comparison to other chronic illnesses. According to available data, asthma is a complicated disorder whose etiology is increasingly thought to result from interactions between environmental exposures, host characteristics, and genetic susceptibility factors (genetic loci associated with asthma). (Air pollution, mold, other aeroallergens, weather, obesity, dietary variables, infections, and hypersensitivity to allergies) (Kim et al., 2017).

Cysteinyl leukotriene antagonists, such as montelukast, have been approved to treat asthma in children and adults (Davidson et al., 2010). In many in vitro studies and on the montelukast label, the key P450 enzymes involved in montelukast metabolism are identified as CYP2C9 and CYP3A4. More recent in vitro research, however, links CYP2C8 to the 36-hydroxylation of montelukast. More proof of the possible role of CYP2C8 comes from monmontelukast'sility to bind firmly into the CYP2C8 enzenzyme's site and to significantly and competitively suppress this enzyme in vitro (Sánchez and Buitrago, 2018).

Currently, the CYP2C8 gene has about 100 non-single nucleotide polymorphisms (SNPs). Among these, CYP2C8*2, CYP2C8*3, and CYP2C8*4 are the most common. It’It'sP2C8*2(Ile269Phe within exon 5) allele, with an allelic frequency of 18%, is predominantly observed in Africans. CYP2C8*3 (Arg139 Lys with Lys399Arg in exons 3 and 8; an allelic frequency of 10-23%) and CYP2C8*4 (Ile264 Met in exon 5; an allelic frequency of 7.5-11%) are primarily carried by Caucasians. In Asians, both of these alleles are comparatively rare (0.5%). Very few additional mutations exist that alter the amino acids. Additionally, SNPs (*1B and *1C) within the CYP2C8 regulatory areas have been found (De Oliveira Cardoso et al., 2015; Sun and Liu, 2019). Compared to Caucasians, Chinese people have a lower percentage of alleles for CYP2C8*1B(rs7909236), only 8% (Sun and Liu, 2019). Previous research has indicated that CYP2C8 is involved with the metabolism of the montelukast (De Oliveira Cardoso et al., 2015).

This study aimed to explore the relationship between the genetic polymorphism in CYP2C8*1B (rs7909236) and the montelukast responses for Iraqi children (De Oliveira Cardoso et al., 2015; Sánchez and Buitrago, 2018; Sun and Liu, 2019).

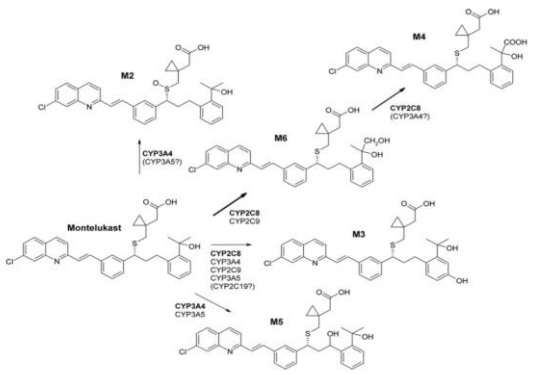

Figure 1: Oxidative Metabolism of Montelukast. The P450 Isoforms Responsible for The Forma- Tion Of the Metabolites M2, M3, M4, M5, And M6 Are Shown According to Chiba Et Al. And the Present Work (Filppula et al., 2011)

2. Material, Patients and Method

2.1. Study Design

This observational cross-sectional study was conducted in the respiratory clinic center of the Kerbala Teaching Hospital of Children between early October 2022 and late September 2023. Children with mild to moderate asthma who exhibited persistent symptoms were included in the trial. One hundred children who were frequent visitors to the hospital's asthma center and had mild to moderate persistent asthma were evacuated, and their responses to Montelukast therapy were evaluated by using ACT (Asthma Control Test), lung function test (PEF, FEV1), and serum total Ig E level.

2.2. Inclusion Criteria Applied for All Patients

1. Children are suffering from mild and modulated asthma.
2. The age range of 6–15.
3. Used montelukast mono-therapy for at least one month regularly.

2.3. Exclusion Criteria Applied for Enrolled Patients

1. A patient experiencing severe acute attacks of asthma.
2. A patient with a history of diabetic mellitus, chronic lung disease, ongoing renal failure, or chronic heart illness. A chest x-ray can diagnose pneumonia and other respiratory conditions (CXR).
3. The patient is using recognized inducers or inhibitors of Montelukast.

2.4. Ethical Approval

The protocol for the study was approved by the research and ethical committee at the University of Kerbala's College of Pharmacy. Before proceeding, the leadership of Kabala Teaching Hospital on Children and the Karbala Health Directorate were consulted. The parents of each patient were requested to obtain permission after being informed about the purpose and nature of the study.

2.5. Samples Collection

Each volunteer's four milliliters of venous blood for the study were divided into two tubes: two milliliters for the total serum Ig E evaluation and two milliliters for genetic testing.

2.6. Childhood Asthma Control Test C-ACT

According to the ACT score, the patients were divided into two groups: controlled asthma (≥20 score) and uncontrolled asthma (≤19 score).

2.7. Assessment **of Lung Function (Pulmonary Function Test)**

The American Thoracic Society (ATS) evaluated lung function through a disposable turbine and Spirometer (Spiro lab I).

2.8. IgE **assessment**

ELISA was performed using the Uroimmun kit to find the total serum IgE level.

2.9. Chemicals

Primers, ethidium bromide, TAE Buffer, absolute Ethanol, Agarose powder, nuclease-free water, DNA extraction kit, Premix kit, and DNA ladder.

2.10. Genotyping of the single nucleotide polymorphisms

In children with asthma, the current study focuses on the CYP2C8*1B gene (rs7909236). Following DNA extraction, allele-specific Polymerase Chain Reactions (AS-PCR) were performed. After that, PCR products were run across a gel electrophoresis enabled via a UV-trans illuminator.

2.11. Primers

The primers were designed by Professor Dr. Hassan Mahmood using Primer Blast software (http:www.ncbi.nih.gov/tools/primer-blast) and sent to Macrogen company for further production. The forward and reverse primers' sequences and product sizes are listed in Table 1

Table 1: Primer Sequence of CYP2C8*1B Enzyme Gene G. -271 C> A

rs7909236	**Sequence (5'->3')**
Forward primer	CAGCACCAGGACCACAAAAG
Allele G	ATCATCACAGCACATTGGAAC
Allele T	ATCATCACAGCACATTGGAAA
Product length 318	

2.12. Optimization of Polymerase Chain Reaction (PCR)Conditions

The desired conditions for CYP2C8*1B (rs7909236) can be achieved using:

1. 6 µL of DNA sample
2. 1 µL of sense primer
3. 1 µL of antisense primer
4. 12 µL of nuclease free water

Table 2: Conditions for CYP2C8*1B Enzyme Gene Optimization

Steps	**Temperature**	**Time**	**Cycles**
Initial Denaturation	95	5 min	1
Denaturation	95	20 sec	35
Annealing	54.1	20 sec	35
Extension	72	35 sec	40
Final extension	72	5 min	1

2.13. Statistical Analysis

IBM's Statistical Programmer for Social Sciences (SPSS) version 24 was used to maintain, process, and analyze the data after it was transferred from research participant data into an electronic database and error and consistency

checked. The results included standard percentage, mean, and standard deviation (SD). The analysis of variance (ANOVA) test was used to assess group differences. The total IgE level was evaluated using the student t-test. Fisher's exact values were calculated for cell counts greater than five. Pearson's correlation was utilized to assess the relationship between clinical parameters (IgE, FEV1, and PEF) and genotype groups in asthmatic children. Only a p-value of less than 0.05 is deemed significant for all statistical tests.

3. Results

One hundred children with mild to moderate asthma who received montelukast medication regularly for at least four weeks before the trial began were enrolled. Table 3 display the outcomes of genetic amplification, Serum Total IgE, and other parameters.

Table 3: Distribution of Different Genotypes of CYP2C8*1B Enzyme G.-271C>A (Rs 7909236) Gene Polymorphism in Asthmatic Children

Patient Genotypes	**N (%)**
Wild homozygous (CC)	74(74%)
Mutant heterozygous (CA)	22(22%)
Mutant homozygous (AA)	4(4%)
Total	100(100%)

As shown in the Table 4 there was no significant association (p value <0.05) between demographic characteristics and different genotypes of CYP2C8*1B enzyme gene g.-271C>A (rs 7909236) polymorphism in asthmatic children.

Table 4*: Association of Some Demographic Characteristics with Different Genotype of CYP2C8*1B Enzyme Gene G.-271C>A (Rs 7909236) Polymorphism in Asthmatic Children

Data express as percent (%), N=100					
Demographic characteristics		**Patient's genotypes**			**P value**
		CC	**CA**	**AA**	
BMI (kg/m^2)	**Underweight**	12(75%)	3(18.8%)	1 (6.2%)	0.62
	Healthy weight	44(71%)	15 (24.2%)	3 (4.8%)	
	Overweight	5(100%)	0 (0)	0 (0%)	
	Obesity	13(76%)	4(23.5%)	0(0%)	
Gender	**Male (n=64)**	43(67%)	18 (28.1%)	3(4.7%)	0.083
	Female (n=36)	31(86%)	4(11.1%)	1(2.8%)	
*Fisher's Exact test was used with a significant P value of less than 0.05.					

The Table 5 Illustrate the Association Between ACT Score and Different Genotypes of CYP2C8*1B Enzyme Gene G.-271C> A Polymorphism in Asthmatic Children. There Was Also No Significant Association (P Value <0.05) Between These Two Parameters.

Table 5: Association of Asthma Control Test with Different Genotypes of CYP2C8*1B Enzyme Gene G.-271C> A Polymorphism in Asthmatic Children

Asthma Control Test (ACT) Score	Patient Genotype (N=100)			P Value
	CC	CA	AA	
Controlled asthma (≥20)	16(76.2%)	5(23.8%)	0(0%)	0.81
Uncontrolled asthma (≤19)	58 (73.4%)	17 (21.5%)	4 (5.1%)	

The Table 6 Show the Mean Distribution of Serum Total Ige In Different Genotypes Of CYP2C8*1B Enzymes Gene G.-271C>A Polymorphism In Asthmatic Children. There Was No Significant Difference of Serum Total Ige Between Each Genotype Groups.

Table 6: Mean Distribution of Serum Total Ige Levels Among Different Genotypes of CYP2C8*1B Enzymes Gene G.-271C>A Polymorphism in Asthmatic Children

Patients Groups	Genotypes of Group 1, N=46			P value
	CC N=33	CA N=11	AA N=2	
Group1, (age6-9) N= 46	176.21±37.39	189.18±40.57	172.5±24.74	0.33
	Genotypes of Groups 2, N=54			P value
	CC N=41	CA N=11	AA N=2	
Group2, (age10-15) N=54	217.46±45.59	205.81±53.13	215±21.21	0.46
Results express as mean ± SD, *The ANOVA test with significant difference (p value <0.05) was used				

As seen in the Table 7 there was no significant difference in mean distribution of PFT parameters among different genotypes of CYP2C8*1B enzymes gene g.-271C>A polymorphism in asthmatic children

Table 7: Distribution of Pulmonary Function Test Among Different Genotypes of CYP2C8*1B Enzymes Gene G.-271C>A Polymorphism in Asthmatic Children

Pulmonary function test Parameters	Patients Genotype N=100			P value
	CC N=74	CA N=22	AA N=4	
FEV1 (L)	85.16±7.98	83.4±6.16	86.25±7.93	0.34
PEF(L/S)	83.28±8.89	81.81±6.17	84±5.77	0. 76
Results were express as mean± SD *ANOVA test with significant p < 0.05 was used				

The Pearson's correlation was done between CYP2C8 enzyme gene g.-271 C>A polymorphism and clinical parameters in asthmatic patients and the result show no significant correlation (p value <0.05) between CYP2C8 enzyme gene g.-271 C>A polymorphism and clinical parameters as described in the Table 8.

Table 8: Pearson's Correlation Between CYP2C8 Enzyme Gene G.-271 C>A Polymorphism and Clinical Parameters in Asthmatic Patients

Clinical parameters	Pearson correlation	P value
Serum total IgE level in group 1	0.089	0.55
Serum total IgE level in group 1	-0.078	0.57
FEV1	-0.48	0.63
PEF	-0.4	0.69

4. Discussion

This study was done to find the correlation between the resistance of ESA and the genetic polymorphism in the SOCs gene (ORAI1). The results showed that TT and TC genotypes were close in percentage, 39.3% and 38.4%, respectively, while the CC genotype represented 22.3%; by comparing these results with previous studies, some similarities presented, for example, the Taiwanese study in which 290 normal controls were included also found that the two prominent groups were TT and TC (41.72% and 43.45% respectively). The CC group was the lowest in percent (14.83%) (Chang et al., 2014). Another Taiwanese study of 579 chronic kidney disease patients showed the following genetic predisposition: TT genotype 40.7%, TC genotype 47.0%, and CC genotype 12.3% (Zhuang et al., 2023). Depending on the results of this study, there is no association between gender differences Genetic variations and neither the duration of the dialysis nor the duration of the treatment has a statistically significant effect on the patients' response to ESA. And there is no considerable variation in erythropoietin levels between the genetic groups.

