Kerbala Journal of Pharmaceutical Sciences

Vol.14 . Issue 23 Jul 2023

P-ISSN: 2221-7207

Semiannual Journal
Pharmaceutical Research
Published by College of Pharmacy
/ University of Kerbala

+964- 7800- 054143

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مجلة كربلاء للعلوم الصيدلانية

المجلد الرابع عشر.العدد الثالث والعشرون , تموز ٢٠٢٣

P-ISSN: 2221-7207

مجلة نصف سنوية محكمة
تعنى بالابحاث الصيدلانية
تصدر عن كلية الصيدلة - جامعة كربلاء

+964- 7800- 054143

المحتويات

Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Loaded of Etodolac on Zinc Oxide Nanoparticles

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Received: 2023/05/01

Accepted: 2023/11/12

Published: 2024/01/04

Keywords: *Etodolac, ZnO nanometers, Pharmaceutical preparation*

DOI:10.62472/kjps.v14.i23.1-10

ABSTRACT

Etodolac, a chiral nonsteroidal anti-inflammatory drug (NSAID), is widely used for pain management and arthritis treatment. However, it suffers from various side effects, including gastrotoxicity and cardiovascular risks. This study investigated the potential of zinc oxide nanoparticles (ZnO NPs) as a delivery platform for etodolac to improve its efficacy and mitigate these adverse effects.

ZnO NPs possess several attractive properties for drug delivery applications, including low toxicity, biodegradability, and the ability to target specific tissues. In this study, etodolac was successfully loaded onto ZnO NPs, and the resulting nanocomposites were characterized using Fourier-transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM).

FT-IR analysis confirmed the successful loading of etodolac onto the ZnO NPs. Additionally, SEM images revealed morphological changes on the surface of the nanocomposites compared to pure ZnO NPs, further indicating successful drug loading.

These findings demonstrate the feasibility of developing etodolac-loaded ZnO NPs as a promising approach for targeted drug delivery. Further studies are warranted to investigate the vivo efficacy and safety of these nanocomposites, paving the way for their potential clinical application.

تحميل الإيتودولاك على جزيئات أكسيد الزنك النانوية

زينب عبد الامير حسين ، رجوان عبد الجبار غزاي ، نهاوند حامد

الملخص

يستخدم الايتودولاك وهو عقار مضاد للالتهابات غير الستيرويدي، على نطاق واسع لإدارة الألم وعلاج التهاب المفاصل. ومع ذلك، فإنه قد يسبب آثار جانبية مختلفة، بما في ذلك تسمم المعدة ومخاطر القلب والأوعية الدموية. بحثت هذه الدراسة في إمكانات استخدام الجسيمات النانوية كمنصة لتوصيل الإيتودولاك لتحسين فعاليته والتخفيف من هذه الآثار الضاره .

تمتلك الجسيمات النانوية لأكسيد الزنك العديد من الخصائص المهمة لتطبيقات توصيل الأدوية، بما في ذلك السمية المنخفضة، والقابلية للتحلل البيولوجي، والقدرة على استهداف أنسجة معينة. تم تحميل الإيتودولاك بنجاح على الجزيئات النانوية لاوكسيد الزنك وتم تشخيص المركبات النانوية الناتجة باستخدام التحليل الطيفي للأشعة تحت الحمراء لتحويل فورييه والمجهر الالكتروني الماسح.

اكد تحليل الاشعة تحت الحمراء على نجاح تحميل الايتودولاك على سطح جزيئات أكسيد الزنك النانوية بالاضافة الى ذلك كشفت صور المجهر الالكتروني عن تغيرات شكلية على سطح المركبات النانوية مقارنة ب جزيئات أكسيد الزنك النانوية النقية مما يشير الى نجاح عملية تحميل الدواء.

توضح هذه النتائج جدوى تطوير جزيئات أكسيد الزنك النانوية المحملة بالإيتودولاك كنهج واعد لتوصيل الأدوية المستهدفة. هناك ما يبرر إجراء المزيد من الدراسات للتحقيق في فعالية وسلامة هذه المركبات النانوية، مما يمهد الطريق لتطبيقها السريري المحتمل.

1. INTRODUCTION

Etodolac 1.8-diethyl- 1,3,4,9-tetrahydropyran (3.4-B)indole-l-acetic acid as shown in figure1. Etodolac is a White crystalline compound, practically insoluble in water but soluble in Alcohol, chloroform, dimethyl sulfoxide and aqueous polyethene glycol(Haldorai and Shim, 2014)The Etodolac main action is blocking the prostaglandin chemokines action which plays an important role in process of inflammatory. Etodolac possesses several unique disposition features mainly due to its stereoselective pharmacokinetics. In plasma, the concentrations of the 'inactive' R-enantiomer are about 10-fold higher than those of the active S-enantiomer, an observation that is novel among the chiral NSAIDs.

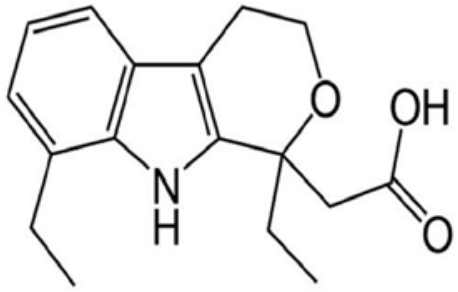

Figure 1. Structural Formula of Etodolac

In common with other NSAIDs, the drug is highly plasma protein bound and undergoes virtually complete biotransformation to oxidised metabolites and acyl-glucuronides. Etodolac is well absorbed, with maximal plasma concentrations attained within 1 to 2 hours in healthy volunteers. The elimination half-life of Etodolac is between 6 and 8 hours in plasma and is similar for both enantiomers (Sutapa *et al.*, 2018)

NSAIDs are used for the management of mild to moderate pain, fever, and inflammation. They work by reducing the levels of prostaglandins, which are chemicals that are responsible for pain and the fever and tenderness that occur with inflammation. Etodolac blocks the cyclooxygenase (COX) enzymes which form prostanoids, resulting in lower concentrations of prostaglandins .(Seay and Elim, 2019)

As with many nonsteroidal anti-inflammatory drugs (NSAIDs), Etodolac has side effects, such as gastrotoxicity, and cardio-Vascular risk. Formulation of etodolac Nanoparticles may reduce these side effects and help to target the active substance for better efficacy (Haldorai and Shim, 2014)

The Nano derives from the Greek word "nanos", which means extremely small.1 . The drug is dissolved, entrapped, encapsulated (or) attached to a nanoparticle matrix. The materials which are used for the preparation of nanoparticles should be nontoxic, biodegradable, sterlizable etc. The types of nanoparticle Nanospheres: Nanospheres are matrix systems in which the drug is physically and uniformly dispersed. Nanocapsules are systems in which the drug is confined to a cavity surrounded by a unique polymer membrane. The goal of using this nano is to control the particle size, surface properties and release of pharmacologically active agents to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen((Çırpanlı *et al.*, 2009), (Brocks and Jamali, 1994)). Nanoparticles are widely used Because of their unique properties and promising applications as anti-cancer and antimicrobial agents the material properties change as their size approaches the Atomic scale. This is due to the surface area to volume ratio increasing, resulting in the material's surface atoms dominating the material's performance. Owing to Their very small size, nanoparticles have a very large Surface area to volume ratio when compared to bulk materials, such as powders, plates and sheets. This Feature enables nanoparticles to possess unexpected Optical, physical and chemical properties, as they are small enough to confine their electrons and produce quantum effects .(Kirkby *et al.*, 2013)

For the past few decades, there has been considerable research interest in the area of drug delivery using particulate delivery systems as carriers for small and large molecules. Particulate systems like nanoparticles have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. They have been used in vivo to protect the drug entity in systemic circulation, restrict access of the drug to the chosen sites and deliver the drug at a controlled and sustained rate to the site of action. Various polymers have been used in the formulation of nanoparticles for drug delivery research to increase therapeutic benefit while minimizing side effects (Biswal, 2020).

One of the most promising directions is to use zinc nanoparticles for molecular diagnostics, target delivery of drugs, and developing new pharmaceutical preparations (Jain, 1997). The zinc nanoparticles possess unique semiconducting, optical, and piezoelectric properties, so it has been investigated for A wide variety of applications. One of the most important features of ZnO nanometers is low toxicity and biodegradability. Biomedical Applications Of ZNO nanoparticle ZnO NPs, as a new type of low-cost and low toxicity. Nanomaterials have attracted tremendous interest in various Biomedical fields, including anticancer, antibacterial; diabetic, and anti-inflammatory activities, as Anti-oxidant well as for drug delivery and bioimaging applications ((Soenen *et al.*, 2015), (Mohanraj and Chen, 2006)). Depending on these facts Etadolac was loaded on the ZnO nanoparticle's surface as a drug delivery system in this research.

2. The Experimental Part

2.1 Materials and Instruments

All the chemicals are of high purity, commercially available AR grade. All the chemicals are of high purity, commercially available AR grade. Ethanol solvent was supplied by Hi-Media, India. The ZnO nanoparticles are purchased from MKnano, Canada. Etodolac from SDI-Samara, Iraq

Table 1: The Instruments

Device	Company	Origin
Electric balance	Sartorius	Germany
Magnatic Stirrce	National	Japan
PH_meter	Mauritius	Germany
Infrared spectroscopy	Shimadzu	Japan
Electronic Microscope	FEI Quanta450	Czech Republic

2.2 Method

Preparation of Etodolac-Loaded Zinc Oxide Nanoparticles

The 0.02g pure Etodolac is dissolved in 50ml ethanol in a beaker and added to 1g of zinc oxide nanoparticles. The pH of the solution is adjusted to 7 by HCl. The ethanolic solution of the Etodolac drug and zinc oxide nanoparticles is stirred for 72 hours in a stirring device. The collected samples are filtered, and the prepared Etodolac conjugate zinc nanoparticles are collected.

2.3 The Fourier Infrared Spectroscopy

To characterize, and determine functional groups and modifications the FTIR spectroscopy performed for pure-ZnO-NPs, Etodolac and ZnO-NPs- Etodolac
as shown in figures (2,3,4).