The Hardy-Weinberg equilibrium test represented that the expected results will be a decrease in CC and TT genotypes with an obvious increase in the TC genotype, which is expected to be the prominent group and is statistically significant.

In this study, the CC genotype showed a statistically significant elevation in hemoglobin level over the TT group, which indicates better response; on the other hand, the CC group has a higher BU level than the other groups, representing poor clinical outcomes. This differs from the results of a previous study that was done in 2021, which showed that the CC/TC genotype has a high risk of erythropoietin resistance (Chang et al., 2014; Zhuang et al., 2023).

5. Conclusion

In ORAI1 genetic polymorphism (rs6486795), the CC genotype may represent the lowest percentage. Furthermore, this group may have a higher Hb level than other groups but may also have negative outcomes. On the other hand, ORAI1 genetic polymorphism has no significant association with erythropoietin resistance in Iraqi patients with CRF on hemodialysis. Despite the presence of the CYP2C8*1B g.-271C> genes in Iraqi asthmatic patients, a genetic polymorphism was non-significantly linked to montelukast responses. More research and a bigger sample size are required to confirm its effects on the montelukast response.

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Signature of IL-33 and Leptin as Early Progression Markers in Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disorder characterized by progressive and irreversible joint damage as a consequence of sustained synovitis. Adipokine levels and interleukin have been reported to be significantly increased in serum and synovial fluid (SF) of RA patients.

The study aimed to investigate the association between the levels of Leptin in the circulation of individuals with rheumatoid arthritis (RA).

Methods and Patients: The present work included a case-control study for a group of (90) samples: (60) patient samples and (30) healthy control samples. Patients with Rheumatoid arthritis were selected from Imam Hassan al-Mujtaba Hospital in Kerbala. The sociodemographic aspects of the patients were collected through the self-reported technique (student questionnaire). All patients underwent clinical history, clinical examination, and relevant laboratory investigations. The degree of rheumatoid was identified based on the evaluation of laboratory measurements for the clinical assessment of rheumatoid arthritis. An enzyme-linked immunosorbent assay system (ELISA) was performed using the sandwich-ELISA method to measure the concentrations of serum IL-33. At the same time, a competitive enzyme immunoassay kit was used to detect human Leptin in serum samples quantitatively. Statistical analysis was performed, and the efficiency of the predicting value was assessed using the receiver operating characteristic (ROC) curve.

Results: Results indicated a significant difference in IL-33 and Leptin hormone levels among the study groups, which increased with increasing age, BMI, and duration of the disease. Both biomarkers showed highly significant differences in such disease and represented a risk factor. Leptin was illustrated to be a three-time risk factor for Rheumatoid arthritis disease compared to IL33. AUC analysis for IL-33 as a diagnostic parameter showed that IL-33 performs well in predicting such cases.

Conclusion: This study confirms a significant association between elevated leptin and IL-33 levels in RA patients, with leptin showing a three-fold higher risk than IL-33. Both biomarkers increase with age, BMI, and disease duration, highlighting their potential role in RA progression and diagnosis.

بصمة IL-33 واللبتين كعلامات مبكرة لتطور التهاب المفاصل الروماتويدي

أحمد جواد، رنا حميد، جاسم حنون هاشم العوضي

الخلاصة

المقدمة: التهاب المفاصل الروماتويدي **(RA)** هو اضطراب مزمن التهابي ذاتي المناعة يتميز بتلف المفاصل التدريجي وغير القابل للعكس نتيجة التهاب الغشاء الزليلي المستمر. تم الإبلاغ عن زيادة ملحوظة في مستويات الأديبوكين والإنترلوكين في مصل الدم والسائل الزليلي لمرضى التهاب المفاصل الروماتويدي.

الهدف: هدفت الدراسة إلى التحقيق في العلاقة بين مستويات هرمون اللبتين في الدورة الدموية للأفراد المصابين بالتهاب المفاصل الروماتويدي**(RA).**

المرضى وطرق العمل: شملت الدراسة الحالية دراسة حالة-شاهد لمجموعة من (90) عينة: (60) عينة من المرضى و(30) عينة ضابطة صحية. تم اختيار المرضى المصابين بالتهاب المفاصل الروماتويدي من مستشفى الإمام حسن المجتبى في كربلاء. تم جمع الجوانب الاجتماعية والديموغرافية للمرضى من خلال تقنية التقرير الذاتي (استبيان الطلاب). خضع جميع المرضى لتاريخ سريري وفحص سريري والتحقيقات المخبرية ذات الصلة. تم تحديد درجة التهاب المفاصل الروماتويدي بناءً على تقييم القياسات المخبرية للتقييم السريري لالتهاب المفاصل الروماتويدي. تم إجراء نظام مقايسة الممتز المناعي المرتبط بالإنزيم **(ELISA)** باستخدام طريقة الساندويتش **ELISA**-لقياس تركيزات **IL-33** في المصل، وفي نفس الوقت، تم استخدام مجموعة مقايسة مناعية إنزيمية تنافسية للكشف عن هرمون اللبتين البشري في عينات المصل بشكل كمي. تم إجراء التحليل الإحصائي وتقييم كفاءة القيمة التنبؤية باستخدام منحنى خاصية التشغيل**.(ROC)**

النتائج :أظهرت النتائج وجود فرق كبير في مستويات هرمون **IL-33** واللبتين بين مجموعات الدراسة، والتي زادت مع زيادة العمر، مؤشر كتلة الجسم **(BMI)**، ومدة المرض. كلا العلامتين البيولوجيتين أظهرتا فروقات ذات دلالة كبيرة في مثل هذا المرض ومثلت عوامل خطر. تبين أن اللبتين يمثل عامل خطر ثلاثي للإصابة بمرض التهاب المفاصل الروماتويدي مقارنة بـ **.IL-33** أظهر تحليل **AUC** لـ **IL-33** كمعامل تشخيصي أن **IL-33** يعمل بشكل جيد في التنبؤ بهذه الحالات.

الاستنتاج: تؤكد هذه الدراسة وجود ارتباط كبير بين ارتفاع مستويات اللبتين والـ **IL-33** لدى مرضى التهاب المفاصل الروماتويدي، حيث يظهر اللبتين خطرًا أعلى بثلاثة أضعاف مقارنةً بالـ **.IL-33** وتزداد مستويات كلا العلامتين الحيويتين مع تقدم العمر وزيادة مؤشر كتلة الجسم ومدة المرض، مما يبرز دورهما المحتمل في تقدم المرض وتشخيصه.

1. Introduction

RA is a systemic disease characterized by a complex pathogenesis involving interactions between various cell types located in synovial compartments and peripheral blood rather than resulting from a single pathogenic factor (Alivernini et al., 2022). These cell populations, comprising fibroblast-like synoviocytes (FLSs), innate and adaptive immune cells, and bone-related cells, change in number, status, and behavior in response to the dynamic microenvironment, which perturbs cytokine secretion, intracellular signaling networks, and homeostasis and consequently leads to corresponding pathology. Multiple dysfunctional cell types and the crosstalk between these pathogenic cells collectively contribute to the onset, progression, and perpetuation of RA (Petrelli et al., 2022)

Under normal physiological conditions, FLSs express components of the extracellular matrix (ECM) and synovial fluid to lubricate and nourish cartilage surfaces, thereby maintaining the homeostasis of joints. However, accumulating studies have identified FLSs as critical players in many pathogenic events in the RA synovium. In pathological conditions, such as RA, FLSs increase rapidly. They are redistributed in the synovium and joints, exhibiting heterogeneity across different locations within the synovium and across other joints (Dakin et al., 2018). FLSs in RA display unique aggressive behavior that arises from their reduced apoptosis rate, deregulated proliferation, migration, and invasion, and improved ability to secrete inflammatory mediators and matrix metalloproteinases (MMPs) into the synovial fluid (Mousavi et al., 2021).

IL-33 was recently described as a new member of the IL-1 family, whose common characteristic is pro-inflammatory activity (Pisetsky, 2023). IL-33 plays an important immune role associated with Th2 response, significantly stimulating the secretion of IL-5 and IL-13 by Th2-polarized cells.

Basophils activated by immunoglobulin E (IgE) produce IL-33 and release histamine. Additionally, basophil migration appears to be regulated by IL-33. These findings aid in the understanding of independent immune responses to antigens present in tissues that express the mRNA of IL-33, such as smooth muscle cells in bronchial tissue and epithelial cells of the airways (Wright et al., 2017).

Mast cells are very responsive to IL-33, which results in increased production of IL-6, IL-13, IL-1beta, TNF, prostaglandin D2, and MCP-1 (Xu et al., 2008). In addition, IL-33 promotes survival, adhesion, and cytokine production in human mast cells and mast cell progenitors (Ali et al., 2007).

Several studies using experimental models of arthritis have evaluated the participation of IL-33 in pictures of joint inflammation. Proposed mechanisms for joint inflammation induction by IL-33 were activation of mast cells, and therefore, the production of inflammatory cytokines; increased secretion of IL-6 and IL-1beta by activated mast cells; or CD4+ cell stimulation that would lead to production of IL-5 and IL-13. This latter mechanism would increase the activation of B cells and immunoglobulin production, worsening the joint inflammation process and stimulating mast cell degranulation and the formation of immune complexes with collagen. These authors also demonstrated that mast cells are important in this experimental model, albeit not essential, for developing arthritis (Trimarchi et al., 2022; Xu et al., 2008).

Adipokine levels have been reported to be significantly increased in serum and synovial fluid (SF) of RA patients (Chihara et al., 2020). Furthermore, several studies outlined the implication of adipokines in the progression and severity of OA and the chronic inflammation in articular joints (Hu et al., 2011).

Leptin is a 16 kDa protein discovered in 1994 by Friedman and collaborators. It belongs to the class I helical cytokine family, which includes growth hormone (GH), leukemia-inhibiting factor (LIF), granulocyte colony-stimulating factor (G-CSF), interleukins (IL) (Zhang et al., 2005). Leptin is the main adipokine secreted by adipose cells. It exerts its role by binding to the long isoform receptor Ob-Rb and transducing the signal through the Janus kinase/signal transducer and activator of the transcription (JAK/STAT) signaling pathway (Boroń et al., 2021). In addition to its evident role in regulating energy homeostasis and food intake, it also has pleiotropic functions (Kelesidis et al., 2010). Leptin is implicated in adaptive and innate immunity. Increasing evidence suggests that Leptin exerts potent modulatory actions in the network of factors involved in the pathophysiology of rheumatic diseases such as OA and RA (Conde et al., 2010). This review recapitulates the most relevant data regarding the involvement of Leptin in these two diseases.

Leptin and its receptor are associated with the stage of OA disease and related pain. Notably, high leptin concentrations in RA patients are correlated with joint pain (Lübbeke et al., 2013). mRNA expression of Leptin and its receptor was more elevated in RA cartilage (Simopoulou et al., 2007) . Leptin has been described as implicating in RA pathogenesis. However, the results of clinical studies comparing serum or SF leptin concentrations in healthy individuals and RA patients still need to be clarified. Many authors have reported significant elevation of serum and SF leptin levels in RA patients compared to healthy controls (18); Popa and Collaborates said, in addition, that plasma leptin concentrations were inversely correlated to inflammatory markers in RA patients, suggesting that chronic inflammation in RA decreases leptin production (Rusu et al., 2012).

The role of Leptin in RA is not only associated with articular tissues; it might also have a potent effect on cell-mediated immune function (Fraser et al., 2000; Popa et al., 2005).

2. Material, Patients and Method

The present work included a case-control study for a group of (90) samples: (60) patient samples and (30) healthy control samples. Patients with Rheumatoid arthritis were selected from Imam Hassan al-Mujtaba Hospital in Kerbala. The sociodemographic aspects of the patients were collected through the self-reported technique (student questionnaire). All patients underwent clinical history, clinical examination, and relevant laboratory investigations. The degree of rheumatoid was identified based on the evaluation of laboratory measurements for the clinical assessment of rheumatoid arthritis.

The leptin-Ab ELISA kit applies the competitive enzyme immunoassay technique, which was used to measure the Leptin level, while the Sandwich-ELISA principle was used to measure the IL-33

Before their inclusion in the study, the hospital administration obtained valid written, signed consent, and each patient and control subject provided valid verbal consent.

Statistical analysis was performed, and the efficiency of the predicting value was assessed using the receiver operating characteristic (ROC) curve.

3. Results

Difference between the level of biological parameters (IL33 and Leptin) in the rheumatic arthritis cases and control group. Generally, patients with rheumatic arthritis disease showed an increasing range of IL-33 and Leptin Hormone levels compared to the healthy control groups.

Results indicated a significant difference in IL-33 and Leptin Hormone levels among groups; the means and standard deviations were presented in Table 1 & Fig.1. The mean level of IL-33 in patients was (950.80±463.07) which was significantly higher than for the Control group (297.10±44.81) ($p \leq 0.001$) and the mean level of Leptin. The hormone was (7.21±1.54) for the patient and (2.78±0.37) for the control,

Table 1: Results of The Analysis of Essential Rheumatic Arthritis Characteristics for Disease with Control Groups.