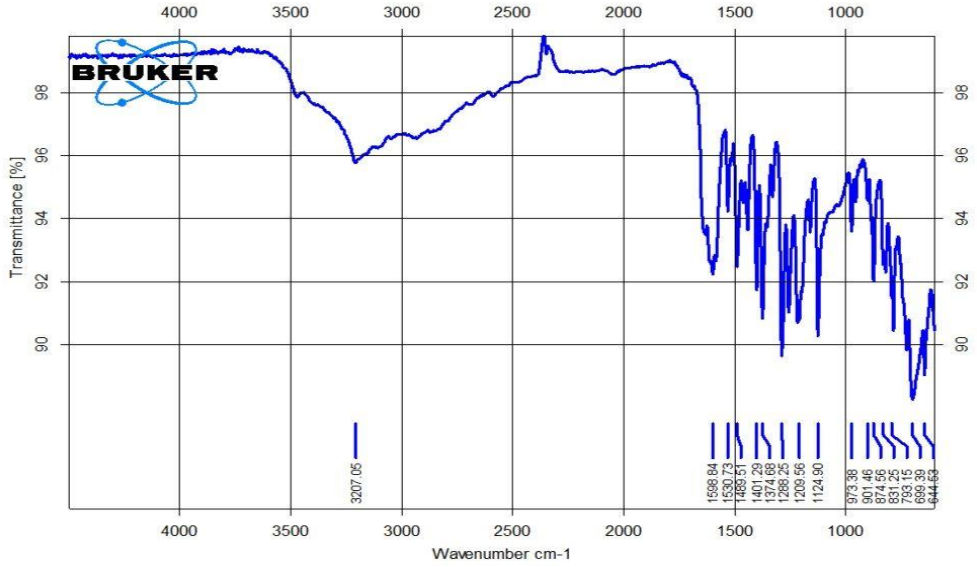

Figure 2: FTIR ZnO-NPs

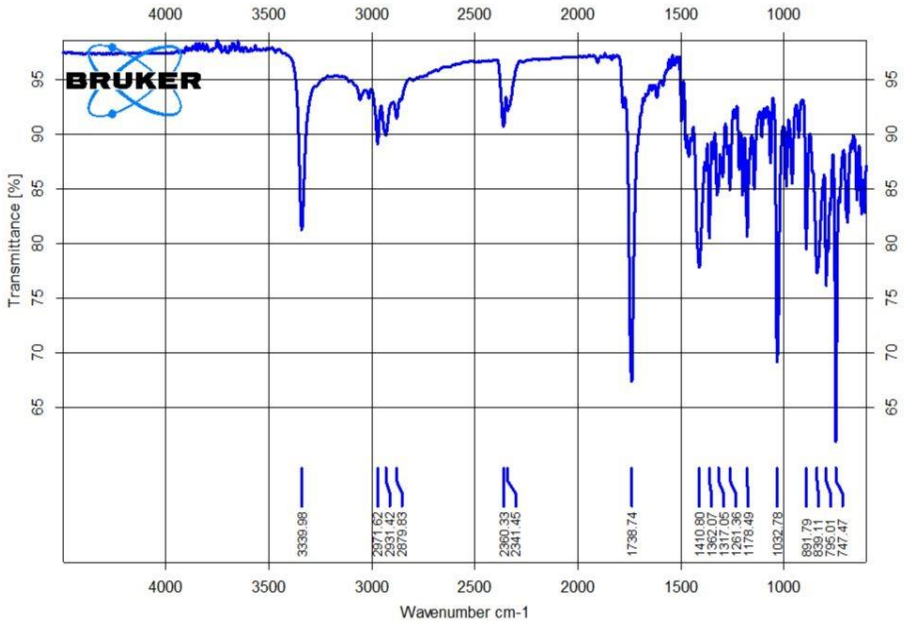

Figure 3: FTIR Etodolac

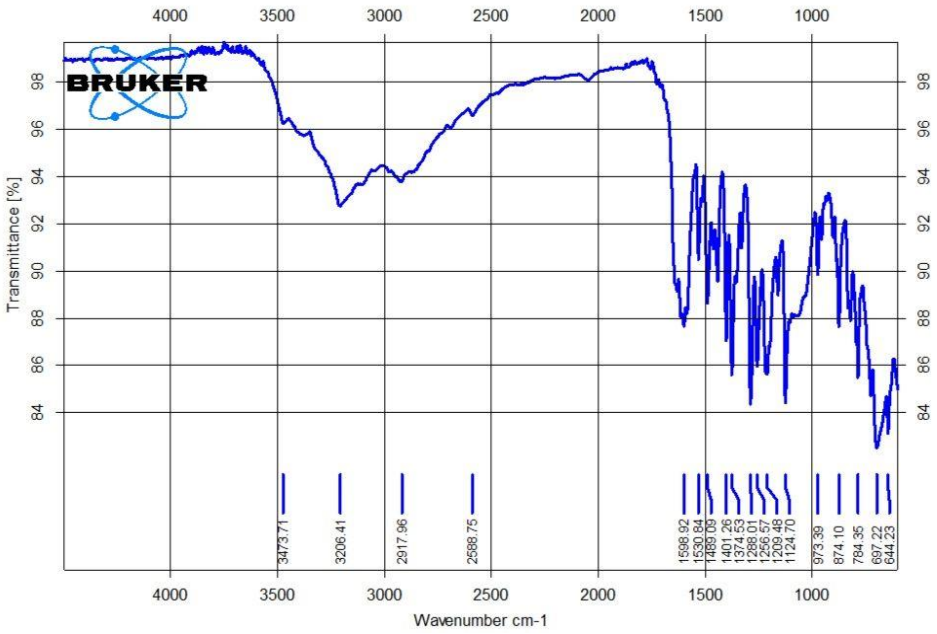

Figure 4: ZnO-NPs + Etodolac

2.4 The Scanning Electron Microscopy (SEM)

It studies the surface of the ZnO-NPs- Etodolac and the pure ZnO-NPs by using scanning electron microscopy to compare between them the results illustrated in Figure 5,6.

2.5 Drug Release%

An accurately weighed quantity of Etodolac conjugate nanoparticles was filed in capsules and placed in a beaker, which was immersed in 900 ml phosphate buffer having pH 7.4. The temperature of the media was maintained stable at 37 °C and stirred at a speed of 75 rpm. At specific time intervals (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 h). The collected samples were filtered and analyzed at 227 nm, using a UV-visible spectrophotometer against the phosphate buffer having pH 7.4 as a blank.

The equation 1 used to calculate the release percentage.

$$\text{Release\%} = \frac{Ct}{CT} \times 100 \text{ -------1}$$

3.Results and Discussion

3.1 The Fourier Infrared Spectroscopy (FTIR)

The Table 2, 3 and 4 explain the FTIR spectrum for Etodolac, ZnO-NPs- Etodolac and the pure ZnO-NPs

Table 2: FTIR Spectrum for Etodolac

Herbicides	**υC-H Aromatic**	**C-O**	**υC=O Acid**	**υC=C Aromatic**	**δC-H Aromatic**	**δN-H**
Etodolac	2879	1261	1738	1410	747	3339

Table 3: FTIR Spectrum for ZnO

Herbicides	**υ (Zn-O)**
ZnO	644

Table 4: FTIR Spectrum for ZnO-NPs

Herbicides	**υC-H Aromatic**	**υ (C=C) Aromatic**	**N-H**	**δ(C-H) Aromatic**	**υ (Zn-O)**
ETO-ZNO	**2917**	**1401**	**3473**	**784**	**644**

The etodolac- ZnO-NPs show new bands that indicate to successful process of inserting the Etodolac between the ZnO nanoparticle's layers.

The broad band at 3443cm-1 refer to starching vibration for the (OH) group. The υC=O disappears in the etodolac-ZnO-NPs and a new band at 644 cm-1 belonging to the Zn-O bond appears ((Ibrahim, Nada and Kamal, 2005), (Cornejo *et al.*, 2000)).

3.2 The Scanning Electron Microscopy (SEM)

Figures 5 and 6 show the difference between the size and the shape of particles for pure Zn-NPs and ZnO NPs-Etodolac that proved the load of Etodolac on ZnO-NPs

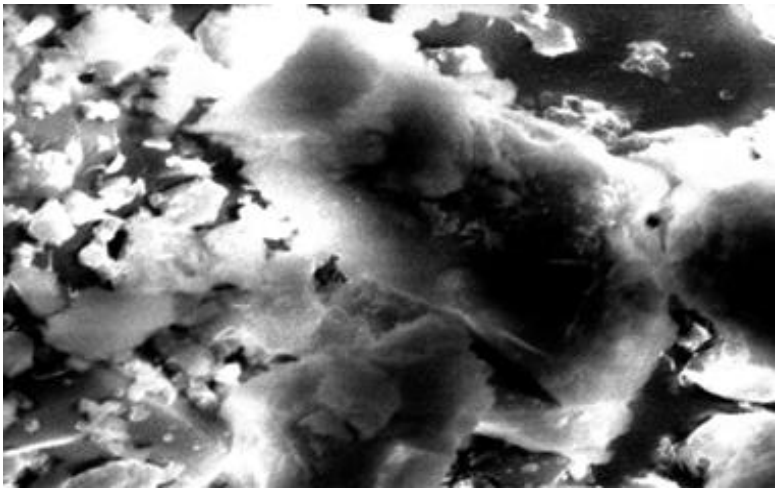

Figure 5: SEM for ZnO- NPs

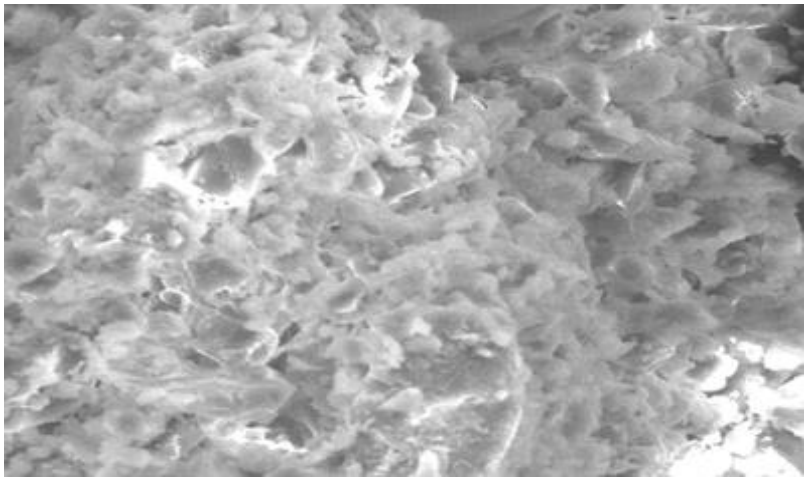

Figure 6: SEM for ZnO + Etodolac

Figure 5 observes the electron microscope images of zinc oxide before insertion, the presence of plate-like structures with low porosity, shapes, and sizes are unknown in the electron microscope images of the hybrid nanocomposite. Figure 6 shows the presence of structures with high porosity between the layers as a result of the attraction between Etodolac and the surface of the zinc oxide nanoparticles, which was shown by previous studies (Rosa *et al.*, 2013). The insertion of the compound into the zinc oxide changes from an irregular shape to nanostructured plates.

3.3 Drug Release

The drug release in Table 5 shows that the highest percent 98% at 50 min.

Table 5: The Etodolac Release with Time

Time / min	Drug Release%
5	15.3
10	24.5
15	33.7
20	42.2
25	55.3
30	62.1
35	73.3
40	82.1
45	90.5
50	98.7
55	98.7
60	98.7

4. Conclusion

ZnO and Etodolac-loaded ZnO nanoparticles were successfully characterized by FTIR and SEM. The analysis confirmed the loading of Etodolac onto the ZnO NP surface. The highest percentage of drug release occurred within 50 minutes. Notably, the incorporation of Etodolac into the zinc oxide structure resulted in a morphological transformation, changing from an irregular shape to nanostructured plates. Furthermore, the disappearance of the υC=O peak in the FTIR spectrum of Etodolac-loaded ZnO NPs provides compelling evidence for the successful formation of these nanocomposites

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Kerbala Journal of Pharmaceutical Sciences

DOI: 1032.25345.v.12-23 / **ISSN**: 2221-7207

Overview of Opportunistic Bacteria

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Received: 2023/05/13

Accepted: 2023/11/12

Published: 2024/01/04

Keywords*: Opportunistic bacteria, Predisposing Factors, Foodborne Opportunistic, waterborne Opportunistic, Airborne Opportunistic*

DOI:10.62472/kjps.v14.i23.11-21

Abstract

The receptive patient and the bacterium serve as the two defining criteria for opportunistic bacteria. Theoretically, no saprophytic or typical commensal microbes can infiltrate a healthy receptive person. Only specific "undesirable" commensal species, "such as Vargues' Specific Pathogenic Bacteria", can infect this person. Several species from the typical commensal flora, or opportunistic bacteria in the wide meaning of the word, “may infiltrate an otherwise healthy host if their immune defences temporarily deteriorate”. Even species that were previously thought to be non-virulent may assault an immunosuppressed patient with a significant and protracted immune system depression, “including various saprophytic and commensal microbes”. Several bacteria that are typically found in water, food, and the air have recently become opportunistic pathogens in both people and animals. The issue is made more difficult by the introduction of many antibiotic-resistant strains of these opportunistic pathogens, which make hospital-acquired infections in susceptible hosts challenging to treat in the setting of illness.

نظرة عامة على البكتيريا الانتهازية

أحمد طالب عبد الكريم، أحمد عباس حسن، حسن رحيم خضر، سيف عابد

الخلاصة

يعتبر المريض المستجيب والبكتيريا المعايير المحددة للبكتيريا الانتهازية. من الناحية النظرية، لا يمكن لأي ميكروبات رمية أو تكافلية نموذجية أن تتسلل إلى شخص صحي مستجيب. فقط أنواع معينة من الأنواع التكافلية "غير المرغوب فيها"، مثل "البكتيريا الممرضة المحددة لفارجس"، يمكنها إصابة هذا الشخص. يمكن لعدة أنواع من الفلورا التكافلية النموذجية، أو البكتيريا الانتهازية بالمعنى الواسع للكلمة، "أن تتسلل إلى مضيف صحي إذا تدهورت دفاعاته المناعية مؤقتاً". حتى الأنواع التي كانت تُعتبر سابقاً غير ممرضة قد تهاجم مريضاً مثبط المناعة يعاني من اكتئاب مناعي كبير وطويل الأمد، "بما في ذلك مختلف الميكروبات الرمية والتكافلية". عدة بكتيريا توجد عادة في الماء والطعام والهواء أصبحت مؤخراً ممرضة انتهازية في كل من البشر والحيوانات. تتعقد المشكلة بإدخال العديد من السلالات المقاومة للمضادات الحيوية من هذه الممرضات الانتهازية، مما يجعل العدوى المكتسبة في المستشفيات لدى المضيفين المستجيبين صعبة العلاج في سياق المرض.

1.Introduction

Opportunistic pathogens (OPs) are often defined as organisms in the medical literature that can become harmful after being perturbed by their host (such as sickness, wound, medicine, past infection, immunodeficiency, and aging). “These opportunists can appear among symbionts” that are typically commensal "such as Streptococcus pneumoniae and Staphylococcus aureus." or from bacteria that have been acquired by environmental exposure “such as Pseudomonas aeruginosa and Burkholderia cepacian”. Many additional diseases are recognized as opportunists in that, in addition to often infecting healthy people, they are also zoonotic and prey on a variety of other hosts (such as the rabies virus and Bacillus anthracis) (Brown et al.,2012). Most pathogens fail to meet these assumptions, with many coexisting relatively peacefully with their human host (i.e., they are not obligately pathogenic) or even exploiting an entirely different environment outside of human hosts(Woolhouse et al.,2001).