Biomarkers	Groups	Mean±SD	P value
IL-33	Patient	950.80±463.07	<0.001
	Control	297.10±44.81	
Leptin. Hormone	Patient	7.21±1.54	0.010
	Control	2.78±0.37	

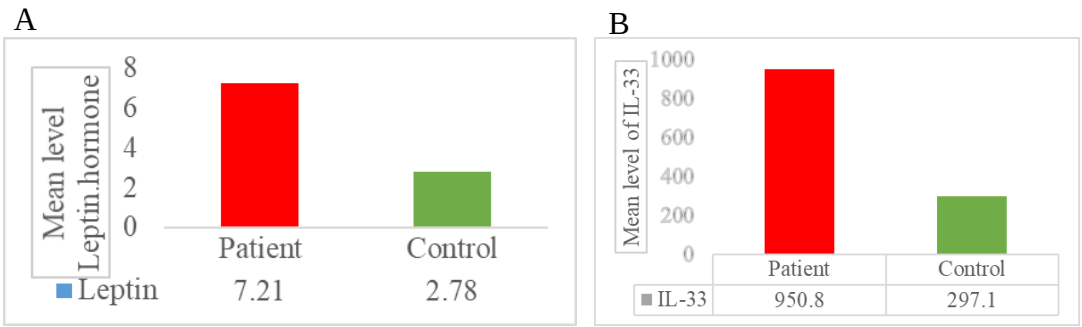

Figure 1: Results of the Analysis of Basic Rheumatic Arthritis for Patients with Control Groups (T-Test Was S= Significant at $P \leq 0.05$, NS= Non-Significant). **A)** Describes Medical Levels of Leptin Hormone. **B)** Refers to The Medical Levels Of IL-33.

Mean Difference of the biological parameters (IL33 and Leptin) based on the duration of disease. Biomarker levels were examined based on the duration of rheumatic arthritis disease. Generally, there were insignificant differences in the mean IL-33 and Leptin Hormone levels. The mean level of IL-33 was increased slightly with increasing the duration of rheumatic arthritis disease through (7-12 Years) compared to the group whose duration was less than one year and the group having a duration of rheumatic arthritis disease (1-6 Years), the differences were insignificant p-value > 0.05, as shown in Table 2 & Fig.2.

Table 2: Mean Difference of Biochemical Parameters for the Duration of Rheumatic Arthritis Disease.

Biomarkers	Groups	Mean±SD	P-value
IL-33	Less than 1 years	953.09±240.58	0.967
	1-6 Years	881.78±612.96	
	7-12 Years	992.01±153.06	
Leptin. Hormone	Less than 1 years	7.08±0.94	0.460
	1-6 Years	7.37±1.80	
	7-12 Years	6.61±0.79	

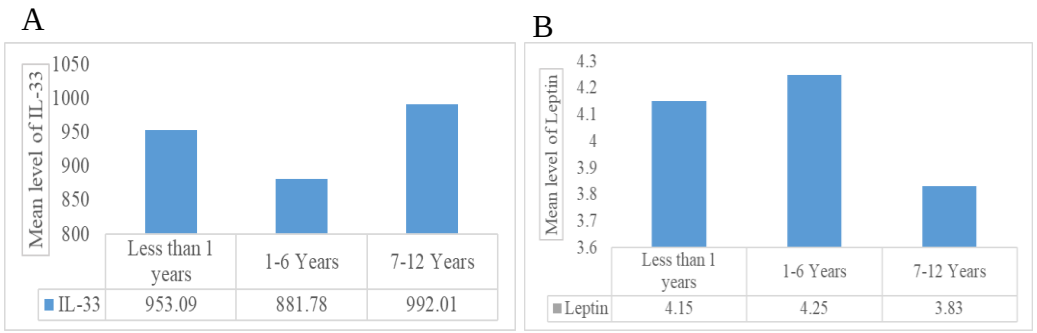

Figure 2: Difference Between Mean Levels of Biomarkers in Rheumatic Arthritis Disease According to Duration of Treatment (T Test Was *: Significant P ≤ 0.05; SD: Standard Deviation; S: Significant; NS= Non-Significant). **A)** Describes Medical Levels of Leptin Hormone. **B)** Refers to The Medical Levels of IL-33.

Table 3: Illustrates the Mean Level of IL-33 and Leptin in the Patients and Control Groups According to Gender

Biomarker	Male			Female		
	Patients Mean±SD N=30	**Control Mean±SD N=15**	**P value**	**Patients Mean±SD N=16**	**Control Mean±SD N=8**	**P value**
IL-33	994.1±458.5	401.3±53.5	<0.001[S]	830.8±540	352.94±93	**<0.001[S]**
Leptin	6.01±0.43	3.80±0.61	<0.001[S]	5.95±0.3	4.54±2.59	**0.003[S]**
T test was *: significant at p ≤ 0.05, N: number of cases; SD: standard deviation; S: significant; NS= Non significant						

Results showed that the levels of IL-33 and Leptin hormone were increased markedly in the patient's group in both males and females compared to the control; p values were <0.001.

In Fig.3. and Fig.4. a comparison of serum levels of IL-33 and Leptin (pg/ml) in different age groups was performed. The levels of IL-33 and Leptin increased significantly within all the age ranges and were highly statistically significant ($p<0.05$).

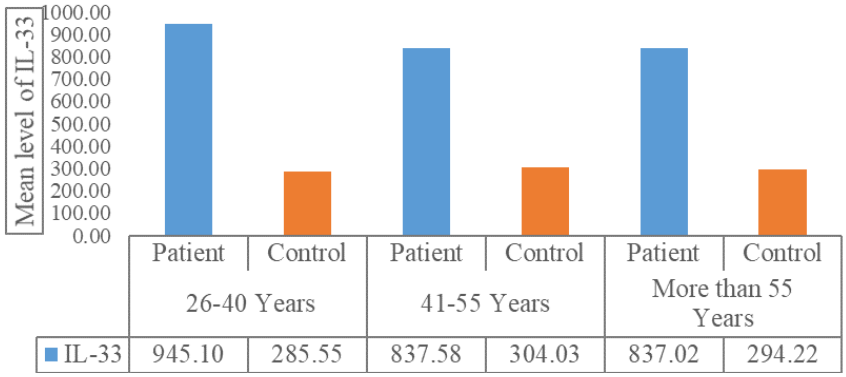

Figure 3: The Effect of Age Groups on the IL-33 According to The Patient and Control Groups

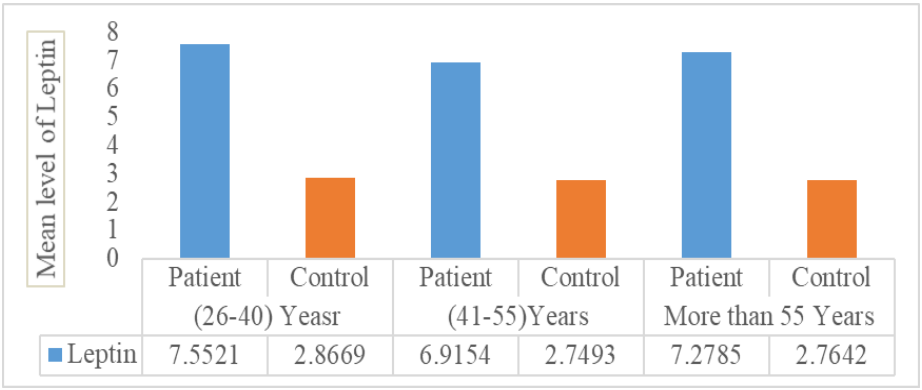

Figure 4: The Effect of Age Groups on Leptin According to the Patient and Control Groups.

In Fig.5. and Fig.6. A Comparison of Serum Levels of Il-33 And Leptin (Pg/Ml) In Different Age Groups Was Performed. Both Levels of Il-33 And Leptin Were Increased Within All the Bmi Ranges And Were Highly Statistically Significant ($P<0.05$).

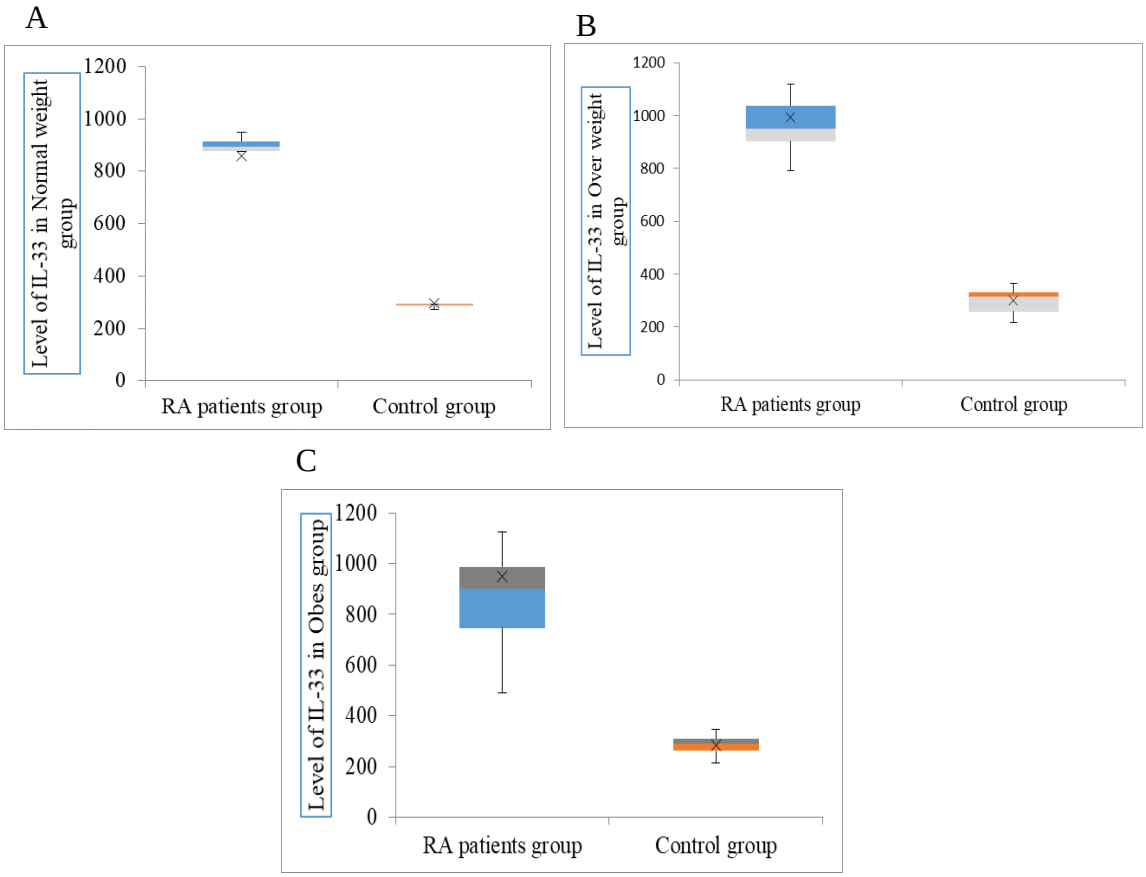

Figure 5: The Effect of BMI Groups on the IL-33 According to The Patient and Control Groups. **A)** According to Normal Weight, **B)** According to Overweight And **C)** According to Obesity.

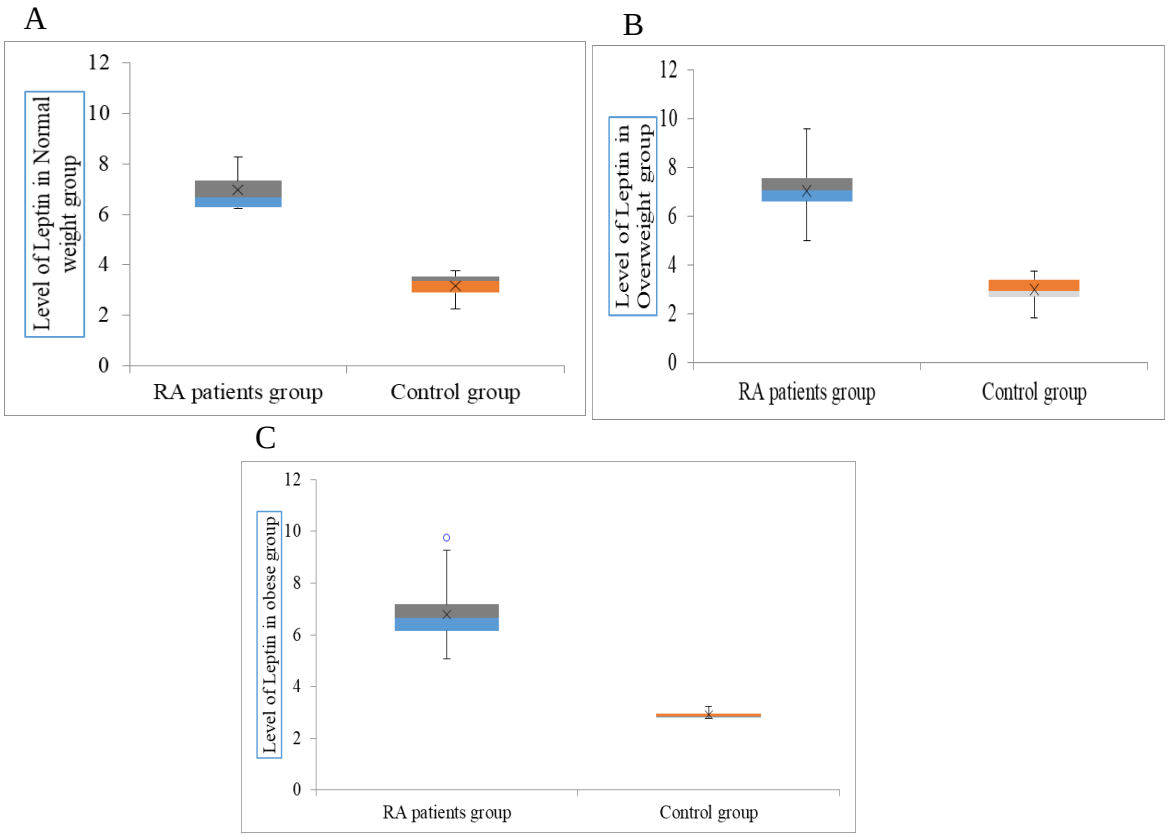

Figure 6: The Effect of BMI Groups on Leptin According to the Patient and Control Groups. **A)** According to Normal Weight, **B)** According to Overweight and **C)** According to Obesity.