Opportunist organisms have three main traits: (1) they typically have low pathogenicity; (2) they typically cause serious infections when the host's defence mechanisms against infection are compromised; and (3) they can act like typical pathogens but may also cause unusual symptoms when given the right circumstances (Fan *et al*., 2019).
Those Hospital settings are the ones where infections brought on by microorganisms resistant to antibiotics are most common, in large part because of comorbidities and problems brought on by medical interventions (Hassan *et al.,* 2015). Patients in particular with weakened immune systems are more prone to infections, which allows opportunistic microorganisms to enter and spread unchecked (Moradali *et al*., 2017). This can happen in individuals who are using immunosuppressants for autoimmune illnesses and other inflammatory problems (Riccardi *et al.,* 2019), as well as other ailments (Kirchgesner *et al*., 2018). Patients with human immunodeficiency virus infections (Buchacz *et al.,* 2016). or as host-parasite interactions are affected by climate in ways that result in greater rates of death (Rohr *et al.,* 2013). Those bacterial commensals that transform into fatal pathogens under specific circumstances seem particularly probable candidates for an expanding involvement in illness and death in light of fast world change and increases in environmental stresses. These organisms are opportunists that may live in hosts for extended periods seemingly unaffected until developing clinical illness when host immunity is weakened or in response to changes in the pathogen. There is proof that commensals can act as opportunistic pathogens. Saiga antelopes had a particularly spectacular case in 2015, which was related to *Pasteurella multocida* serotype B in conjunction with environmental factors (Kock *et al*.. 2018).

2. Opportunistic bacteria Predisposing Factors

2.1 Long-term use of antibiotic According to research (José *et al.,* 2020), prolonged use of antibiotics may cause changes in the oral microbiota of the body's natural inhabitants and may lead to a rise in the prevalence of opportunistic microorganisms such as enteric bacilli, Pseudomonas, Staphylococcus, and yeasts. As a commensal fungus, Candida spp. is found in the oral cavity. According to Komiyama *et al.,* (2004), *Candida albicans*, which makes up 60 to 70% of all isolations, is the prevalent species. *Candida tropicalis* and *Candida glabrata* are the next most common isolates. Despite not being considered pathogenic in healthy humans, bacteria from the families Enterobacteriaceae and

Pseudomonas spp. have undergone extensive study. However, the oral cavity may act as a reservoir for these microorganisms, endangering the lives of people with disabilities (Kaklamanos *et al.,* 2005). “Staphylococci bacteria” are not regarded to be a normal component of the human oral microbiota, but they can behave as opportunistic pathogens in patients receiving long-term systemic antibiotics or immunosuppressive therapy (Zaatout, 2021).

When administered properly, antibiotics lower all-cause mortality and save children's lives from serious infectious infections (Keenan *et al.,* 2019). Despite this, they are routinely administered improperly (Fink *et al.,* 2020) and have a variety of unintended consequences in human hosts, in particular. The short- and long-term maturation of the gut microbiome in children is considerably altered by antibiotic exposure (D'Souza *et al.,* 2020). It is unclear how these modifications vary by antibiotic class and how they affect various populations' clinical outcomes and illness risks. It is well known that antibiotics can modify the microbiota, decreasing colonization resistance and increasing vulnerability to some opportunistic (entero-) infections including Salmonella and *Clostridium difficile* (Wu *et al.,* 2020). On the other hand, it has been demonstrated that mass azithromycin treatment considerably lowers pediatric mortality, maybe because of a decrease in enteropathogenic load (Keenan *et al.,* 2019). Asthma (Marra *et al.,* 2009), diabetes (Chen *et al.,* 2021) and obesity (Block *et al.,* 2019) are only a few of the children's disorders that have been linked to antibiotic-driven changes in early microbiome colonization in high-income environments.

2.2 Weakness of Immune System

According to Wasef *et al.* (2014), immunocompromised people are particularly susceptible to opportunistic parasite infections. Tekle *et al.,* (2008), found that immunosuppression creates an environment that allows opportunistic parasites to thrive against the host system and cause clinical illnesses. Various disease conditions, including cancer, AIDS, organ transplants, corticosteroid chemotherapy, autoimmune and metabolic diseases, irradiation, malnutrition, environmental factors, as well as in the elderly and young children, are associated with impaired host immune systems (Hassanein and Fanaky, 2021).

A-**Human Immunodeficiency Virus (HIV) and Other Virus**

Opportunistic infections are illnesses that affect patients with compromised immune systems, most often those infected with the Human Immunodeficiency Virus (Podlekareva *et al.,* 2006). Although the prevalence and incidence of HIV-associated opportunistic infections (OIs) varies significantly (Low *et al.,* 2016), all patients with HIV are prone to developing a variety of opportunistic illnesses. Opportunistic infections continued to be a key source of considerable morbidity and death among HIV/AIDS patients in low and medium-income countries (LMIC) despite improvements in HIV diagnosis and therapy (IeDEA, 2014). “HIV impairs immune function and increases the risk of contracting opportunistic infections, which can accelerate the progression of the disease and its transmission” (Edwards,2015). Low-income and industrialized nations have different rates of the most common opportunistic infection (OI); TB and recurring bacterial infections are more frequently seen in the former than in the latter (Seyler *et al.,* 2007). The virion binds to CD4+ and chemokine receptors to begin the HIV life cycle. Following the fusion of the HIV membrane with the cell membrane, the viral DNA enters the cytoplasm. Reverse transcriptase facilitates proviral DNA synthesis, which results in provirus integration into the cell genome. The cell is then activated by cytokines, the HIV genome is transcribed, and spliced and unspliced RNAs are transported to the cytoplasm. HIV protein synthesis

and assembly then occur, followed by gp120/gp41 expression on the cell surface and mature virion budding. The infectious cycle is accelerated by an infected cell's abundance of virions, which can attack CD4+ cells, macrophages, and neurons.HIV is characterized by a steady decline in CD4+ T cells, which destroys both cell-mediated and antibody-mediated immune systems (Vaillant & Naik, 2023).

However, a primary infection caused by the Varicella-Zoster Virus (VZV) in a patient (most often a child) receiving immunosuppressive medications for rheumatoid arthritis should not be considered an OI because that infection would have likely developed regardless of the immune suppression, even in the absence of a severe clinical picture or complications related to the VZV. For primary TB, similar factors might be taken into account. The common cold, conjunctivitis, or upper respiratory infection caused by an adenovirus, for instance, can strike both immunocompetent children and previously healthy people. However, these infections may be classified as opportunistic infections if they become persistently localized and/or widespread. (Ison & Hayden,2016).

B- **Immunocompromised Patients**

According to (Riccardi *et al.*, 2019), opportunistic infections (OIs) are illnesses caused by bacteria, fungi, viruses, or parasites that ordinarily do not cause disease but become harmful when the body's defensive system is compromised. Additionally, iatrogenic immune suppression of various types, grades, and timings as well as genetic host patterns might influence the risk as well as the clinical characteristics of opportunistic infections (Polvi *et al.*, 2015). In the context of epidemiological studies in a specific patient group with a congenital or acquired condition (such as HIV illness, antineoplastic treatment, transplant, etc.), Opportunistic infections frequently make the news of immune system dysfunction. Unfortunately, pre-defined categories of opportunistic infections are generally not used, but any infections encountered may be recorded as opportunistic infections, especially in clinical trials with the administration of immunosuppressive or cytotoxic medications (B-Lajoie *et al.*, 2016).

2.3 Climate Changing

The natural environment and constructed environment, especially the aquatic ecosystems found in towns and cities, are projected to be significantly impacted by climate change. In-built infrastructure, opportunistic pathogens like Legionella, Mycobacteria, and Pseudomonas spp. can live in water distribution lines, premises plumbing, pools, spas, and green infrastructure, which can lead to serious infections and disease outbreaks in exposed, susceptible people. Ponds, ditches, and even roadside puddles can serve as reservoirs for the growth and eventual dispersal of these species. Only legionellosis, which includes Pontiac fever and Legionnaires' disease (LD), is a notifiable OP-related illness in Canada. Numerous terms are used to describe waterborne pathogens that colonize building water systems and infect exposed susceptible people. Legionellosis cases in Canada have increased from an average of 0.29 per 100,000 people before 2010 to over 1.7 in 2018 and 2019 (Government of Canada, 2021). These are also known as drinking water-associated microorganisms that cause illness, "opportunistic premise plumbing pathogens." (Proctor *et al.*, 2022). The opportunistic pathogens naturally occur in soils, surface waters, and groundwater but thrive in the built environment in distribution and premise plumbing systems (*Schwake et al.*, 2021). Opportunistic organisms frequently prefer warm water (e.g., 25–40 °C), have limited tolerance for disinfection and increased temperatures (e.g., 50–60 °C), and have the capacity to develop or join biofilms inside pipes and plumbing fittings (Hayward *et al.*, 2022).

Opportunistic diseases can colonize plumbing thanks to biofilms, which are collections of microbial cells, polysaccharides, minerals, nutrients, detritus, and silt (Donlan, 2002). Opportunistic pathogens (OPs) may also reside inside free-living amoeba, which gives them mobility while protecting them from disinfection (Atanasova *et al.,* 2018). When growth conditions are poor, certain opportunistic infections can transition into a viable but uncultivable form, enabling bacteria to endure challenging circumstances and avoid detection (Hayward *et al.,* 2022).

3. Foodborne Opportunistic Bacteria

The endospheres and rhizospheres of plants have been reported as the significant reservoirs for emerging opportunistic pathogens like *Escherichia coli* pathotypes, Enterobacter, Burkholderia, Ralstonia, Pseudomonas, Staphylococcus, Serratia, Stenotrophomonas, the multi-drug resistant multi-resistant species of Pseudomonas and Stenotrophomonas, resulting in disease outbreaks (Zope *et al.,* 2014). It should be emphasized that if plants serve as a natural reservoir for Enterobacteriaceae, then these bacteria may appear to be a natural part of the human diet. "Opportunistic infections with a broad evolutionary background" commonly conquer natural habitats or are connected with eukaryotic hosts maintaining an endophytic existence, which is extremely helpful for immunocompetent hosts.; Opportunistic pathogens can cause serious infections such as pneumonia, bloodstream infections, urinary tract infections, surgical site infections, and diarrhoea in immunocompromised persons (Berg *et al.,* 2014). A study using whole genome sequencing (WGS) discovered a link between *Cronobacter sakazakii* (a member of the Enterobacteriaceae family) and food-borne acute gastroenteritis (AGE) in neonates, infants, and adults with Cronobacter spp. (McCusker & Warrington, 2011). However, the toxicity and epidemiology of this species are still poorly understood. Probiotics are an intriguing field of investigation for opportunistic bacteria. The Lactobacillus genus complex (LGC), which is commonly found in fermented foods (or as a supplement), is known to colonize the mouth, gastrointestinal (GI) tract, and female genitourinary system of humans due to its numerous positive effects. Surprisingly, food-borne lactobacilli illnesses such as bacteremia, endocarditis and pleuropneumonia have lately been observed. (Rossi *et al.,* 2019).

4. Waterborne Opportunistic Bacteria

Opportunistic bacteria and other microbes identified from man-made and natural recreational water reservoirs pose the risk of infection or disease transmission. The harm to human health from non-potable water, particularly from natural sources, is currently unknown. Surface water quality is prone to rapid, dramatic, and even deadly changes in microbiological quality as a result of several human and animal activities. These changes are induced by the discharge of urban wastewater and precipitation runoff, as well as pollution from farmlands and animal husbandry into bodies of water such as rivers and lakes (Edokpayi *et al.,* 2017). Non-fecal pathogens such as *Legionella pneumophila*, *Mycobacterium avium* complex, and *Pseudomonas aeruginosa* can be spread through water (DeFlorio-Barker *et al.,* 2016). According to (José *et al*.,2020*)* the infections stated above should be referred to as "water-based" rather than "waterborne," because diseases of fecal origin are referred to as "waterborne" Waterborne microbes" are sometimes known as "opportunistic pathogens." Pathogens that live in water usually flourish and thrive in both natural and manmade water systems (Salvat *et al.,* 2020).

5. Airborne Opportunistic Bacteria

Bioaerosols are abundant in human living and natural settings, and they mostly consist of bacteria, fungi, archaea, viruses, pollen, and endotoxins (Zhai *et al.*, 2018). Bioaerosols have an essential function in atmospheric chemistry and climate change as a key component of atmospheric particulate matter (Dong *et al.*, 2016). Bacteria are the major component of bioaerosols, accounting for 80% of the microbial components in the atmosphere (Zhai *et al.*, 2018). High concentrations of airborne bacteria, particularly pathogenic bacteria, "can cause a variety of ailments, including respiratory, digestive, and cardiovascular problems. (Riggs *et al.*, 2018). As a result, the influence of microorganisms in the environment on human health is progressively becoming recognized. Pathogenic bacteria are frequently found in airborne germs, posing a substantial hazard to human health. For example, *Acinetobacter baumannii* airborne transmission in hospitals can cause respiratory infections, bacteremia, and meningitis, among other disorders (Gao *et al.*, 2014). *Staphylococcus aureus*, an important human pathogen, is extensively prevalent in wastewater treatment plant bioaerosols and can cause bacteremia and skin infections in people (Talepour *et al.*, 2020). Several investigations have revealed the detection of a substantial number of harmful bacteria in the outdoor air environment(Fan *et al.*, 2019). Pathogenic bacteria with antibiotic resistance can make treating bacterial infections more difficult and extend hospital stays, as well as raise treatment costs (French, 2005). "Micrococcus, Sphingomonas, Enterococcus, Rhodococcus, and Stenotrophomonas have also been found as bioaerosol threats to human health " (Yan *et al.*, 2021)

6. Conclusion

Opportunistic microorganisms are non-pathogenic germs that act as pathogens under particular conditions. "They remain latent for extended periods until the host's immune system is weakened, at which point they become active "Several microorganisms found in foods, water, and air have evolved as opportunistic pathogens in people and animals.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Serum Adiponectin Marker in Women with Polycystic Ovary Syndrome

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Received: 2023/06/25

Accepted: 2023/07/20

Published: 2024/01/04

Keywords: *Adiponectin, polycystic ovarian syndrome and insulin resistance*

DOI:10.62472/kjps.v14.i23.22-30

Abstract

Background: One of the leading causes of female infertility is polycystic ovarian syndrome (PCOS). Adiponectin is one of many adipokines adipose tissue releases that affect insulin resistance (IR).