Multinominal Logistic Regression Was Performed to Analyze the Association Between Il-33 And Leptin Hormone with Rheumatoid Arthritis Disease. It Was Found That Both Biomarkers Were Shown Highly Significant Differences in Such Disease and Represented A Risk Factor. Leptin Was Illustrated to Be A Three-Time Risk Factor for Rheumatoid Arthritis Disease Compared to Il33. The Odd Ratio of Il33 Was (Or 1.487; 95% Ci: (0.778-2.841), And for Leptin Hormone Was (Or: 3.796; 95% Ci: 1.21-5.632), As Shown in Table 4.

Table 4: The Binary Logistic Regression of Rheumatic Arthritis Disease (RA) with Levels of Biomarkers

Biomarkers	OR (Lower-Upper)	P value
Leptin Hormone	3.796(1.21-5.632)	0.004
IL-33	1.487 (0.778-2.841)	0.001

ROC curve and AUC analysis for the Leptin for rheumatic arthritis disease ROC curve and AUC analysis were performed for the IL-33 patients compared to the control group. Results of the receiver operating curve (ROC) curve and AUC analysis for the IL-33 as a diagnostic parameter showed that IL-33 has a good performance for predicting such cases; data are presented in Table 5 & Fig.7. For IL-33 levels: (sensitivity 83.3 %, specificity 97%) at a level = 593. The p-values of the AUC were <0.001 and highly statistically significant. Results sensitivity & Specificity results were confirmed using Youden's J statistics to find parameters.

Table 5: Receiver Operating Characteristic Curve Showing Sensitivity and Specificity Of IL-33 In Patients Compared to Control

Test Result Variable(s)	IL-33
AUP	88.5%
Sensitivity %	83.3%
Specificity %	97%
Youden index	0.803
Cut-off points	593.464
CI (95%)	0.811-0.959
PPV	99%
NPV	75%
Accuracy	88.8%
P value	<0.001

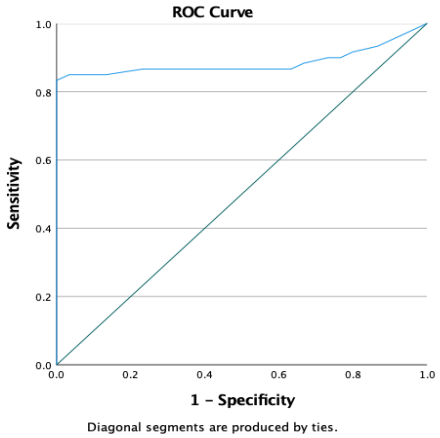

Figure 7: Receiver Operating Characteristics (ROC) Curve Analysis of Il33 Levels in Rheumatoid Arthritis Cases

Roc Curve and Auc Analysis for The Leptin for Rheumatic Arthritis Disease. ROC curve and AUC analysis for the IL-33 patients compared to the control group were performed. Results of the receiver operating curve (ROC) curve and AUC analysis for Leptin as a diagnostic parameter showed that Leptin performs well in predicting such cases; data are presented in Table 6 and Fig.8. For Leptin levels: (sensitivity 98.3%, specificity 99%) at a level = 5.0387. The p-values of the AUC were <0.001 and highly statistically significant. The Sensitivity & Specificity results were confirmed using Youden's J statistics to the parameters.

Table 6: Receiver Operating Characteristic Curve Showing Sensitivity and Specificity of Leptin In Patients Compared To Control.

Test Result Variable(s)	Leptin
AUP	98.3%
Sensitivity %	98.3%
Specificity %	99%
Youden index	0.883
Cut-off points	5.0387
CI (95%)	0.962-1.000
PPV	99%
NPV	75%
Accuracy	88.8%
P value	<0.001[S]

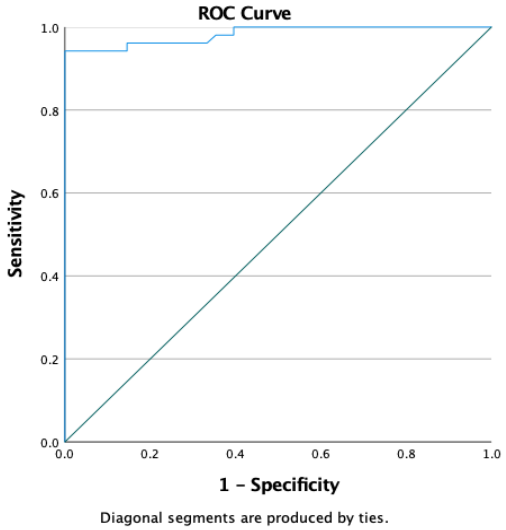

Figure 8: Receiver Operating Characteristics (ROC) Curve Analysis of Leptin Levels in Rheumatoid Arthritis Cases

4. Discussion

Rheumatoid arthritis is a severe chronic and progressive autoimmune disorder characterized by synovium inflammation (Lai et al., 2017). Although the pathological mechanisms involved in RA are different, the onset and progression of both diseases are associated with inflammation, immune mechanisms, and metabolic factors (Francisco et al., 2018a; Lai et al., 2017). Mechanical loading and inflammatory mediators such as adipose-tissue-derived cytokines (adipokines) have been reported as a link between obesity and RA(Smekal and Vaclavik, 2017) . Adipokines, including Leptin, are secreted principally by white adipose tissue (WAT) (Carrión et al., 2019). Through their endocrine, autocrine, or paracrine actions, they are implicated in several physiological and pathological processes

and lead to a low-grade inflammatory state (Lago et al., 2007). Indeed, they are demonstrated to be involved in the pathogenesis of rheumatic diseases by the modulation of the inflammatory process in the joint, the imbalance between catabolic and anabolic factors, and the remodelling of bone and cartilage (La Cava, 2017).

In this study, Leptin levels have significantly increased in the serum of RA patients. Leptin is implicated in both innate and adaptive immune responses. It promotes the synthesis and secretion of pro-inflammatory cytokines. It enhances T-cell proliferation and memory-T-cells differentiation to T-helper (Th1), inhibits regulatory-T-cell (Treg) proliferation, (Carrión et al., 2019; La Cava, 2017; Lago et al., 2007)

In RA, Autoantibodies and rheumatoid factor (RF) are the first immune abnormalities detected, followed by joint damage starting in the synovial membrane. Synovium inflammation appears in the early stages of the disease after activation of endothelial cells that express adhesion molecules and chemokines following the infiltration of leukocytes through the synovium; leptin has been described as implicated in RA pathogenesis(Alam et al., 2017; Radu and Bungau, 2021) .

Leptin has been described as modulating bone homeostasis through locally and centrally mediated mechanisms. It inhibits osteoclast differentiation in peripheral blood mononuclear cells (PBMCs) and murine spleen cells in bone culture via the RANKL/RANK/OPG system, thus contributing to the inhibition of bone resorption (Holloway et al., 2002).

Increasing levels of Leptin were confirmed due to their role in activated macrophage inducing the release of IL-6 and TNF-α. In vitro macrophage chemotactic activity is associated with Leptin induction. In autoimmune diseases, the deregulated immune response of cells is affected by the alteration of metabolic processes within these cells (Francisco et al., 2018b; La Cava et al., 2004) because Leptin binds to its long isoform receptor (Ob-RB) to induce its biological and physiological effect through the JAK/STAT signalling pathway. JAK/STAT signal transduction is caused by the involvement of Janus kinase 2 (JAK2), activators of transcription (STAT), and transducers found on more extended receptor isoform (Ob-Rb) (Stofkova, 2009).

In addition to Leptin, this signalling pathway requires the interaction between complex molecules, including node-like receptor pyrin domain-containing protein 3 (NLRP3) and IL-33(Giles et al., 2011) .

Throughout the roc analysis, leptin level was found to be a reliable surrogate biomarker of RA disease progression. Our results were consistent with those of others (Francisco et al., 2018b; Giles et al., 2011; Holloway et al., 2002; La Cava et al., 2004; Stofkova, 2009) who reported that the predictive value of Leptin showed an excellent diagnostic value for RA.

Since Leptin is a pro-inflammatory factor that stimulates the innate and acquired immune response, and its concentration increases during infection and inflammation, this study analyzed the cut-off point of Leptin in patients with RA. Given that this value has been defined in the Kerbala population compared to controls, it is worth highlighting the values to identify a more sensitive value for the other disease and population.

Conclusion

This study confirms the significant role of leptin as a pro-inflammatory factor in rheumatoid arthritis (RA). Elevated leptin levels were strongly associated with RA progression, as demonstrated through ROC analysis, highlighting its diagnostic value. Leptin's involvement in both innate and adaptive immune responses, particularly its influence on cytokine production and T-cell differentiation, underscores its contribution to RA pathogenesis. Furthermore, the identification of a leptin cut-off point specific to the Kerbala population suggests the potential for more sensitive diagnostic markers tailored to different populations.

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Extraction, Isolation, Purification and Identification of Caffeine in Zamioculcas Zamiifolia L. Leaves Cultivated in Iraq

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Abstract

Background: Phytochemical analysis of *Zamioculcas zamiifolia* has revealed the presence of alkaloids, phenols, flavonoids, endogenous metabolites, vitamins, carotenoids, and tannins. phytochemical profile of *Zamioculcas zamiifolia* holds promise for discovering new therapeutic agents and natural products. Caffeine is a purine alkaloid, its trimethyl xanthine compound that has stimulant effect on central nervous system.

Materials and Methods: the fresh leaves of plant were extracted by harborne extraction method in reflux apparatus by using ethyl acetate, chloroform, chloroform- methanol, and distilled water and then determine compounds preliminary by using specific tests through phytochemical assay and TLC. Further phytochemical investigation of compounds performed by using GC-MS, FT-IR, melting point, HPLC, and Mass spectrometer.

Results: the results exhibit different fractions as ethyl acetate, chloroform, chloroform: methanol, chloroform and distilled water weighed 0.92, 0.19,1.51, and 28.65 gram respectively, also the presence of alkaloids specifically purine alkaloids, terpenoids, and phenolic compounds which show in leaves extract, in addition to the isolation of pure caffeine from chloroform-methanol fraction and this study was the first one that isolate caffeine from *Zamioculcas zamiifolia* in the world.

Conclusion: The phytochemical analyses results exhibit the extract fractions of *Zamioculcas zamiifolia* leaves indicate the plant considered as an important source of active compounds that might feeding the modern medicine with drugs especially caffeine discovering in plant. Therefore, additional researches are required to confirm the antimicrobial, anthelminthic, anti-hyperglycemic, and anti-inflammatory activities. As well as, compounds isolation, purification and even characterization are essential to make the plant has a novel important study.

عزل وتنقية وتشخيص الكافيين في نبات الزاميا القلقاسية المستزرع في العراق

استبرق حسين ناصر

الخلاصة

المقدمة: كشف التحليل الكيميائي النباتي لنبات الزاميا القلقاسية عن وجود قلويدات وفينولات وفلافونويدات ومستقلبات داخلية وفيتامينات وكاروتينات وعفص. يحمل الملف الكيميائي النباتي لنبات الزاميا القلقاسية وعدًا باكتشاف عوامل علاجية ومنتجات طبيعية جديدة. الكافيين هو قلويد البيورين، ومركبه ثلاثي ميثيل الزانثين له تأثير منبه على الجهاز العصبي المركزي.

المواد والطرق: تم استخلاص أوراق النبات الطازجة بطريقة الاستخلاص هاربورن في جهاز الارتجاع باستخدام أسيتات الإيثيل، والكلوروفورم، والكلوروفورم-الميثانول، والماء المقطر ومن ثم تحديد المركبات الأولية باستخدام اختبارات محددة من خلال التحليل الكيميائي النباتي والتصوير المقطعي بالبلورات السائلة، ثم تم إجراء مزيد من التحقيق الكيميائي النباتي للمركبات باستخدام GC-MS وFT-IR ودرجة الانصهار وHPLC وMass spectrometer.

النتائج: أظهرت النتائج وجود اجزاء مختلفة من خلات الإيثيل، الكلوروفورم، الكلوروفورم، الميثانول، الكلوروفورم والماء المقطر بوزن 0.92، 0.19، 1.51، و28.65 جرام على التوالي، كما أظهرت وجود قلويدات وخاصة قلويدات البيورين، التربينويدات، والمركبات الفينولية والتي تظهر في مستخلص الأوراق، بالإضافة إلى عزل الكافيين النقي من جزء الكلوروفورم-الميثانول وهذه الدراسة هي الأولى من نوعها لعزل الكافيين من نبات الزاميا القلقاسية في العالم

الاستنتاج: أظهرت نتائج التحليلات الكيميائية النباتية أن مستخلصات أوراق نبات الزاميا تشير إلى أن النبات يعتبر مصدرًا مهمًا للمركبات النشطة التي قد تغذي الطب الحديث بالأدوية خصوصا اكتشاف وجود الكافيين في النبات. لذلك هناك حاجة إلى أبحاث إضافية لتأكيد الأنشطة المضادة للميكروبات ومضادات الديدان ومضادات ارتفاع السكر في الدم ومضادات الالتهابات. كما أن عزل المركبات وتنقيتها وحتى توصيفها أمر ضروري لجعل النبات موضوعًا لدراسة جديدة مهمة.

1. Introduction

Zamioculcas zamiifolia is a monocotyledonous perennial flowering and ornamental plant that belongs to family Araceae (Badizadegan et al., 2023; Croat and Ortiz, 2020; Krömer et al., 2019). Phytochemical analysis of *Z. zamiifolia* has revealed the presence of alkaloids, phenols, flavonoids, endogenous metabolites, vitamins, carotenoids, and tannins (Belakhdar et al., 2015). The traditional use of *Z. zamiifolia* in folk medicine for treating various disease such as external use of leaves by people of Malawia to treat children's earache, for the moment roots are used by people of Sukuma to treat gastric problems in Tanzania (dos Santos et al., 2022). The health beneficial properties of medicinal plants are relatively attributed to antioxidant actions of their phytochemical components (Shang et al., 2022; Xu et al., 2017).

Iraq, with its unique ecological diversity and rich botanical heritage, offers an intriguing setting for the study of Z. *zamiifolia* and its phytochemical constituents. However, despite its widespread cultivation in the region, there remains a paucity of scientific literature regarding the phytochemical composition and pharmacological potential of *Z. zamiifolia* specimens cultivated in Iraq (Sapiun et al., 2020; Zuecco et al., 2022). This study focusses to bridge this knowledge gap by conducting a comprehensive phytochemical investigation of *Z. zamiifolia* plants grown in Iraqi soil. By employing advanced analytical techniques such as chromatography and spectroscopy (El-Emary, 2021; Türkyılmaz, 2022).

Taxonomy of *Zamioculcas zamiifolia* (Pourhassan et al., 2023)

- **Kingdom**: Plantae
- **Division**: Angiosperms (flowering plant)
- **Class**: Liliopsida (monocotyledonous)
- **Order**: Arales
- **Family**: Araceae
- **Genus**: *Zamioculcas*
- **Species**: *Z. zamiifolia*

Phytochemical investigation of *Z zamiifolia's* leaves and petioles extract exhibits seven compounds, the major constituents of leaves is apigenin 6-C-(6″-(3-hydroxy-3-methyl-glutaroyl)-ß-glucopyranoside), as in Fig.1 (Price, 1985). *Zamioculcas zamiifolia* has antibacterial activity and the plant considered as a potential source of antibiotic compounds that significant to attack the antibiotic-resistant bacteria (Sasidharan et al., 2011; Seneviratne et al., 2020). Hence, this study seeks to extract, isolate, purify, identify and characterize the bioactive compounds present in *Z. zamiifolia* extracts, elucidate their chemical structures through a systematic analysis of the phytochemical composition of *Z. zamiifolia* cultivated in Iraq.

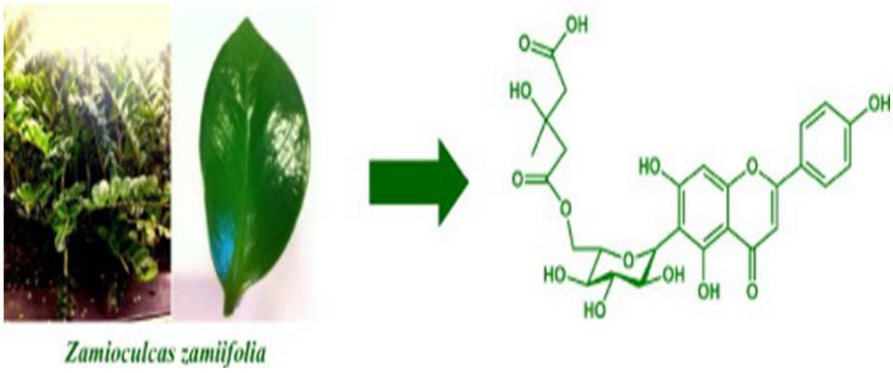

Figure 1: Describes Apigenin 6-C-(6″-(3-Hydroxy-3-Methyl-Glutaroyl)-ß-Glucopyranoside) Structure in *Zamioculcas Zamiifolia* Leaves (Price, 1985)

2. Materials, Patients and Methods

2.1. Experimental Work

The fresh leaves from *Zamioculcas zamiifolia* L. plant were collected from the garden during October, November in 2023. The plant leaves were washed thoroughly with tap water and then with distilled water and used as raw materials for the extraction of phytochemical compounds from the plant.

2.2. Preparation of Plant Extract

The extract was prepared by using 200 g of fresh leaves that homogenized in methanol: water (4:1) for 24 hrs. and extracted according to Harborne (Paterson, 1999; Richardson and Harborne, 1990) by using reflux apparatus, as in Fig.2. Preliminary phytochemical investigations of active compounds detected according to alkaloids, terpenoids, purine alkaloids, and phenolic compounds tests in the crude extract (Naser and Kathem, 2023). Chemical identification performed by GC-MS, FT-IR, melting point, TLC, and HPLC.

2.3. Chemical Identification by GC-MS

The investigation by GC-MS was performed by GC-MS Perkin Elmer Clarus 500 apparatus. Capillary column (30.0 m × 0.32 mm × 1.80 µm). the carrier gas was helium (99.9995% purity) at a constant flow rate of 1.61 mL/min with injection volume of 2 µL was employed in a split mode (El Hafidi et al., 2023). The temperature of injector was preserved at 280°C, and the column temperature was automated to 60°C (isothermal for 2 min) with an increase in temperature from 10°C/min to 280°C (isothermal for 6 min). 200°C for ion source temperature and 280°C for interface temperature were sustained. The mass spectra were gained through ionization energy of 70 eV in the EI mode. about 30 min was needed to run GC-MS. The compounds were recognized by comparison of their mass spectra with national libraries (NIST - 11) (Amudha et al., 2018; Munda et al., 2019; Sasikala and Chandra Mohan, 2014).

2.4. FT-IR (Fourier Transform Infrared Spectroscopy)

The FT-IR spectrum of isolated component was determined at pharmaceutics department in pharmacy College/ Kerbala University by a SHIMADZU apparatus. The structural duties have been connected for

characteristic bands of different chemical groups, many functional groups can be determined by their characteristic vibration frequency this makes the spectrums obtained from IR the simplest and often the mostreliable method of assigning a chemical substance to its class (Abd-Elhafeez et al., 2024; Bonfilio et al., 2010; Mudigiri and Jorige, 2023; Siddiqui et al., 2017; Zagade et al., 2020).

2.5. Melting Point Measurement

Melting point of an isolated compound was measured and matched with the reference standard, using electro–thermal melting point apparatus (Stuart / UK) at University of Kerbala/ College of Pharmacy.

2.6. Qualitative and Quantitative Identification of Analysed Fraction by High Performance Liquid Chromatography

Qualitative estimations occur using LC800-0101, USA. Diode array detector 2.1L, and C18 (150X4.6) 5 µm particles size from water corporation, USA. The mobile phase used was water: methanol (60:40) both are in HPLC grade water, standard compound was caffeine, flow rate was 1 ml / min, and the HPLC chromatogram was detected using a photo diode array UV detector at (275 nm) (Braz et al., 2012) at AL- Ameed university in Kerbala. Identification was made by comparing retention times obtained at identical chromatographic condition of analyzed sample and authentic standard (Gupta and Garg, 2014).

2.7. Mass Spectrometer

Isolated caffeine mass was measured at Al-Zahrawi University College, Karbala, Iraq by using Advion's expression® Compact mass spectrometer apparatus, USA. The measured mass then matched with standard caffeine mass by interpreted the fragmentation peaks.

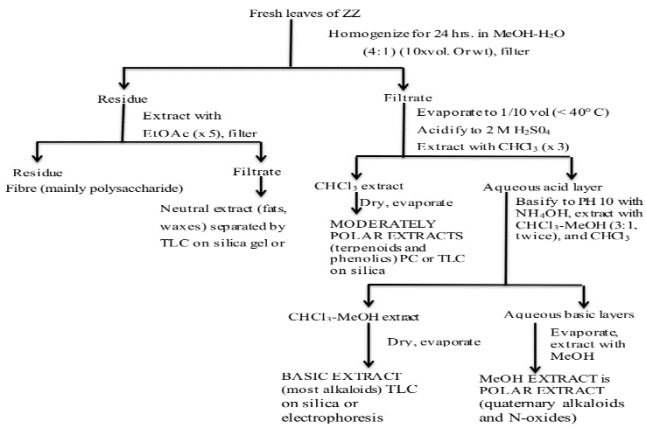

Figure 2: Extraction procedure of *Zamioculcas zamiifolia plant* leaves

3. Results

3.1. The extraction of *Zamioculcas zamiifolia* leaves yield four different fractions weighed as in Table 1 and undergoing phytochemical tests as in Table 2.

Table 1: Weight Yield of Each Fraction Obtained from Extraction Method

Extraction method	Weight of crude extract	Yield%
Ethylacetate fraction	0.92 g	0.46
$CHCl_3$ fraction	0.19 g	0.1
$CHCl_3$:MeOH fraction	1.51 g	0.76
Aqueous fraction	28.65 g	14.33

Table 2: Phytochemical Tests of Alkaloids, Purines Alkaloids, Terpenoids, And Phenolic Compounds of *Zamioculcas Zamiifolia* Leaves

Test Name in Plant Fraction	Result
Alkaloids test in aqueous fraction	+
Purines alkaloids test in CHCl3- MeOH fraction	+
Terpenoids test in Ethylacetate fraction	+
Phenolic compounds in $CHCl_3$ (1) fraction	+
(Absent –, Presence of compound +)	

3.2. GC.MS Of Phytochemical Constituents of *Z. Zamiifolia*

The analysis of ethylacetate fraction carried out by GC-MS apparatus, exhibited about nine different compounds in *Z. zamiifolia* leaves fractions based on the extraction method as shown in Fig.3. and recorded in Table 3.

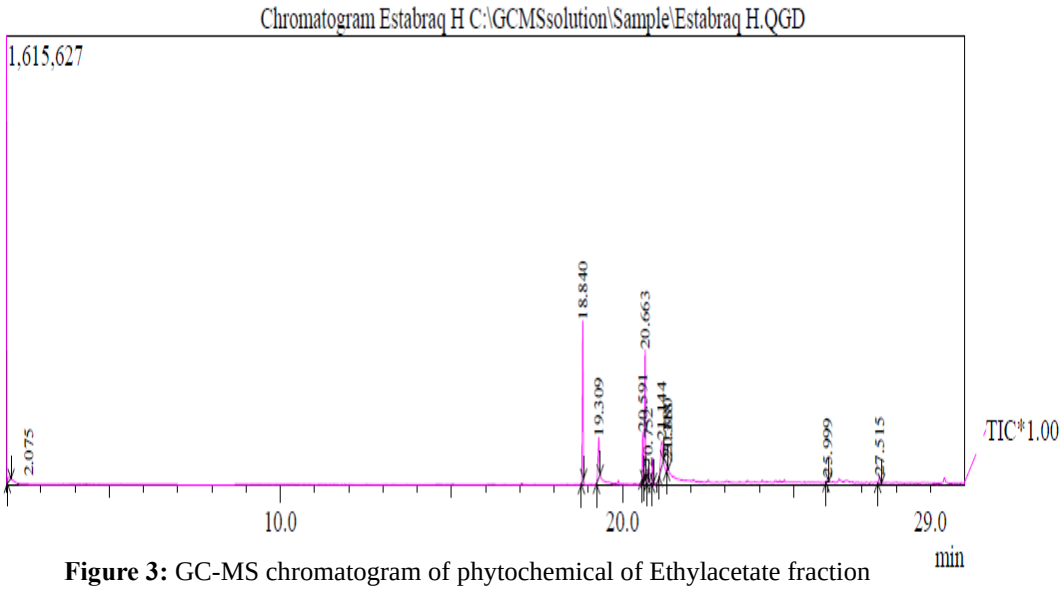

Figure 3: GC-MS chromatogram of phytochemical of Ethylacetate fraction

Table 3: Chemical components in leaves of *Z. zamiifolia detected* by GC.MS

NO.	Compound name	Chemical formula	SI	RT
1	Peroxide, bis(1-methylpropyl)	C8H18O2	81	2.075
2	Methyl 4-hydroxybutanoate	C5H10O3	62	18.842
3	1-Butanol, 3-methyl-, formate	C6H12O2	68	19.308
4	2,4-Pentadien-1-ol, 3-ethyl-, (2Z)-	C7H12O	75	20.667
5	Oxalic acid, isobutyl pentyl ester	C11H20O4	84	20.750
6	9-Oxabicyclo[6.1.0]nonane, cis-	C8H14O	74	21.142
7	1-Pentanol	C5H12O	61	21.317
8	Furost-5-en-3-ol, 22,26-epithio-, (3.beta.,22.alpha.,25R)-	C27H42O2S	77	26.000
9	1,3,5,7,9-Pentaethylbicyclo[5.3.1]pentasiloxane	C10H28O6Si5	51	27.517

3.3. Fourier Transforms Infrared (FT-IR) Spectra

The FT-IR of the isolated caffeine constituent showed identical spectrum to that of standard caffeine as reported in literature (Bansode et al., 2016; Mudigiri and Jorige, 2023) and shown in Fig.4. The characteristic IR absorption bands of isolated caffeine constituent are recorded in Table 4.