Objective: To measure PCOS participants' IR, adiponectin, and serum insulin levels.

Materials and procedures: a case-control study involving 80 patients with PCOS (40 obese and 40 nonobese) and 80 controls (40 obesity and 40 nonobese). Adiponectin was measured using an enzyme-linked immunosorbent assay, while serum levels of insulin and FBS were measured using a chemiluminescent automated immunoassay system (ECL).

Results: Regardless of body mass index and PCOS, women had lower levels of adiponectin and higher levels of blood fasting glucose, insulin, and IR compared to the relevant with a p-value of ≤0.001 for controls.

Conclusion: According to weight, blood adiponectin levels inversely correspond to IR. It can, therefore, act as an indicator in PCOS-affected women.

مؤشر الأديبونكتين في مصل الدم لدى النساء المصابات بمتلازمة تكيس المبايض

ياسمين عماد كاظم؛ فاضل جواد ال طعمة؛ نورا صباح رسول

الخلاصة

المقدمة: أحد الأسباب الرئيسية للعقم عند النساء هو متلازمة المبي

ض المتعدد الكيسات (PCOS). الأديبونيكتين هو واحد من العديد من الأديبوكينات التي تفرزها الأنسجة الدهنية والتي تؤثر على

مقاومة الأنسولين (IR).

الهدف: قياس مستويات مقاومة الانسولين والأديبونيكتين

والأنسولين في الدم لدى المشاركين في متلازمة تكيس المبايض.

المواد والإجراءات: دراسة الحالات والشواهد التي تشمل ٨٠ مريضاً يعانون من متلازمة تكيس المبايض (٤٠ يعانون من السمنة المفرطة و٤٠ من غير المصابين بالسمنة) و٨٠ من الضوابط (٤٠ من السمنة و٤٠ من غير المصابين بالسمنة). تم قياس الأديبونيكتين باستخدام مقايسة الامتصاص المناعي المرتبط بالإنزيم، في حين تم قياس مستويات مصل الأنسولين ومستويات سكر الصيام باستخدام نظام المقايسة المناعية الآلي الكيميائي.(ECL)

النتائج: بغض النظر عن مؤشر كتلة الجسم ومتلازمة تكيس المبايض، كان لدى النساء مستويات أقل من الأديبونيكتين ومستويات أعلى من الجلوكوز في الدم أثناء الصيام، والأنسولين، ومقاومة الانسولين مقارنة مع القيمة الاحتمالية ٠,٠٠١≥ للضوابط.

الاستنتاج: وفقا للوزن، فإن مستويات الأديبونيكتين في الدم تتوافق عكسيا مع الأشعة تحت الحمراء. وبالتالي يمكن أن يكون بمثابة مؤشرات لدى النساء المصابات بمتلازمة تكيس المبايض.

1. Introduction

The majority frequent reason for hyperandrogenism and anovulatory infertility in women of reproductive age is polycystic ovary syndrome (PCOS), a common heterogeneous condition. In addition to impaired ovarian steroidogenesis, complicated pathogenesis includes: (a) hypothalamic-pituitary gonadotropin secretion disruptions in particularly elevated levels of LH; (b) diminished ovarian steroidogenesis; and (c) compensatory hyperinsulinemia brought on by insulin resistance (IR), which increases androgen production and decreases the synthesis of sex hormone-binding globulin (SHBG), contributing to PCOS-associated **(Khan, Stas and Kurukulasuriya, 2006)**. Women with PCOS have a five to ten times higher than average chance of developing type 2 diabetes mellitus. **(Ovalle and Azziz, 2002)**. Within the etiology of PCOS-related hyperandrogenism, insulin has both direct and indirect functions. LH (luteinizing hormone) and insulin work together to increase the production of androgen in theca cells. **(Ehrmann *et al.*, 2005)**. Both thin and fat women with PCOS have IR, however, obesity and PCOS may have an independent

impact on IR**(Dunaif *et al.*, 1989)**. Target tissues include skeletal muscles and adipose tissue, as well as the liver fails to respond appropriately to normal plasma insulin concentrations in IR**(Longo, 2012)**. In industrialized nations, obesity is a disease that is spreading quickly and causes both adipocyte hyperplasia and hypertrophy**(Kahn and Flier, 2000)**. Women with PCOS are more likely to have central obesity (50%) and more elevated peripheral IR. Several adipokines, including leptin, adiponectin, resistin, and vaspin, are released by visceral adipose tissue**(Fukuhara *et al.*, 2005)**, A protein known almost solely as adiponectin produced via adipocytes, is thought to have insulin-sensitizing, anti-inflammatory, anti-diabetic, and anti-atherogenic effects at high levels, whereas low amounts are linked to obesity, insulin resistance, metabolic syndrome, type 2 diabetes, and cardiovascular disease (CVD) **(Toulis *et al.*, 2009)**. In the current study, we compare obese and non-obese PCOS patients with the corresponding controls in terms of blood adiponectin levels, insulin, and IR.

2. Material and Methods

From November 2022 to April 2023, a case-control study was conducted in the Chemistry and Biochemistry Department at Kerbala College of Medicine, Iraq. It included 80 PCOS women with a diagnosis (40 obese and 40 non-obese) and 80 controls (40 obese and 40 non-obese) between the ages of 18 and 40.

Women who are younger than 18, those with any known illnesses, infections, or inflammatory problems, Cushing's syndrome, hyperprolactinemia, and congenital adrenal hyperplasia, or who were any of the medication had been prohibited from participating in the current investigation. The institutional ethics committee received permission from the ethical committee. Participants' informed consent was acquired. Each subject underwent a physical examination. Each person's height and weight were recorded. By using kg/m2, the body mass index (BMI) was computed.

According to the Rotterdam ESHRE (European Society of Human Reproductive Medicine) updated consensus 2003 **(Fauser *et al.*, 2012),** PCOS was diagnosed.

Each participant in the trial provided a fasting blood sample of 6 ml. A chemiluminescent automated immunoassay system (ECL) (Cobas e 411, Roche Diagnostic, Germany) was used to test fasting serum glucose and insulin. The enzyme-linked immunosorbent test was used to quantify serum adiponectin. The following formula was used to calculate IR by the homeostasis model assessment (HOMA): Fasting insulin (µIU/ml) × fasting glucose (mg/dl)/405 **(Nestler *et al.*, 2002)**.

Statistic evaluation

An SPSS (Statistical Package for the Social Sciences) Statistics student t-test software, version 28.0 (IBM, SPSS, Chicago, Illinois, USA), was used to compare the mean and SD. The p values of ≤0.05 and≤0.001 are regarded as statistically significant and highly significant, respectively.

3. Results

Glucose levels were higher in obese PCOS women (93.06±9.51) and non-obese PCOS women (93.61±10.48) when compared with controls (87.20±4.65 and 84.6±84.43), with a p-value of <0.001 **(Table 1).**

IR in obese PCOS patients 3.81±0.94 and non-obese PCOS 2.06±0.76 were high when compared with controls 1.54±0.26 and 1.28±0.27, with p values of <0.001, 0.002 respectively **(Table 1).** Serum insulin concentration in obese PCOS females (15.71±4.56) and in non-obese women with PCOS (13.21±8.10) were high when contrasted to their controls (7.29±1.31 and 7.13±1.62), as appropriate, with a p-value of <0.001.

With a p-value of≤0.001, obese PCOS females (7.87±2.50) and non-obese females (9.40±2.56) had lower serum levels of adiponectin than their respective controls (11.86±3.59 and 11.71±3.33, respectively) **(Figure 1).**

Table 1: Characteristics of Obese and non-obese PCOS and Control

Variables	Non-obese		P-value	Obese		P-value
	Case	Control		Case	Control	
BMI	24.77±2.34	23.71±1.69	**0.02***	33.40±3.71	33.77±3.52	0.64
Insulin (µU/ml)	13.21±8.10	7.13±1.62	**<0.001***	15.71±4.56	7.29±1.31	**<0.001***
FBS (mg/ml)	93.61±10.48	84.68±4.43	**<0.001***	93.06±9.51	87.20±4.65	**<0.001***
HOMA-IR	2.06±0.76	1.28±0.27	**0.002***	3.81±0.94	1.54±0.26	**<0.001***

Data expressed as mean±SD. BMI= body max index; FBS= fasting blood sugar; HOMA-IR= homeostasis model assessment-estimated insulin resistance. Statistical significance is defined as * p≤0.05.

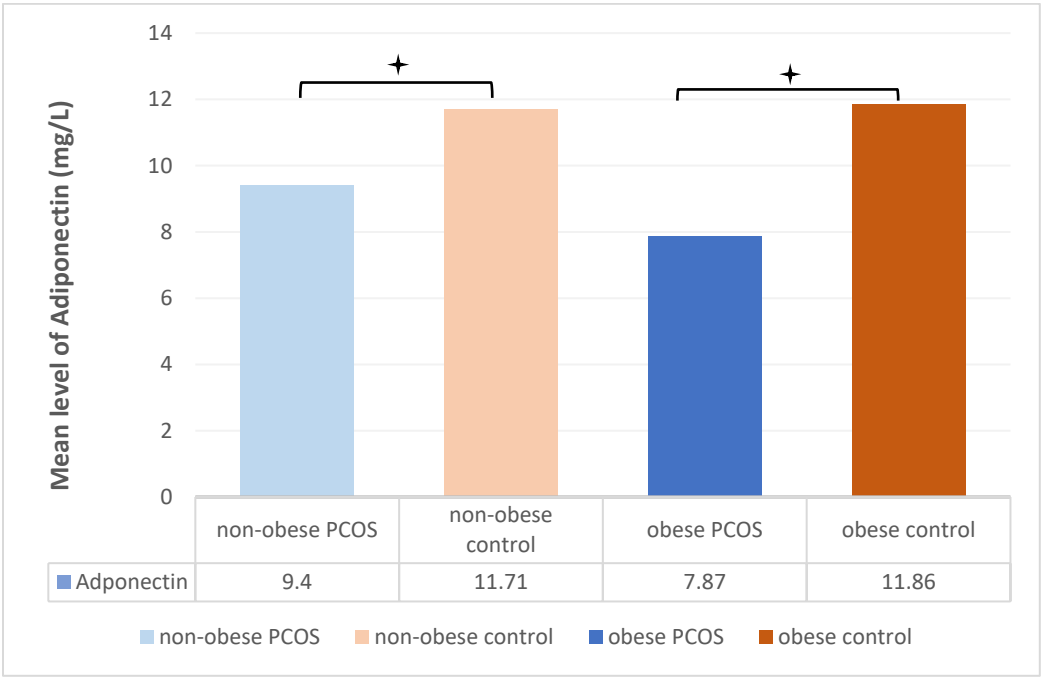

Figure 1: Comparison of Adiponectin in Non-Obese PCOS, Non-Obese Controls, Obese PCOS and Obese Controls. Statistically Significant at $^{*}p \leq 0.001$

4. Discussion

In our investigation, both obese and non-obese PCOS subjects had significantly higher mean fasting blood glucose levels compared to controls, combined with significantly higher serum levels of insulin and IR. Zuo et al. reported making comparable findings **(Zuo, Zhu and Xu, 2016)**. The primary mechanism in the etiology of PCOS in both obese and non-obese people is thought to be hyperinsulinemia and IR, Yun et al **(Yeon Lee *et al.*, 2010)** have demonstrated that obese PCOS women with elevated blood glucose and BMI of over 27 develop diabetes. The percentage of obese PCOS patients was 31% compared to 10.3% of thin PCOS patients and 7.5% compared to 1.5% of PCOS patients who are slim, respectively.

In PCOS patients, insulin signalling that is mediated by a protein tyrosine kinase receptor has been studied; Dunaif and his team **(Dunaif *et al.*, 1989)** revealed increased insulin receptor serine phosphorylation in insulin-resistant PCOS patients, which prevents insulin receptor tyrosine kinase function. It also influences the P450c17 enzyme's activity, which in women with PCOS results in hyperandrogenism. Additionally, hyperinsulinemia amplifies the actions of LH on theca interstitial cells, increasing the synthesis of androgen **(Yeon Lee *et al.*, 2010)**.