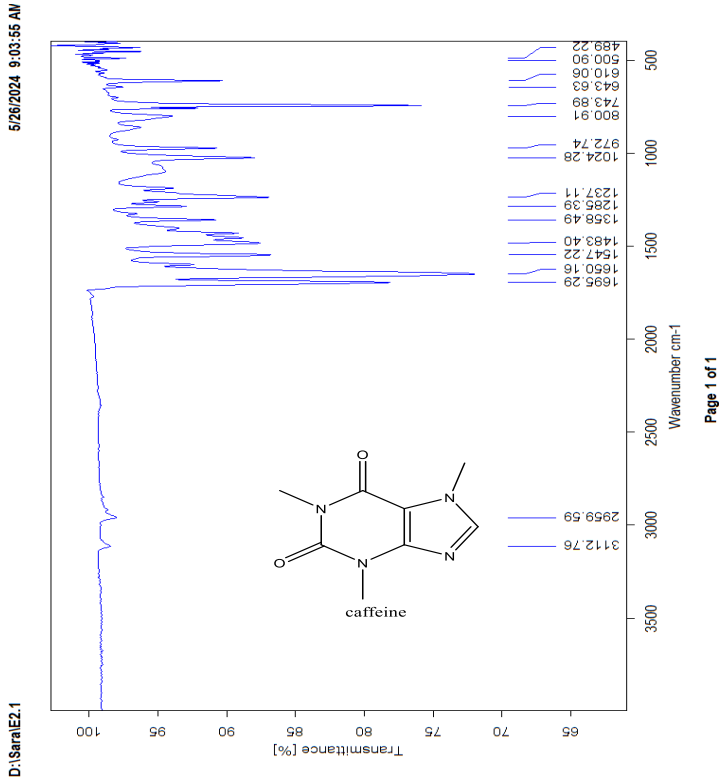

Figure 4: FT-IR spectra of isolated caffeine compound

Table 4: Characteristic FTIR Absorption Bands (Cm^{-1}) of the Isolated Caffeine Constituent

Functional group	Group frequency wave number (in cm^{-1})		Main attributed
	Isolated caffeine	Standard caffeine	
N–H	3112.76	3111	N-H stretching
C—H	2959.59	2953	C-H stretching
C=O	1695	1703	C=O stretching (conjugation and H- bonding)
C—N	1547.22	1546	C-N stretching
C-H	1358.49	1361	C-H bending of CH2
C–C	972.74	974	C-C stretching

3.4. Melting Point Measurement

The melting point of isolated caffeine was 235-238°C, compared to standard caffeine 238°C.

3.5. Qualitative and Quantitative Analysis of Caffeine by High Performance Liquid Chromatography (HPLC)

The qualitative identification of the active constituent was carried out by contrasting the retention periods of analysed sample and authentic standard (caffeine) obtained at identical chromatographic conditions, as in Fig.5 and Fig.6. The quantitative identification carried out by calculating Area under the curve (AUC) versus five concentration levels of caffeine sample and its standard was used to plot the calibration curve for quantification studies. The concentration of the analyte was determined using a straight-line equation, as illustrated in Fig.7.

3.6. TLC

In analytical TLC, the isolated caffeine constituent appeared as a single spot with the same colour and R_f value as that of standard caffeine (R_f = 0.41) after devolved in Ethylacetate: Methanol: water (100:13.5:10) (Lederer, 1985; Makin, 1985; Thorburn Burns, 1986; Wagner and Bladt, 1996)and detected at UV 254nm as shown in Fig.8.

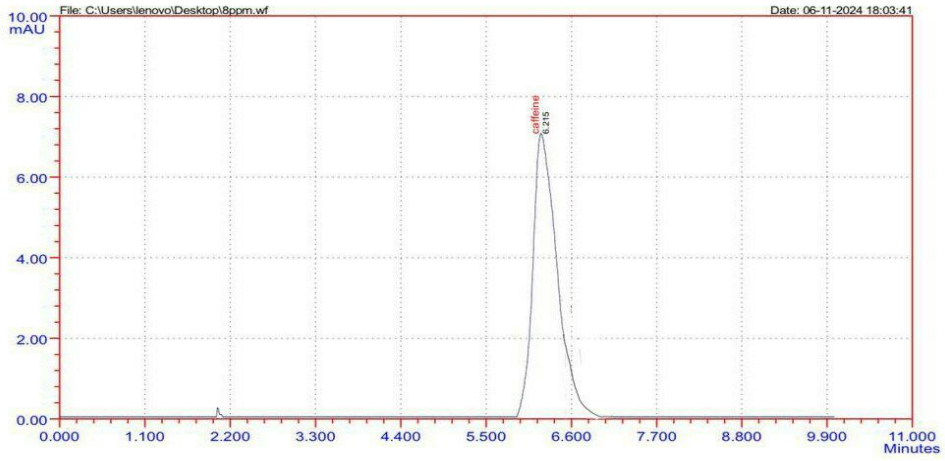

Figure 5: HPLC Chromatogram of Standard Caffeine

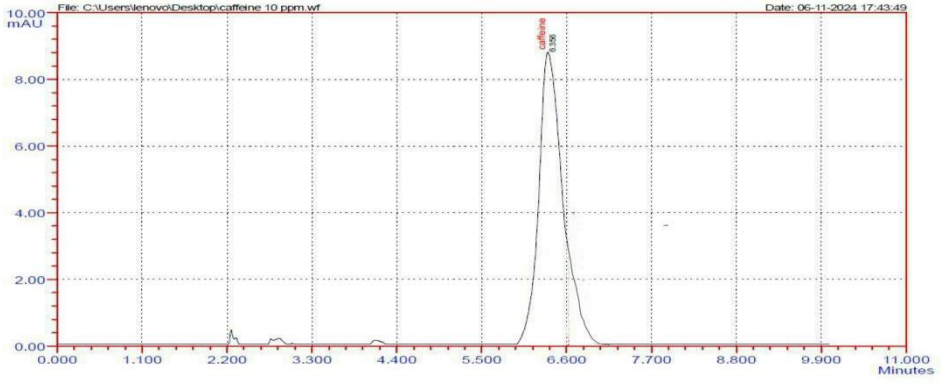

Figure 6: HPLC Chromatogram of Isolated Caffeine

concentration stander (ppm)	Area stander	conce sample ppm
0	0	y = 17.653x + 6.5953
2	45.4	45.56057502
5	98.7	
8	157.2	
10	173	

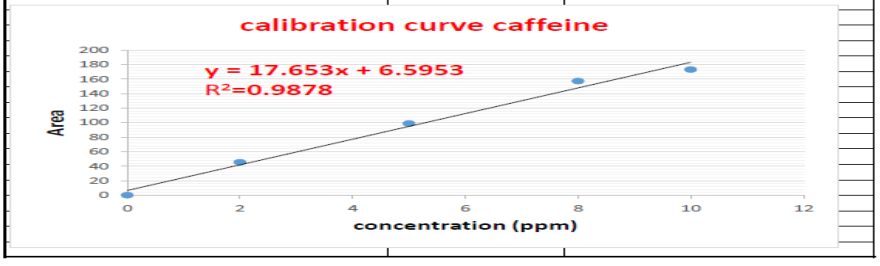

Figure 7: Calibration Curve of Caffeine

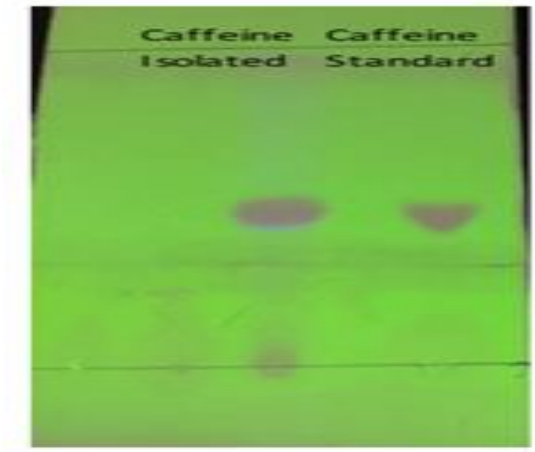

Figure 8: TLC Chromatogram of Isolated and Standard Caffeine at 254 nm.

3.7. Mass Spectrometer

The measured mass of isolated caffeine was 195.2 m/z that matched with standard caffeine mass by interpreted the fragmentation peaks according to literature (Dubale et al., 2023; Muharini et al., 2018; Salim et al., 2024) as in Fig.9.

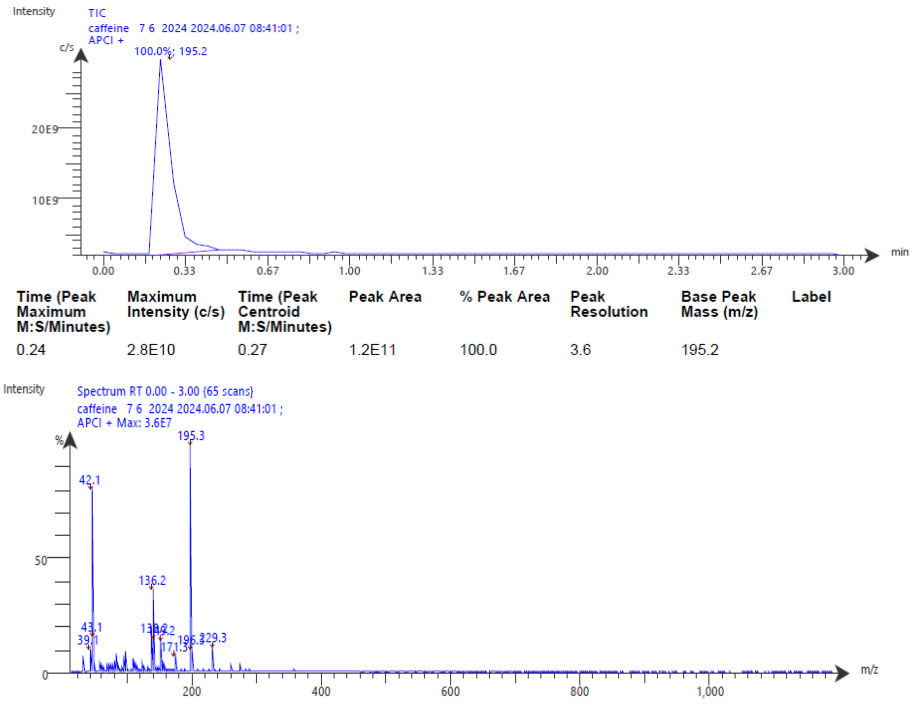

Figure 9: Representative Full Scan Product Ion Mass Fragmentation Spectra of Isolated Caffeine