Patients with PCOS have an IR rate between 50% and 70%. IR promotes Oxidative stress because reactive oxygen species (ROS) are produced due to hyperglycemia and increased levels of free fatty acids. According to several studies, the degree of clinical presentation and hyperinsulinemia severity are associated **(Yeon Lee *et al.*, 2010)**.

Patients with PCOS have shown signs of oxidative stress brought on by hyperglycemia, IR, and ongoing inflammation. Due to the excess generation of ROS caused by hyperglycemia and more significant amounts of free fatty acids, IR increases Oxidative stress. By causing multinucleated cells to produce tumour necrosis factor (TNF a), hyperglycemia also contributes to inflammation. Excess testosterone enhances the production of ROS from leukocytes, the expression of the p47phox gene, and the development of MDA, according to studies done on lean, healthy women of reproductive age who also had hyperglycemia. It's possible that diet-induced Oxidative stress, with hyperandrogenism as the progenitor, is the cause of OS being present in the lack of obesity. Chronic inflammation is exacerbated by Oxidative stress and vice versa **(Deba *et al.*, 2017)**.

Independent of IR, the current investigation demonstrated that adiponectin levels were considerably lower in obese and not obese women with PCOS than in their controls, showing a negative connection between adiponectin and obesity indexes (BMI). Vardhana et al. reported making comparable observations **(Vardhana *et al.*,**

2009). Nevertheless, several research studies have indicated the opposite **(Spranger *et al.*, 2004)**, and Lewandowski et al. said variable adiponectin levels**(Lewandowski *et al.*, 2005).**

A 247 amino acid polypeptide called adiponectin, mainly released by adipose tissues, has an antagonistic relationship with obesity, metabolic syndrome, and IR **(Shin, Lee and Lee, 2011)**. Adiponectin is recognized to play essential roles in the control of lipid and glucose metabolism through the promotion of oxidation of fatty acids, inhibition of liver glucose production, and increased skeletal muscle and liver insulin sensitivity. Women with PCOS who are lean or obese possess a larger ratio of trunk to peripheral fat than those who are not. The absence of a correlation between insulin sensitivity and body weight may be explained by this effect **(Svendsen *et al.*, 2008)**. According to Groth **(Groth, 2010)**, IR may not be the only possible answer. High androgen levels could be a contributing factor.

In obesity, the expression of adiponectin receptors (adipo R1 and 2) is reduced **(Kadowaki and Yamauchi, 2005)**. Such receptors are, however, both visceral and subcutaneous fat tissue, which are upregulated in PCOS patients. AdipoR1 expression is favourably connected with insulin, testosterone/SHBG (Sex hormone biding-globin) 100, and the androgen index in both forms of fat in all women but negatively correlated with SHBG **(Kalish *et al.*, 2003)**. According to a previous study **(Sir-Petermann *et al.*, 2007)**, It's probable that PCOS-related metabolic problems existed before hyperandrogenism, and adiponectin may be employed as a susceptibility biomarker for females who are in danger of developing PCOS.

5. Conclusion

Within the current study, women with PCOS, regardless of BMI, had decreased adiponectin levels in serum. However, the values in PCOS patients who were obese were lower than those in PCOS patients who were not obese. Fasting insulin and HOMA-IR were negatively correlated with adiponectin. Therefore, regardless of BMI status, it can become a biomarker for women at risk of developing PCOS.

Acknowledgements

The authors thank Kerbala University in Kerbala, Iraq, for supporting this endeavour (www.uokerbala.edu.iq). The authors thank the study's healthy control group and sick participants.

Compliance with Ethical Standards

Conflict of Interest

The authors warrant that they don’t have any competing interests to declare.

Ethical Approval

All procedures involving human subjects in research projects were carried out by Kerbala University's research committee's ethical standards, the 1964 Helsinki Declaration, and any updates or additional ethical guidelines deemed equivalent.

Informed Consent

Each participant in the study gave their consent in writing.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Association Between Serum Level of Interleukin-15 and Severity of Pediatric Asthma

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Received: 2023/07/12

Accepted: 2023/10/22

Published: 2024/01/04

Keywords: *Pediatric asthma , Interleukin-15, IgE, ELISA test.*

DOI:10.62472/kjps.v14.i23.31-43

Abstract

Background: A chronic inflammatory process of the airways is a common characteristic of asthma, representing a primary health problem. This disease can infect people of all ages, about 3.5-20 % of the population, but it usually begins in childhood.

Objective: To evaluate the relationship between interleukin-15 and pediatric asthma severity.

Methods: The current study is a case-control study that includes 60 asthmatic participants as the patient group and 60 non-asthmatic participants as the control group. Sandwich ELISA using an ELISA kit can be used for the measurement of IL-15 and IgE serum levels.

Results: Elevated serum level of IgE in the patients group compared with a control group with significant (*P= 0.005*) was a result of this study, and there were no significant differences between patients and control about IL-15 serum levels with (*P= 0.968*) in asthmatic children.

Conclusions: According to the present study, there was a non-significant association between the severity of asthma and the serum level of IL-15 in children.

العلاقة بين مستوى الإنترلوكين ١٥ في مصل الدم وشدة الربو لدى الأطفال

الخلاصة

الخلفية : يعد الالتهاب المزمن في الشعب الهوائية من الخصائص الشائعة لمرض الربو، والذي يمثل مشكلة صحية أولية، يمكن أن يصاب الناس بهذا المرض في جميع الأعمار بنسبة ٣,٥-٢٠٪ من السكان، لكنه يبدأ عادة في مرحلة الطفولة.

الهدف : تقييم العلاقة بين مستوى الانترلوكين ١٥ وشدة الربو عند الأطفال.

الطرق : نوع الدراسة الحالية هو دراسة الحالات والشواهد التي شملت ٦٠ مشاركا من مرضى الربو كمجموعة مرضى و ٦٠ من غير المصابين بالربو كمجموعة سيطره. يمكن استخدام اختبار الاليزا باستخدام كت الاليزا لقياس مستويات الانترلوكين ١٥والامينوكلوبيولين E في المصل.

النتائج : ارتفاع مستوى الامينوكلوبيولين E في مجموعة المرضى مقارنة مع مجموعة السيطرة ذات دلالة احصائية ($P=0.005$) نتيجة لهذه الدراسة, ولم تكن هناك فروق ذات دلالة إحصائية ($P=0.968$) بين المرضى ومجموعة السيطرة حول مستوى الانترلوكين ١٥ في المصل مع في الأطفال المصابين بالربو.

الاستنتاجات : كان هناك ارتباط غير معنوي مع شدة الربو لدى الأطفال فيما يتعلق بمستوى مصل الانترلوكين ١٥ وفقا لهذه الدراسة.

1. Introduction

A chronic inflammatory disorder of the airways is a general characteristic of Asthma, in which mast cells and eosinophils play a vital role. It's the predominant child respiratory disease, affecting 235 million individuals worldwide **(1).** Childhood and adult-onset asthma share many of the exact causes. The interactions between environmental and other intrinsic factors, such as genetics and atopy, were the primary evidence to potentially cause asthma (Dharmage, Perret and Custovic, 2019)**.** An allergic phenotype is the majority of childhood-onset asthma, while a non-allergic phenotype is predominant in adult-onset asthma. However, both allergic and non-allergic airborne triggers such as animal hair, dander, pollen, tobacco smoke, or other pollutant exposures were associated with allergic and non-allergic asthma (Jacquemin *et al.*, 2012)**.**

Eosinophilic hyper-infiltration, overproduction of mucus in airways, hyper-reactivity, and eventually remodelling of airways were the most characteristic of patients with asthma. T-helper 2 (Th2) cells and their related cytokines, including Interleukins (IL) 4, 5 and 13, are associated with pathological changes in asthma (Larché, Robinson and Kay, 2003)**.**In other asthma phenotypes like severe asthma, in addition to eosinophils, neutrophils can also accumulate in the airways (Louis *et al.*, 2000)**.** Asthma can be classified into four phenotypes depending on the number of different cells in the sputum that are induced by hypertonic saline: eosinophilic asthma, neutrophilic, mixed granulocytic asthma and asthma with normal sputum eosinophil and neutrophil **(**(Simpson *et al.*, 2006)**,** (Porsbjerg *et al.*, 2009)**).**

The functioning of several immune and structural cells involved in the pathogenesis of chronic allergic inflammation can be influenced by the complex biological role of IgE. The influence of the cell activity by the effect of IgE is related to the interaction with specific receptors. The prominent cytokine that regards asthma is Interleukin (IL) -15. This protein is structurally similar to a growth factor and modulator of T lymphocytes, which isIL-2, and natural killer (NK) cells; both immune marker levels were increased in bronchoalveolar lavage cells in asthmatic patients, essentially those with steroid-resistant asthma (McInnes and Gracie, 2004)**.**

2. Materials and Methods

One hundred twenty participants' blood samples were divided into 60 clinically diagnosed asthmatic cases and 60 non-asthmatic as a control group. These samples were taken from both sexes, aged between 4 months to 14 years old, attending the asthma clinic of Imam AL-Hussein Medical City, Karbala /Iraq, during the period extended from November (2021) to March (2022). Patients ' exclusion criteria were autoimmune disease, tumour and chronic obstructive pulmonary disease. One ml of blood was collected from each participant in an EDTA tube and was used for absolute eosinophil count determination. Using Sysmex XN-350, five differential automated haematology analyzers, four ml of venous blood was collected from patients and controlled in gel tubes; serum was separated and stored at -20 C˚ for subsequent analysis. The immune markersIL-15 and IgE serum levels were determined by classic sandwich-ELISA using kits (Bioassay Technology, China).

Ethical approval for the study was acquired from the Kerbala Health Directorate Committee. Also, verbal approval was obtained from the parents of the children before the sample was taken.

3. Statistical Analyses

All statistical tests were calculated using SPSS. *A p-value* less than 0.05 was regarded as statistically significant.

4. Results

The current study showed that the (mean ± SE) of eosinophils count were (0.40 ± 0.06) and (2.14 ± 0.24) in patients and control, respectively. There was a significant difference between patients and control in eosinophil counts (*p =0.005*). Also, this study found that the IgE serum levels were (126.21 ±14.57) and (37.65 ±5.14) in patients and control, respectively. There was a significant difference between patients and controls in IgE serum levels (*p =0.005*). The serum levels of IL-15 were (39.69 ±8.91) and (39.15 ±9.89) in patients and control, respectively. There was no significant difference between patients and control in IL-15 serum level (*p = 0.968*), as clarified in Table (1).

Table 1: Mean Differences of IgE and IL-15 Serum Levels and EOS Count Among the Patients and Control

Variables	**Group**				***P value***
	Patients		**Control**		
	Mean	**SE**	**Mean**	**SE**	
EOS count	0.40	0.06	2.14	0.24	**0.005***
IgE	126.21	14.57	37.65	5.14	**0.005***
IL-15	39.69	8.91	39.15	9.89	0.968

**** p value* is significant *(P < 0.05)*, Student's t-test, EOS: Eosinophils**

In this study (4.3%) of moderate patients were positive for direct contact with cats, while (100 %) and (95.7%) of both mild and moderate asthmatic patients had negative for direct contact with cats; there were no significant differences between mild and moderate asthma (*P value = 0.427*). Also, in the present study (4.3%) of moderate asthma patients were positive for direct contact with dogs. In comparison (100%) and (95.7%) of both mild and moderate asthmatic patients had negative for direct contact with dogs; there were no significant differences between mild and moderate asthma (*P value = 0.585*).

In addition, 14.3 % and 10.9% of mild and moderate asthma patients were positive for direct contact with birds. In comparison, 85.7% and 89.1% of both soft and medium asthmatic patients had negative for direct contact with birds; there were no significant differences between mild and moderate asthma (*P value = 0.727)*.

The study also showed that 64.3 and 30.4 of both mild and moderate asthmatic patients, respectively, were highly aggravated by upper respiratory inflammation, while 35.7% and 69.6% of both soft and medium asthmatic patients, respectively, were not; there were significant differences between mild and moderate asthma regarding aggravating by upper respiratory inflammation (*P value = 0.031*).

Regarding aggravated by dust, the study showed that 35.7% and 32.6% of mild and moderate asthmatic patients are favourable. In comparison, 64.3% and 67.4% of both soft and medium asthmatic patients had negative results; there was no significant difference between the severity of asthma and aggravating by dust (*P value = 0.535*). Also, this study showed that 64.3% and 50% of both mild and moderate asthmatic patients, respectively, are aggravated by playing or exercising, while 35.7% and 50% of both soft and medium asthmatic patients, respectively, were not; there was no significant difference between the severity of asthma and aggravating by physical activities (*P value = 0.379*).

Regarding treatment,57.1% and 19.6% of the mild and moderate asthmatic patients were under no treatment. 35.7% and 56.5% were taking Montelukast as primary treatment, while 7.1 % and 19.6% were taking inhaled corticosteroids (ICS)as the immediate treatment. Only 4.3 of the moderate were under mixed treatment of ICS and Montelukast. There was a significant difference in treatment between mild and moderate asthmatic patients (*P value = 0.049*).