4. Discussion

Zamioculcas zamiifolia extracted with solvents (chloroform, Ethylacetate, methanol-chloroform, and distilled water) according to differences in polarity, from Ethylacetate which is semi polar to extract fat and waxes or nonpolar substances and residue extract by solvent with increase polarity gradually, chloroform to extract moderately polar substances, that don't extracted with Ethylacetate like terpenoids and phenolic compounds, increase polarity as by using chloroform- methanol to extract basic compounds like purine alkaloids, increase polarity to distilled water mixed with methanol solvent to extract quaternary alkaloids and N-oxide. Fractions of crude extract then tested by using chemical test to identifying substances that present in extract solutions like alkaloids, terpenoids, phenolic compounds, and purine alkaloids as in literature (Gupta and Maurya, 2023; Koina et al., 2023). The result showed that the percent of active compounds by these solvents were almost different in the weight of final crude extract, Ethylacetate fraction was 0.92 g, chloroform was 0.19, chloroform- methanol was 1.51 g, and distilled water mixed with methanol fraction was 28.65 g, these different weights explain that the plant mostly contain polar compounds like quaternary alkaloids and N-oxide. The chemical components in Ethylacetate fraction of the leaves of *Z zamiifolia* which identified by GC-MS as tabulated in Fig.4. exhibit nine compounds including Peroxide, bis(1-methylpropyl); Methyl 4-hydroxybutanoate; 1-Butanol, 3-methyl-, formate; 2,4-Pentadien-1-ol, 3-ethyl-, (2Z)-; Oxalic acid, isobutyl pentyl ester; 9-Oxabicyclo[6.1.0]nonane, cis-; 1-Pentanol; Furost-5-en-3-ol, 22,26-epithio-, (3.beta.,22.alpha.,25R)-; 1,3,5,7,9-Pentaethylbicyclo[5.3.1]pentasiloxane. The predominant constituents were Methyl 4-hydroxybutanoate 28.99%; 1-Butanol, 3-methyl-, formate 12.45%; Oxalic acid, isobutyl pentyl ester 24.03%; and 9-Oxabicyclo[6.1.0]nonane, cis- 11.85%. The differences in these percent explained by the rule of like dissolve like. The chloroform- methanol fraction show the presence of pure caffeine when compared with standard one on TLC plate, melting point, FT-IR, HPLC, and mass spectrometer and this is the first study that isolate caffeine from *Z. zamiifolia* plant fresh leaves. The R_f value of isolated and standard caffeine was the same 0.41, as in Fig.8. The melting point of isolated caffeine was 235-238 as in literature (Dyulgerov et al., 2023). FT-IR spectrum peaks were the same spectrum when matched with standard caffeine as in literature (Arimurti et al., 2020; Nugrahani et al., 2019), HPLC results showed the same peak retention time of isolated caffeine that appear at 6.356 min. while the standard one appear at 6.215min., the calibration curve of different concentrations of standard caffeine (0,2,5,8,10) give straight line according to equation Y= a X +b, Where Y: is the response factor (AUC), a: is the slope of the curve (slop=y/x), x :is the concentration in part per million (ppm) or in (mg/ml), and b: is the y-intercept (Mohammed and Al-Bayati, 2009; Priyadi and Saifudin, 2023). Mass of isolated caffeine was matched with the standard one 195.2 m/z as in literature (Bianco et al., 2009).

5. Conclusion

Zamioculcas zamiifolia is considered as very interesting plant of secondary metabolites that may provide a new source of drugs to medicine. The plant biological activities such as antimicrobial, anthelminthic, anti-hyperglycemic, and anti-inflammatory should be studied. As well as, compounds isolation, purification and even characterization are essential to make the plant has a novel important study. Additional studies are needed to uncover other compounds in this plant that have yet to be discovered utilizing different portions of the plant. Other pharmacological studies are required to elucidate other reported and or unreported pharmacological activities.

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Bacterial Profile and Evaluation of Cxcl10 Level in Urine Among People Suffering from Urinary Tract Infections

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Abstract

A common bacterial infection that affects millions of people worldwide every year are infections of the urinary tract. The most frequent causes of urinary tract infections, both simple and serious, are *Escherichia coli* and *Klebsiella pneumonia*. *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis, Staphylococcus aureus*, and saprophylococcus *saprophyticus.* In the early 1970s and late 1980s, chemokines were discovered. These positively charged cytokines have molecular weights ranging from 8 to 10 kDA. Since they control immune cell infiltration and inflammatory mediator release., they are essential to the immune system. The 10 kDa interferon-gamma inducible protein of the inflammatory chemokine IP-10, (IP-10) is too referred as C-X-C motif chemokine ligand 10 (CXCL10). Assess the concentration of CXCl10 in the urine of individuals with urinary tract infections. Biomarker (CXCL10), had high-level in-patient group compared to control group which suggests an inflammatory state in these patients. the Sensitivity % of (CXCL10) marker 55.6% and the Specificity 82.2% can be castoff as markers of inflammation, progression, and complications in patients with UTI.

البيانات البكتيرية وتقييم مستوى الكيموكاين 10 في الادرار بين الاشخاص الذين يعانون من التهاب المسالك البولية

ألق علي عبد الحسين، مي الجيلاوي، مسار رياض رشيد

عدوى المسالك البولية هي عدوى بكتيرية شائعة تؤثر على الملايين من الناس في جميع أنحاء العالم كل عام. تعتبر الإشريكية القولونية والكلبسيلة الرئوية الأسباب الأكثر شيوعًا لعدوى المسالك البولية، سواء كانت بسيطة أو خطيرة. تشمل أيضًا البكتيريا المسببة لعدوى المسالك البولية بروتيوس ميرابيليس، الزائفة الزنجارية، المكورات المعوية، المكورات العنقودية الذهبية، والمكورات العنقودية الرمية. في أوائل السبعينيات وأواخر الثمانينيات، تم اكتشاف الكيموكينات. هذه السيتوكينات موجبة الشحنة تتراوح أوزانها الجزيئية بين 8 إلى 10 كيلودالتون. نظرًا لأنها تتحكم في تسلل الخلايا المناعية وإطلاق الوسطاء الالتهابيين، فهي ضرورية لجهاز المناعة. بروتين IP-10 *الكيموكيني الالتهابي ذو الوزن الجزيئي 10 كيلودالتون* (IP-10يُعرف أيضًا باسم الكيموكين ذو الدافع C-X-C (CXCL10). *تم تقييم تركيز* CXCL10 *في البول لدى الأفراد المصابين بعدوى المسالك البولية. وُجد أن هذا المؤشر الحيوي* (CXCL10) *مرتفع في مجموعة المرضى مقارنة بمجموعة التحكم، مما يشير إلى حالة التهابية في هؤلاء المرضى. كانت حساسية مؤشر* 55.6% (CXCL10) ، *والخصوصية 82.2%، ويمكن استخدامه كعلامة على الالتهاب، وتقدم الحالة، والمضاعفات لدى المرضى المصابين بعدوى المسالك البولية.*

1. Introduction

A common bacterial infection that affects millions of people worldwide every year are urinary tract infections (UTIs) (De Gaetano et al., 2023) . Bacteria are the main cause of UTIs, while viruses and fungi have also been shown to cause infections on occasion (Mancuso et al., 2023). Bacteria classified as Gram-positive or Gram-negative contribute to the development of infections. T The most frequent reasons of urinary tract infections, both simple and serious, are *Escherichia coli* and *Klebsiella pneumonia. Proteus mirabilis, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus,* and staphylococcus saprophyticus (Flores-Mireles et al., 2015) .UTIs typically affect the bladder. The bladder epithelium has strong barriers, and BECs have antimicrobial properties. Despite its benefits, UPEC frequently ignores BECs and the bladder epithelium(Wu et al., 2017) . Naturally, the cytokine response of the cells in response to an appropriate stimulus can serve as a means of both acquired and innate immunity to destroy or protect against pathogens (Pirdel and Pirdel, 2022). Early in the 1970s and late in the 1980s, chemokines were discovered. These positively charged cytokines have molecular weights ranging from 8 to 10 kDA. Since they control immune cell infiltration and inflammatory mediator release, they are essential to the immune system (Li et al., 2023). The activation, adherence, and recruitment of many types of white blood cells to areas of inflammation rely on chemokines and their corresponding receptors. Activation of type-1 helper (Th1) in an inflammatory environment leads to the production of IFN-γ and TNF-α. Consequently, several cells including lymphocytes, erythrocytes, keratinocytes, fibroblasts, neutrophils, monocytes, and endothelial cells release CXCL10. (Gao et al., 2020). Both IP-10 and CXCL10 refer to the same inflammatory chemokine, IP-10, which is an inducible protein of 10 kDa. IP-10 is linked to infectious agents such as fungi, viruses, bacteria, and parasites. (Hussein, 2021).

2. Material and Method

2.1. Ethical Approval

Before the sample was collected, written permission was obtained from each study participant, and all subjects involved in this experiment were informed. The University of Kerbala's College of Medicine's Publication Ethics Committee gave its approval to this work.

2.2. Study Design

Between October 2023 and January 2024, a total of 100 urine samples from both sexes (male and female) who were hospitalized and visited Al-Hussein Teaching Hospital / Laboratory Microbiology in Karbala, Iraq, with urinary tract infections were collected.

2.3. Clinical Samples

Usually, patients with UTIs provided the specimens for collection. Urine samples from midstream were collected and stored in sterile screw-cap containers. Following inoculation on culture media, the urine samples were cultured aerobically at 37°C for duration of 24 hours.

2.4. Phenotypic Identification of the Isolates

The isolated from pure colonies was phenotypically identified using GN cards (ID) and GN cards (ID) of the VITEK 2 system (Biomérieux, France), based on morphological, cultural, and biochemical properties(Bitew et al., 2017).

2.5. Measurement Chemokine 10 (CXCL10)

Chemokine 10 levels in urine were evaluated using ELISA research kits and a conventional sandwich-ELISA method (Human CXC -chemokine Ligand 10, CXCL10 ELISA KIT, BT LAB, China, CAT.No. E3800Hu).

2.5.1. Sandwich ELISA Method Principle

ELISA, when combined with an antibody, could be the most efficient immunosorbent method for detecting antigens. because their sensitivity is typically two to five times higher than methods where the antigen is directly attached to the solid surface. To detect antigen, test solutions containing antigen are incubated after a specific (capture) antibody is coated on micro titer plate wells. Following the removal of the unbound antigen, incubation is continued using a second antibody specific to the antigen that has been coupled to an enzyme (developing reagent). After removing the unbound conjugate, substrate is added. Following a second incubation, the degree of substrate hydrolysis is evaluated. There is a direct correlation between the amount of hydrolyzed substrate and the amount of antigen present in the test solution (Hussein, 2017).

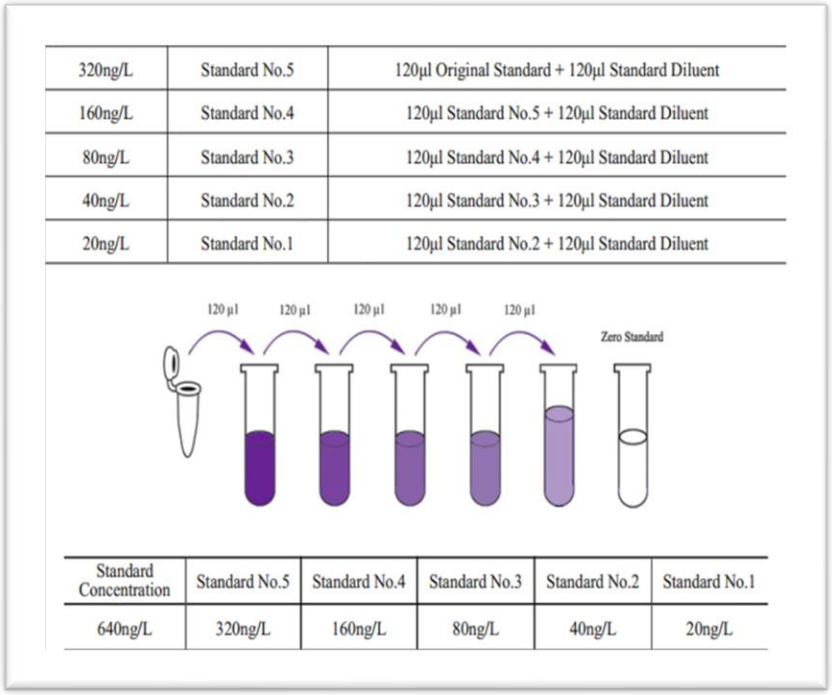
2.5.2. Test Principle

The plate has been pre-coated with a human CXCL10 antibody. Upon introduction into the sample, CXCL10 forms a binding interaction with the antibodies that have been immobilised on the surface of the wells. Subsequently, the sample's CXCL10 was attached by introducing biotinylated human CXCL10 antibody. The biotinylated CXCL10 antibody was subsequently bound by the addition of streptavidin-HRP. After incubation, any streptavidin-HRP that was not bound was eliminated using a washing process. Upon the addition of the substrate solution, the colour evolved in direct correlation to the concentration of human CXCL10. The process was halted by introducing an acidic stop solution, and the absorbance at 450 nm was subsequently quantified, consider specifying that the absorbance was measured using a microplate reader.

2.5.3. Assay Procedure

1. Before beginning the assay procedure, all of the reagents were prepared Fig.1.
2. Each sample well received 50µl of standards, 40µl of sample, and 10µl of biotinylated antibody; the standard well did not get biotinylated antibody since it was present in the standard solution.
3. Each well (standard and sample wells) received 50µl of Streptavidin-HRP reagent; the blank well received no additions. The wells were then covered with a seal plate, gently shaken, and incubated for 60 minutes at 37C.
4. The color developed as follows: 50µl of Substrate Solution A was added to each well first, followed by the addition of 50µl of Substrate Solution B to each well. Shake well to combine. incubated in a dark, 37°C environment for ten minutes in order for color to develop.

5. 50µl Stop Solution to each well had been added to stop the reaction (The blue color immediately became yellow.
6. After applying the stop solution, the optical density (OD value) was measured at 450 nm in less than ten minutes.



320ng/L	Standard No.5	120µl Original Standard + 120µl Standard Diluent
160ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
80ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
40ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
20ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
640ng/L	320ng/L	160ng/L	80ng/L	40ng/L	20ng/L

Figure 1: Concentration of Standards of cxcl10

2.5.4. Calculation of Results

The results were calculated according to the standard curve shown in Fig.2.

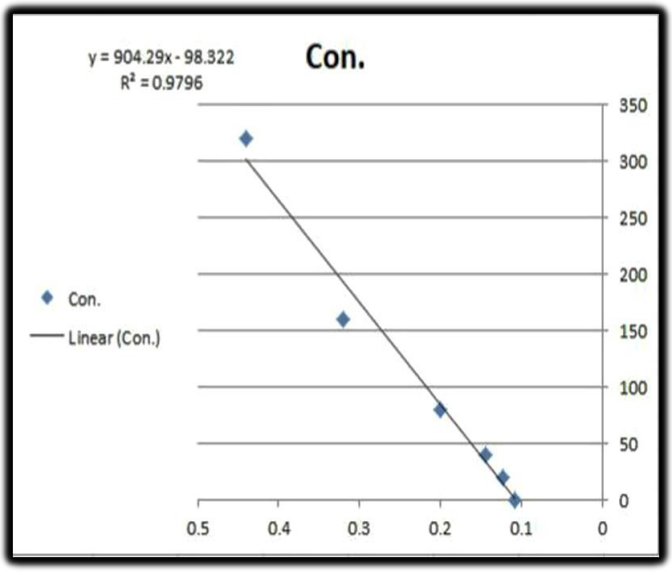


Figure 2: CXCL10 Standard Curve

2.6. Statistical Analysis

The statistical analysis of this study was conducted using IBM SPSS V27.0 software. Data were presented as the Mean ± standard Error of the mean. Chi Square was employed for comparison, and a ROC curve was utilized to identify the appropriate cut-off value for diagnosing the presence or absence of a disease.

3. Results

3.1. Demographic Characteristics of Study

The study samples as presented in Table 1conducted that People who live in the rural areas suffered from urinary tract infections more than those who live in urban areas, where the percentage in rural areas was (66.6) and in urban areas it was (33.3). People suffer from urinary tract infections more in the summer season than in the winter season, where the percentage in summer (68.8) and in winter was (31.1). The distribution of UTI cases based on age is as follows: 42.2% were in the (15-30) year age group, 26% were in the (46-60) year age group, 20% were in the (31-45) year age group, and 11.1% were in the >60-year age group.

Table 1: Demographic Characteristics of Study

Variables		Patients	Percentage
		Count	%
Sex	Male	7	15.6
	Female	38	84.4
	Total	45	100
Residency	Urban	15	33.3
	Rural	30	66.6
	Total	45	100.0
Season	summer	31	68.8
	Winter	14	31.1
	Total	45	100.00
Age	15-30	19	42.2
	31-45	9	20
	46-60	12	26.6
	>60	5	11.1
	Total	**45**	**100.00**

3.2. Bacterial Isolation Percentage

The microbiology data obtained for the current study showed that the Gram-positive bacteria were 23 isolates (51.11%) represented by *Coagulase -ve staphylococcus* species (20%) were the commonest isolated genera, followed by *Staphylococcus haemolyticus* (8.8%), *Staphylococcus aureus* (8.8%), *Staphylococcus epidermidis* (4.4), *Staphylococcus hominis* (2.2%), *Enterococcus faccalis* (2.2%), while 22 isolates (48.88%) were Gram negative bacteria represented by *Escherichia coli*.as in Fig.3.

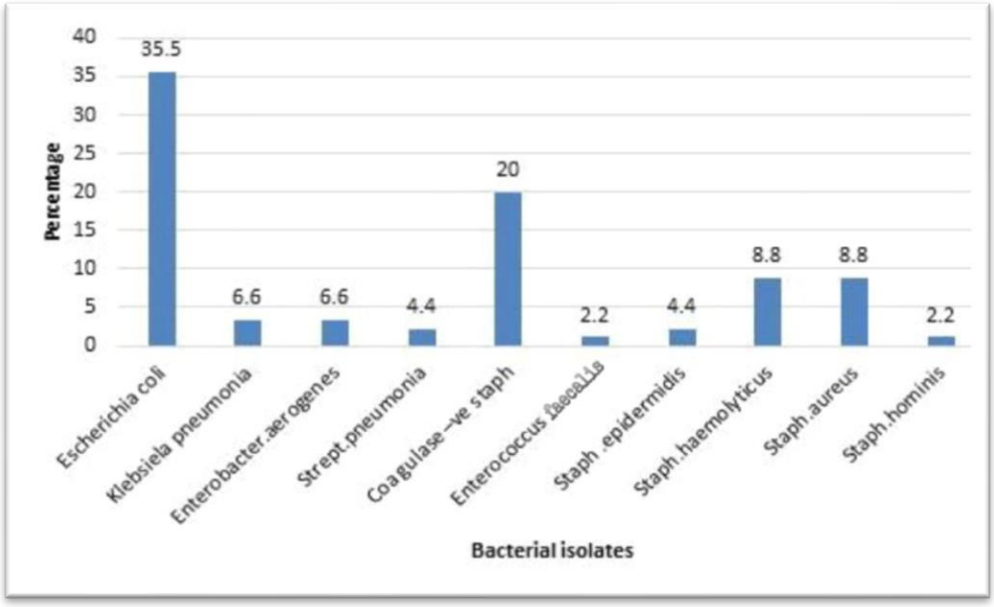


Figure 3: Percentage of Bacterial Isolates Studied

3.3. Study of the Immune System

3.3.1. Level CXCL10 in Patients and Control Group

The results summarized in Table 3. The analysis revealed a statistically significant difference ($P < 0.001$*) between the total number of controls and the total number of patients.

Table 3: Level CXCL10 In Patients and Control Group

Parameter	CXCL-10 level mean ± SE (pg/ml)		Probability
	Patients	Control	
CXCL10	137.02 ± 6.42	107.28 ± 4.99	P < 0.001*

3.3.2. ROC Curve and AUC Analysis for the CXCL10 In Patients with Urinary Tract Infection Compared to Control Groups

For the CXCL10, ROC curve and AUC analysis were carried out for patients in comparison to the control group. Platelets perform well in predicting such cases, according to the results of the receiver operating curve (ROC) curve and AUC analysis for CXCL10 as a diagnostic parameter; the data are shown in Table 4 and Fig.4. For CXCL10 levels, at a level of 131.795, the sensitivity is 55.6% and the specificity is 82.2%. The AUC's p-value was statistically significant, at less than 0.001. Youden's J statistics were used to validate the Sensitivity and Specificity results for the given values.

Table 4: Receiver operating characteristic curve showing sensitivity and specificity of CXCL10 in patients with Urinary tract infection compared to control groups.

Test Result Variable(s)	**CXCL10**
AUC	71.4%
Sensitivity %	55.6%
Specificity %	82.2%
Youden index	0.378
Cut-off points	131.795
CI (95%)	0.607-0.822
PPV	76%
NPV	65%
Accuracy	70%
P value	**<0.001[S]**
S= Significant, PPV= Positive protective value, NPV= Negative predictive value, AUC= Area under curve, CI= confidence interval	

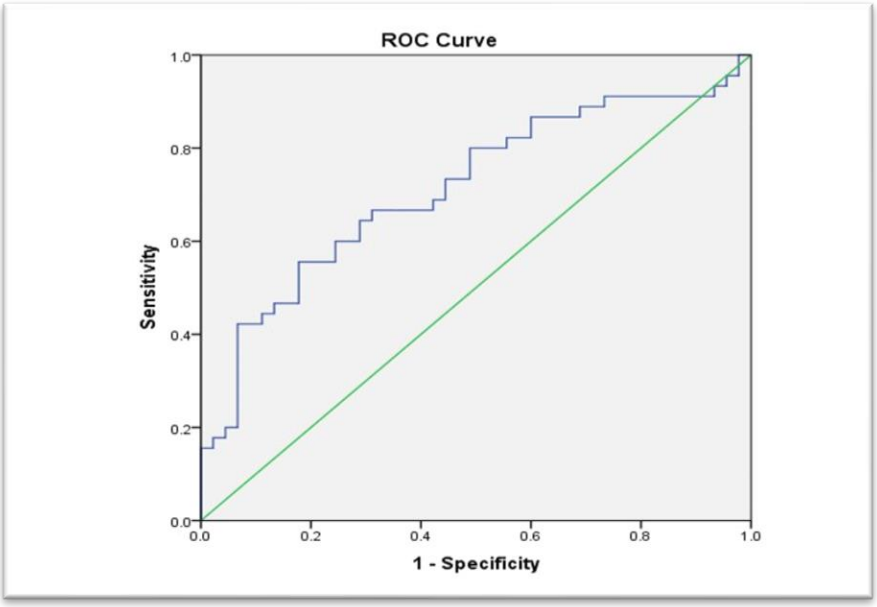


Figure 4: Receiver operating characteristics (ROC) curve analysis was used to compare the levels of CXCL10 in patients with urinary tract infection to those in control groups.

4. Discussion

Urinary tract infections are the most frequent Microbial diseases caused by bacteria encountered by health care professionals (Spaulding and Hultgren, 2016). In current study noticed the Females had a higher prevalence and incidence of urinary tract infections than males The closer distance between the urethra and the anus, the broader and shorter urethra, sexual activity, incontinence, and the surface of the vagina has a lower acidity level and unclean living conditions (Quaiser et al., 2015). Males are more prone to UTIs after the age of fifty, when they are also more likely to experience prostate issues as a result of less prostatic fluid. Additionally, a larger prostate gland may restrict and obstruct urine flow, increasing the risk of infection. According to Nicolle's (2008) observations, males who are not circumcised also have a higher likelihood of contracting UTIs because germs can more readily accumulate in the folds of the additional skin of the penis (John et al., 2016). There is evidence from studies that show how a community's socioeconomic status and geographic location might affect the prevalence of urinary tract infections (UTIs) (Ayoyi et al., 2017).

A higher proportion of individuals residing in rural regions experienced urinary tract infections, with 66.6% in rural areas compared to 33.3% in urban areas. Current study agreement with results of (Seifu and Gebissa, 2018) in Shashemene referral hospital, Ethiopia ,where the percentage of prevalence of urinary tract infections in Urban area (37.1)% ,and in Rural (62.9)%.

Individual-level risk factors for urinary tract infections (UTIs) include sexual activity, female sex, prior history of UTIs, and possibly insufficient fluid intake or dehydration (Simmering et al., 2018) . There may be environmental risk factors for UTIs in addition to risk factors at the human level. Indeed, single-center studies have shown that UTIs are more common during the summer, with a seasonal increase in incidence (Czaja et al., 2007).

In the study finding,the percentage people who sufferd UTI in summer season was higher agreemant with results (Simmering et al., 2018). Studies on infections in a range of age groups were few (Alwan et al., 2023). The present study showed that among males and females, the age group of 15-30 years had the highest number of infected people ,where the percentage 42.2% . According to results (Almukhtar, 2019),58.4% of patients were in the age range of 21-30 years agree with current study. Numerous studies revealed that the Enterobacteriaceae family was the most common cause of urinary tract infections in people (Odoki et al., 2019) These studies are in agreement with current study. In current study showed the E.coli was most common pathogen in UTI,were the percentage of *E.coli* was 35.5%.Result of (Alwan et al., 2023). agreement with current study.Results of disagreement with current study where Klebsiella spp. was the most prevalent microorganism in UTI patients. Cytokines and chemokines produced by the detrusor smooth muscle cells during the inflammatory process in the bladder wall may be discharged into the urine (Yu et al., 2022)According to recent research, a wide range of nonhematopoietic cells, such as urothelium and detrusor cells, express chemokines and their receptors(Bouchelouche et al., 2006). Chemokines are used for more than only inflammatory cell infiltration.

The secreted proteins in the chemokine superfamily have molecular weights between 8 and 10 kDa (Ragnarsdóttir and Svanborg, 2012)and work by interacting with G protein-coupled receptors that are found on

glycosaminoglycans in endothelial cell layers. Chemokines are known for their promiscuity, meaning that they can connect to multiple receptors simultaneously, and that receptors can bind to multiple chemokines(Ragnarsdóttir and Svanborg, 2012) . The result of current study showed elevated CXCL10 level in UTI patient.The result of (Tyagi et al., 2016)showed elevation level of CXCL10 corroborates with current study. No statistically significant differences in the urinary levels CXCL10 were discovered in convalescent phases or, the patients in the acute UTI, or in the healthy controls (Gorczyca et al., 2014).

5. Conclusions

Coagulase-negative staphylococcus was the predominant pathogen among the gram-positive isolates, while Escherichia coli was the most common pathogen among the gram-negative isolates, according to the bacteriological profile of urinary tract infections (UTIs). The biomarker investigation revealed that the patient group had significantly higher levels of the biomarker (CXCL10) compared to the control group, indicating the presence of an inflammatory condition in these patients. The marker CXCL10 has a sensitivity of 55.6% and a specificity of 82.2%. It can be used as an indicator of inflammation, progression, and complications in patients with UTI. Further studies with large sample size should be conducted to confirm the significant association of CXCL10 as indicator of severity UTIs patients. assessment the concentration of CXCl10 in the serum of individuals with urinary tract infections and compare concentration in urine.

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