Also, in this study, 42.9 % and 45.7% of mild and moderate asthmatic patients were diagnosed as early wheezier, while 7.1% and 6.5% were diagnosed with allergic asthma. 7.1% and 6.5% of which were diagnosed with non-allergic asthma. 14.3% and 13% were adolescent (obese) asthma, 14.3% and 8.7% of both mild and moderate asthmatic patients, respectively, were diagnosed as late-onset asthma. In comparison, 14.3% and 19.6% of soft and medium

asthmatic patients were diagnosed as persistent wheezier. A non-significant difference regarding the asthma phenotype between mild and moderate asthmatic patients (*P = 0.991*) is demonstrated in Table 2.

Table 2: The Relation of Severity with Aggravating Factors, Treatment and Phenotypes in Asthmatic Patients

Variables			Severity				*P value*
			Mild		Moderate		
			Count	%	Count	%	
Pets	Cats	+ve	0	0.0%	2	4.3%	0.427
		-ve	14	100.0%	44	95.7%	
	Dogs	+ve	0	0.0%	2	4.3%	0.585
		-ve	14	100.0%	44	95.7%	
	Birds	+ve	2	14.3%	5	10.9%	0.727
		-ve	12	85.7%	41	89.1%	
Aggravating by upper respiratory inflammation		+ve	9	64.3%	14	30.4%	**0.031***
		-ve	5	35.7%	32	69.6%	
Aggravating by dust		+ve	5	35.7%	15	32.6%	0.535
		-ve	9	64.3%	31	67.4%	
Aggravating by physical activities		+ve	9	64.3%	23	50.0%	0.379
		-ve	5	35.7%	23	50.0%	
Treatment		No treatment	8	57.1%	9	19.6%	**0.049***
		Montelukast	5	35.7%	26	56.5%	
		ICS	1	7.1%	9	19.6%	
		Mixed	0	0.0%	2	4.3%	
Asthma phenotypes		Early wheeze	6	42.9%	21	45.7%	0.991
		Allergic asthma	1	7.1%	3	6.5%	
		Non allergic	1	7.1%	3	6.5%	
		Adolescent & obesity	2	14.3%	6	13.0%	
		Late onset	2	14.3%	4	8.7%	
		Persist wheezier	2	14.3%	9	19.6%	

**** p value* is significant*(P<0.05)*, Chi-square test, Inhaled Corticosteroids (ICS)**

Figure 1 below shows the correlation between the serum IL-15 level and the eosinophil count. No significant positive correlation was found between IL-15 serum level and eosinophil count, r= +0.112 (*p* = *0.395*).

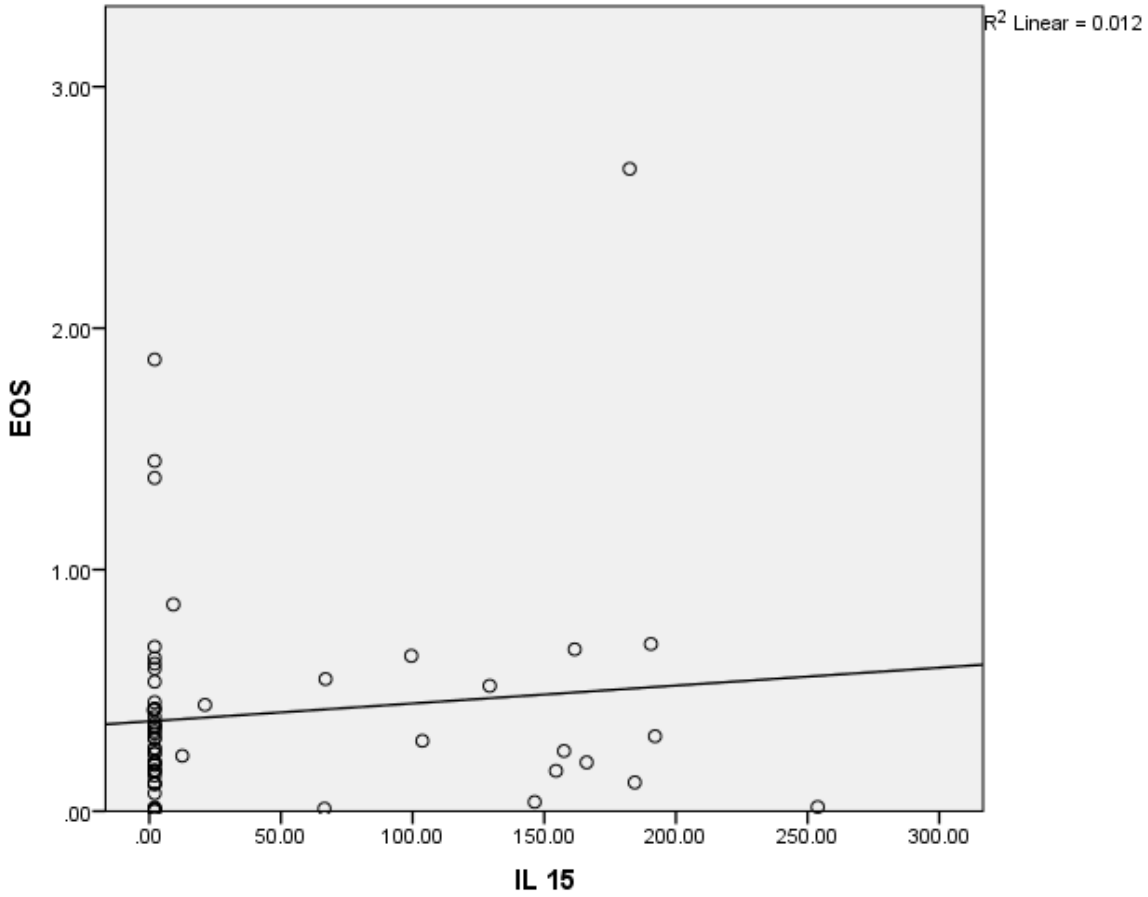

Figure 1: Correlation ofIL-15 Serum Level with Theeosinophil Count pg/ml. Pearson Correlation Coefficient: r= +0.112 (*p= 0.395*).

Figures 2 below showed the correlation between the IL-15 and IgE serum levels. No significant negative correlation were found between IL-15 and IgE serum levels,r= -0.136 (*p = 0.301*)**.**

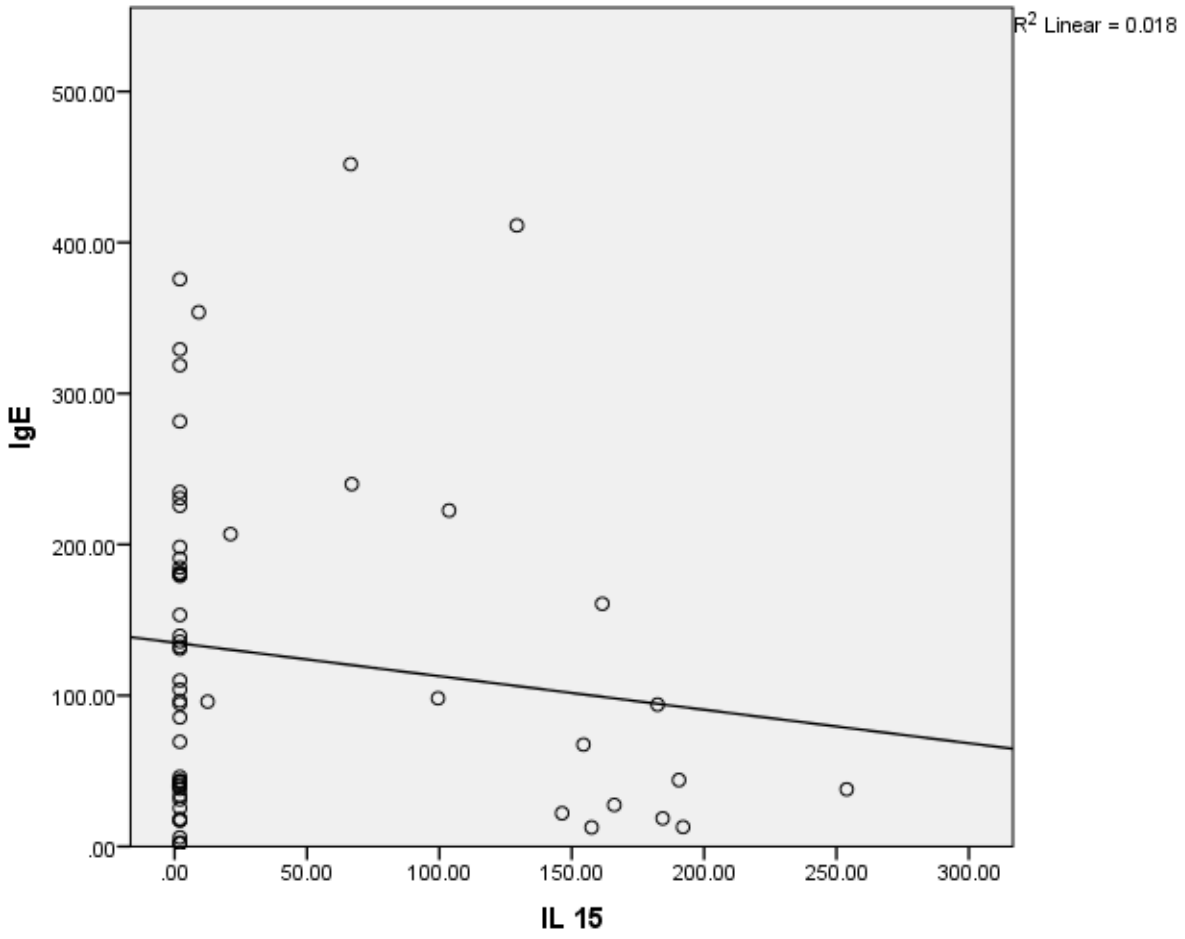

Figure 2: Correlation of IL-15 Serum Level with the Serum IgE pg/ml. Pearson Correlation Coefficient: r= -0.136 (*p= 0.301*)

Figure 3 shows the correlation between IL-15 serum level and disease duration in months of asthmatic patients. No significant correlation was found between IL-15 serum level and disease duration, r= 0.055 (*p = 0.675*).

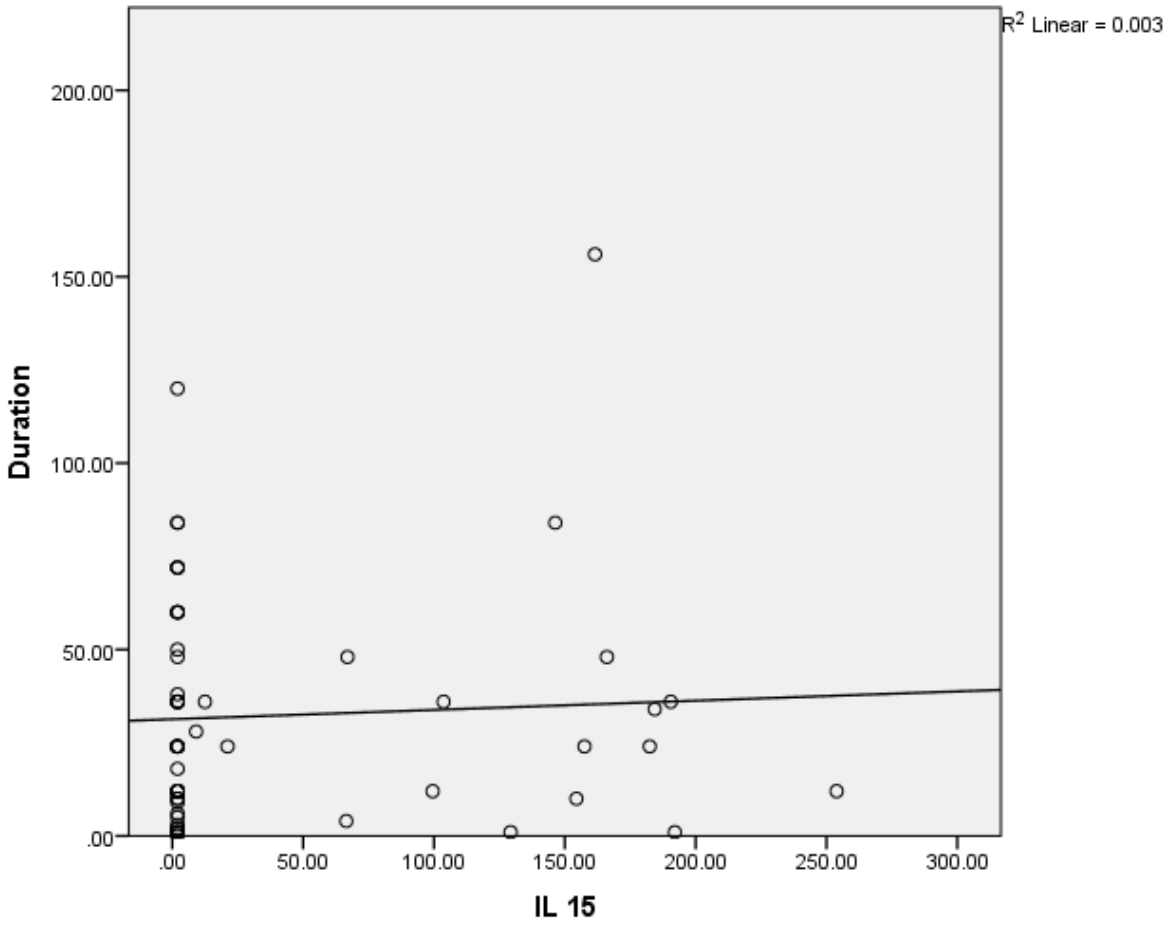

Figure 3: Correlation of IL-15 Serum Level with the Duration of Asthma in Months. Pearson Correlation Coefficient: r= 0.055 (*p = 0.675*)

The current study showed a non-significant difference in the severity of asthma, regarding the EOS count, IgE and IL-15levels between the mild and moderate asthmatic children with (*P value* = 0.344, 0.391 and 0.283) respectively and mean about (0.30, 154.32 and 57.16) in the mild asthma compared with mean about (0.43, 117.65 and 34.37) in the moderate asthma respectively. At the same time, the present study showed a significant difference (*P value*=0.046) in the severity of asthma depending on the duration, which was higher in moderate asthma than the mild asthmatic patients with a mean (of 36.70) and (17.86) respectively, as clarified in Table (3).

Table 3: The Relation of the Studied Markers and Duration with the Severity of Asthma

Variables	Severity				*P value*
	Mild		Moderate		
	Mean	SE	Mean	SE	
EOS	0.30	0.06	0.43	0.07	0.344
IgE	154.32	38.94	117.65	14.92	0.391
IL 15	57.16	18.94	34.37	10.08	0.283
Duration	17.86	4.34	36.70	4.90	**0.046***

***p value* is significant*(P<0.05)*, Student's t-test**

5. Discussion

In the present study, a significant association *(P= 0.005)* existed between eosinophil count in control and asthmatic patients, as shown in (Table). Other studies indicated a non-significant eosinophil count in asthmatic patients, such as a study (Ullmann *et al.*, 2013) that showed that (86%) of asthmatic patients had an average eosinophil count. Also, (Amin *et al.*, 2000) led to low eosinophils in non-atopic asthma. Contrary to the current study, (Foster *et al.*, 2008) revealed that the recruitment of eosinophils has long been recognized as a hallmark of the inflammatory response in asthma. The differences might be due to the small sample size and different procedures.

This study had a significant association *(P=0.005)* between serum IgE levels in asthmatic patients compared with control. This result is related to previous studies, such as a study (Strømgaard *et al.*, 2011)**,** which found a strong positive relationship between total serum IgE level and asthma in children**.** Also, a survey (Maneechotesuwan, Sujaritwongsanon and Suthamsmai, 2011) revealed that serum total IgE concentrations were significantly higher in patients with uncontrolled asthma. This aligns with (AN Abood, RI Ghazal and M Al-Musawi, 2013), who mentioned that (48.7%) of asthmatic patients showed positive IgE screening.

Regarding the IL-15 serum level, this study found no significant differences between asthmatic patients and controls *(P value = 0.968*). This result agreed with a survey achieved by (Stoner *et al.*, 2019) who showed similar IL-15 responses in exacerbating asthmatics and control. Contrary to this result, a study conducted by (Bierbaum *et al.*, 2006) confirmed the association of IL-15 with asthma by running a genotyping of all IL-15 polymorphisms within the promotor and coding region of IL-15, which were identified by single-strand conformation polymorphism (SSCP) analyses and sequencing in the initial study.

The patients in the present study were classified into mild and moderate groups according to their asthma severity. The number of patients with moderate asthma was higher than those with mild asthma, as shown in Table (2). These results agreed with a previous study conducted by (Hinks *et al.*, 2015)**,** who demonstrated that moderate asthma was more prevalent than mild and severe asthma and a survey conducted by (Dolan *et al.*, 2004), who showed that the percentage of moderate asthma was (48%) more than soft but equal with severe asthma, while other studies reported by **(**(Jabbar Rahi, 2011)**,** (Fleming *et al.*, 2015)**,** (Abood *et al.*, 2020)) inconsistent with current research. The differences in these results may be because these studies were designed with different methodologies, and the selection of their study population was not randomized.

Regarding aggravating by pets, there were no significant differences between mild and moderate asthmatic patients regarding aggravating by cats *(p*=0.427); this study was inconsistent with the result of (Simoneti *et al.*, 2018), who demonstrated that exposure to cats was associated with increased asthma risk. Also, this study showed no significant differences *(P=0.585) or (P*=0.727*)* between mild and moderate asthmatic patients regarding aggravation by dogs and birds, respectively. This study was incompatible with a survey conducted by (Weber-Chrysochoou *et al.*, 2014)**,** who confirmed that birds may exacerbate asthma severity. This variety may be attributable to the difference in sample group or differences in study geographic location and also socio-cultural variations of study subjects.

This study found a significant association between aggravation by upper respiratory inflammation and mild asthma compared with moderate asthma (*P=0.031)*. This study related to (Grissell *et al.,* 2005)**,** who found that respiratory infection can provoke asthma. Along the same line, a study was conducted by (Corne *et al.,* 2002)**,** who confirmed that upper respiratory inflammation causes an extended duration of illness and increased severity of lower respiratory symptoms in individuals with asthma.

In this study, there were no significant differences in the severity of asthma regarding the aggravation by dust between mild and moderate asthma (*P= 0.535*). Contrary to this result, a study achieved by (Cadelis, Tourres and Molinie, 2014) revealed that dust represents a high risk of asthma and increases the severity of asthmatic patients. This discrepancy might be attributed to the differences in sample size between this study and theirs.

In this study, there were no significant differences in the severity of asthma regarding aggravating by physical activities *(P= 0.379*). This study was inconsistent with a study reported by (Paggiaro and Bacci, 2011)**,** which revealed that exercise-induced bronchospasm is frequent in children and young patients with mild asthma.

Regarding the controlling therapy, this study revealed a significant association (*P = 0.049)* between the no treatment administrating and mild asthma compared with moderate asthma and an association between the patients who were controlling their asthma with montelukast and moderate asthma compared with mild asthma. The result of the present study was compatible with a study accomplished by (Paggiaro and Bacci, 2011), who reported that montelukast improved symptoms, rescue medication use and pulmonary function and reduced the rate of exacerbation and the level of blood eosinophils in mild-to-moderate asthmatics not treated with ICS.

Regarding asthma phenotype, there were no significant differences (*P= 0.991*) between asthma phenotype and disease severity. A study by (Fitzpatrick and Moore, 2017) found that despite global differences between severe and non-severe asthma in the Severe Asthma Research Program (SARP), significant heterogeneity was present in both groups, prompting further exploration of phenotypes irrespective of asthma severity definitions.

This study showed no significant association between IL-15 level and EOS count (*p = 0.395*) with a non-significant positive correlation (r=0.112); this might be as described by a study conducted by **(**(Huilan *et al.*, 2010)**,** (Zhu *et al.*, 2011)**),** which demonstrated that IL-15 activated STAT5 and CD4+ T cells to produce cytokines that act on eosinophils. Also, (Hoontrakoon *et al.,* 2002) found that IL-15 plays an essential role in allergic diseases by inhibiting eosinophil apoptosis. Its anti-apoptotic effects appear to be mediated through autocrine production of GM-CSF and ultimately by NF-κB activation.

In addition, this study showed no significant association between IL-15 level and IgE level (*p = 0.301*) with a non-significant negative correlation (r=-0.136). This result was agreed with a study achieved by (Ong *et al.,* 2002)**,** who demonstrated that IL-15 suppressed IgE synthesis. Therefore, a decrease in IL-15 may contribute to the elevation of IgE levels in atopic disease. Also, this study was compatible with a study confirmed by (Huilan *et al.,* 2010)**,** who demonstrated that decreasing the IL-15 serum level induces the increasing expression of IgE produced by B cells.

About IL-15, this result showed no significant association between the IL-15 serum level and duration of asthma (*p = 0.675*), as in Figure 3. This might be because of treatment admission; a study conducted by (Komai-Koma *et al.,* 2001) confirmed that IL-15 production appears to be reciprocally regulated by steroid therapy in asthma patients.

6. Conclusion

There was a non-significant association between the IL-15 serum level and the severity of asthma. Therefore, further studies with large sample sizes are needed to confirm the role of this interleukin as an immune marker for the diagnosis of asthma.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Evaluation and Correlation of Urinary Micro Albumin in Early Diagnosis of Patients with Hypertension Related Chronic Kidney Disease

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Received: 2023/09/12

Accepted: 2023/11/16

Published: 2024/01/04

Keywords: Urinary microalbumin, Hypertension, Chronic Kidney Disease, Urea, Creatinine.

DOI:10.62472/kjps.v14.i23.44-53

Abstract

One of the main organs targeted by hypertension, which is now understood by science to be an inflammatory disease, is the kidney. Urinary microalbumin, serum blood urea nitrogen, and creatinine are examples of clinical biochemical indicators for kidney illness that are often employed. This study aimed to study the role of Urinary microalbumin as a marker of inflammation in hypertensive chronic kidney disease patients. Evaluate the association of Urinary microalbumin levels in chronic kidney disease with/without hypertension. Possible usage of this marker as a predictive index for regular kidney disease occurrence. A case–control study was conducted on 100 participants, including 48 individuals identified as male and 52 individuals identified as female; in addition, their ages ranged from (20 to 70), these 100 participants, including (35) chronic kidney disease patients with hypertension (35) chronic kidney disease patients without hypertension, and (30) healthy control group, were evaluated concerning Urinary Micro Albumin using an immunochromatographic test kit. The results illustrated that Urinary microalbumin, Urea and Creatinine in CKD with the HBP group showed a highly significant increase ($p<0.05$) compared to the control group according to case/ control, sex and age. In contrast, the most negligible value was in the Control group.

In conclusion

The research findings indicate a correlation between hypertension and chronic kidney disease (CKD). The presence of hypertension resulted in elevated levels of Urinary microalbumin, urea, and creatinine in patients as compared to the control group. Urinary microalbumin has the potential to serve as a marker for the assessment of glomerular and tubular function in adults. It performs comparably to the Cr-based estimating equations as an indicator of renal function.

تقييم وارتباط الزلال البولي الدقيق في التشخيص المبكر للمرضى الذين يعانون من مرض الكلى المزمن المرتبط بارتفاع ضغط الدم

زهراء راضي جبر، إسراء سعيد عباس، علي جاسم محيميد

الملخص

أحد الأعضاء الرئيسية المستهدفة بارتفاع ضغط الدم، والذي يُفهم الآن على أنه مرض التهابي، هو الكلية. الميكروبومين البولي، نيتروجين اليوريا في مصل الدم، والكرياتينين هي أمثلة على المؤشرات الكيميائية الحيوية السريرية لأمراض الكلى. كان الهدف من هذه الدراسة هو دراسة دور الميكروبومين البولي كعلامة على الالتهاب في مرضى أمراض الكلى المزمنة المصابين بارتفاع ضغط الدم، وتقييم ارتباط مستوى الميكروبومين البولي في أمراض الكلى المزمنة مع أو بدون ارتفاع ضغط الدم، واستخدامه كمؤشر تنبؤي لحدوث أمراض الكلى المزمنة. أجريت دراسة حالات مراقبة على ١٠٠ مشارك، بينهم ٤٨ ذكور و٥٢ إناث، تراوحت أعمارهم بين ٢٠ و٧٠ سنة. تضمنت الدراسة ٣٥ مريضًا بأمراض الكلى المزمنة المصابين بارتفاع ضغط الدم، ٣٥ مريضًا بأمراض الكلى المزمنة بدون ارتفاع ضغط الدم، و٣٠ فردًا كمجموعة تحكم صحية. تم قياس الميكروبومين البولي باستخدام اختبار مناعي. أظهرت النتائج أن الميكروبومين البولي، اليوريا، والكرياتينين كانت مرتفعة بشكل كبير ($p<0.05$) في مجموعة مرضى الكلى المزمنة مع ارتفاع ضغط الدم مقارنة بمجموعة مرضى الكلى المزمنة بدون ارتفاع ضغط الدم، بناءً على الحالة/السيطرة، الجنس، والعمر، بينما كانت أقل قيمة في مجموعة التحكم.

1. Introduction

Hypertension is one of the top five global killers and a significant risk factor for cardiovascular and renal illnesses, which kill more than 40% of people worldwide (Nahimana et al., 2018).

Hypertension is a frequent condition encountered during kidney disease development and a leading cause of its progression. Multiple crosstalk mechanisms are involved in sustaining the inevitable high blood pressure (BP) state in CKD, and these play an essential role in the pathogenesis of increased cardiovascular (CV) events associated with CKD (Ameer, 2022).

Primary hypertension, sometimes known as essential hypertension, is the prevailing kind of hypertension. Secondary hypertension may be ascribed to several reasons, including chronic kidney disease, renal artery stenosis, and sleep apnea. The symptoms of hypertension are often not apparent until severe or chronic signs mark the disorder. *Sphygmomanometry* is a diagnostic procedure used in medical settings. Testing may be performed to ascertain the aetiology, evaluate the extent of organ impairment, and ascertain other risk factors associated with cardiovascular well-being(Carey et al., 2018).

A kidney typically contains around one million nephrons, contributing to the glomerular filtration rate (GFR). In cases of kidney damage, the kidney may maintain a glomerular filtration rate (GFR) via the mechanisms of hyperfiltration and compensatory hypertrophy of the unaffected nephrons (C. Shima et al., 2022).

Signs of kidney damage (albuminuria and structural abnormalities on ultrasonography) high urea and creatinine levels and were used to identify chronic kidney disease (Eka et al., 2021)

Microalbumin, with a molecular weight of 68,000 Daltons, indicates aberrant permeability in the glomerulus. One of the first signs of chronic kidney disease (CKD) is the presence of urine albumin excretion ranging from 20 to 200 mg/L. This particular range is also associated with an increased susceptibility to metabolic diseases, cardiovascular illness, and death from any cause (Gaeini et al.,2022).

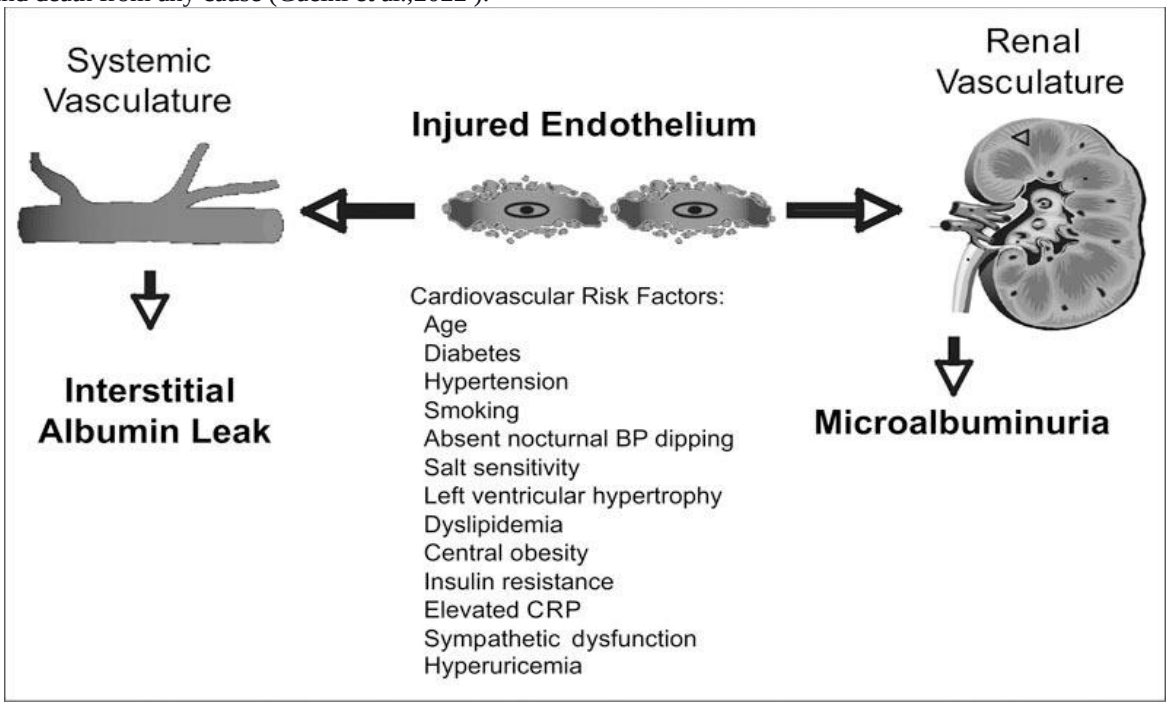

Figure 1: Microalbuminuria: Manifestation of Diffuse Endothelial Cell Injury. BF=Blood Pressure; CRF=C-Reactive Protein (Toto, 2004)

Numerous obstacles impede the passage of albumin through the glomerular filtration system of the nephron. At pH, characteristic of physiological conditions, the glomerular capillary wall and endothelial cells exhibit a repulsive interaction with albumin due to opposing charges on their surfaces. , Its porous nature characterizes the glomerular basement

membrane (GBM), yet the size of these openings is often insufficient to allow the passage of albumin. Furthermore, the megalincubulin complex is responsible for the albumin degradation inside the nephron, especially in the proximal convoluted tubule. The primary purpose of this mechanism is to save amino acids for subsequent use while concurrently serving as an additional means of impeding the translocation of albumin. The presence of albumin in urine may be attributed to the malfunction of the glomerular basement membrane (GBM) filtration barrier, and the quantity of albumin excreted has significance (Waghmare & Goswam, 2016).

The Steno hypothesis proposes that the initiation of microalbuminuria and cardiovascular disease is attributed to systemic vascular endothelial dysfunction since a robust link exists among these three variables. Hence, the presence of comorbidities that induce endothelium degradation is regarded as a risk factor. The factors contributing to this phenomenon include hypertension, advanced age, insulin resistance and dyslipidemia (Prasad et al., 2023).

Therefore, in addition to the renal system, albumin has the potential to either extravasate from or permeate into the artery wall at many vascular sites. In instances of occurrence, albumin has the potential to incite inflammation, lipid buildup, and atherosclerosis, ultimately leading to persistent albuminuria and a decline in renal function (Abdelhafiz et al., 2011).

The primary purpose of assessing the urine microalbumin level is to examine the patient's potential susceptibility to future issues. Nevertheless, it is essential for healthcare practitioners to not just see microalbuminuria as a simple indicator of kidney damage but rather as a prognosticator of the pace at which kidney dysfunction may proceed, as well as an indicator of the impact of systemic illnesses on renal function (Waghmare & Goswami, 2016).

The Study Aims to evaluate and correlate urinary microalbumin (mAlb) in chronic kidney disease patients with/without hypertension.

2. Materials and Methods

The case-control study has been conducted at Al-Imam Al-Sadiq Teaching Hospital in Babylon Governorate, spanning from January

2023 to April 2023. The study population consisted of seventy individuals diagnosed with chronic kidney disease (CKD), with 35 of them presenting with CKD and Hypertension, while the remaining 35 had CKD without Hypertension. Additionally, a control group of 30 healthy individuals was included for comparison. The ages of the individuals ranged from 20 to 70 years. Regarding sex, there were 48 individuals identified as male and 52 individuals identified as female.

The urinary microglobulin level of participants was measured by immunochromatographic test kit after the urine was collected using the traditional technique and conversed in a screw cup. The fluorecare® mAlb is a diagnostic tool that utilizes the immunochromatographic assay concept to measure the content of mAlb in human urine using a competitive immunodetection approach. The urine sample is introduced into the designated aperture for specimen addition. Within the specimens intended for analysis, the monoclonal anti-mAlb antibody interacts with the fluorescently labelled mAlb antibody on the bonding pad, forming the mAlb antibody complex—the diffusion of the mAlb antibody complex along the nitrocellulose membrane results from the chromatographic action.

3. Exclusion Criteria

Patients were excluded from the study if they had any of the following: hepatitis, autoimmune disease, pregnant women, elevated blood pressure due to cancer, diabetic patients, kidney stones disease and other types of renal diseases, except CKD with hypertension.

4. Inclusion Criteria

CKD patients with/without hypertension served as the case group, and Healthy subjects served as the control group.

5. Ethics Approved

The College of Applied Medical Sciences Ethical Committee, which has its headquarters in Karbala, gave the study's protocol their stamp of approval. Patients or their guardians provided their approval for samples to be collected. Additionally, a questionnaire designed by the researcher was used to interview participants.

6. Statistical Analysis

Independent T-Test and Mann-Whitney U tests have been utilized to conduct a comparative analysis between two groups on the same continuous variable and the
results have been
considered to have
statistical significance at ($p \leq 0.05$).

7. Results

1-Correlation of Urinary Microalbumin on study groups

The findings shown in Table (1) demonstrate a highly significant statistical distinction ($P<0.05$) in the levels of urine albumin between the CKD with HBP group and the CKD without HBP group, as compared to the control group. This distinction was seen when considering factors such as case, sex, and age.

The group with chronic kidney disease (CKD) and high blood pressure (HBP) exhibited the most significant Urinary microalbumin level, with a mean value of 114.49 in the case/control group. The group with CKD followed this without HBP, with a mean level of 56.62 in the same category. The average degree of control is 8.68.

Furthermore, it was observed that the concentration of Urinary microalbumin was found to be the greatest in both male and female individuals belonging to the chronic kidney disease (CKD) with high blood pressure (HBP) category. The mean values for this group were recorded as 113.41 and 115.52, respectively. In contrast, the mean levels of urinary Microalbumin in the CKD without HBP category were lower, with values of 51.80 and 60.68 for males and females, respectively. The mean degree of control is 8.51 and 8.77, respectively.

Table 1: Relation of Sex and Age Group on Microalbumin in the Patients Compared with the Control Group

Disease Status	Variable	Variable Level	Patients		Control		P. Value
			Mean	Std. Deviation	Mean	Std. Deviation	
CKD with HBP	**Case**	**Case/ Control**	**114.49**	**32.98**	**23.42**	**8.68**	**0.001****
	Sex	**Male**	**113.41**	**41.56**	**21.65**	**8.51**	**0.007****
		Female	**115.52**	**23.40**	**25.20**	**8.77**	**0.003****
	Age Group	**20 - 40**	**107.18**	**36.72**	**٢٦.٢٠**	**9.٢٨**	**0.007****
		41 - 70	**118.31**	**31.03**	**٢٢.٠٣**	**٨.٢٥**	**0.007****
CKD without HBP	**Case**	**Case/ Control**	**56.62**	**31.97**	**23.42**	**8.68**	**0.007****
	Sex	**Male**	**51.80**	**32.59**	**21.65**	**8.51**	**0.002****
		Female	**60.68**	**31.74**	**25.20**	**8.77**	**0.001****
	Age Group	**20 - 40**	**49.66**	**29.52**	**٢٦.٢٠**	**9.٢٨**	**0.004****
		41 - 70	**61.27**	**33.39**	**٢٢.٠٣**	**٨.٢٥**	**0.001****

-Independent T-Test and Mann-Whitney U tests have been used to compare two groups on the same continuous variable.

-****** The mean difference is significant at the 0.05 level

2-Correlation of Biochemical Parameters (Urea and Creatinine) on study groups

Table (2) illustrated that Urea and Creatinine in CKD with the HBP group showed a highly significant increase ($p<0.05$) when compared with the control group according to case/ control, sex and age. In contrast, the most negligible value was in the Control group.

Also, Urea and Creatinine in CKD without the HBP group showed a highly significant increase ($p<0.05$) compared to the control group according to case/ control, sex and age. In contrast, the most negligible value was in the Control group.

Generally, in comparisons among all groups of chronic kidney disease, the results showed significantly higher differences ($p<0.05$), as illustrated in the table below.

Table 2: Relation of Sex and Age Group on the Biomarker Levels (Urea, Creatinine) in the Patients Compared with the Control Group

Disease Status	Parameters	Variable Level	Patients		Control		P. Value
			Mean	Std. Deviation	Mean	Std. Deviation	
CKD with HBP	Urea	Case/Control	22.82	10.99	4.62	1.40	0.005**
		Male	25.86	11.83	4.77	1.24	0.002**
		Female	19.95	9.59	4.47	1.58	0.002**
		20 - 40	23.43	10.98	٤.٢٦	١.١٦	0.00٧**
		41 - 70	22.51	11.23	٤.٨0	١.٥٥	0.00١**
	Creatinine	Case/Control	216.05	114.93	65.82	11.51	0.005**
		Male	247.79	125.69	66.73	13.28	0.002**
		Female	186.08	97.93	64.90	9.81	0.006**
		21 - 40	235.49	133.58	٦٦.٩٧	١٠.٥٥	0.001**
		42 - 70	205.91	105.73	٦٥.٢٤	١٢.١٨	0.002**
CKD without HBP	Urea	Case/Control	19.64	10.17	4.62	1.40	0.003**
		Male	21.07	11.89	4.77	1.24	0.006**
		Female	18.43	8.61	4.47	1.58	0.001**
		20 - 40	20.58	11.16	٤.٢٦	١.١٦	0.002**
		41 - 70	19.01	9.68	٤.٨0	١.٥٥	0.001**
	Creatinine	Case/Control	213.46	99.64	65.82	11.51	0.003**
		Male	221.14	126.44	66.73	13.28	0.005**
		Female	206.99	73.03	64.90	9.81	0.009**
		20 - 40	216.69	96.68	٦٦.٩٧	١٠.٥٥	0.005**
		41 - 70	211.31	103.87	٦٥.٢٤	١٢.١٨	0.003**

Independent T-Test and Mann-Whitney U test have been used to compare two groups on the same continuous variable.

-. The mean difference is significant at the 0.05 level.**

3-The Relationship Between Research Parameters in Patients with CKD and Their Controls

Table (3) demonstrates that the levels of Urea and Creatinine were significantly higher ($p<0.05$) in the group with Chronic Kidney Disease and High Blood Pressure. In contrast, the Control group had the lowest values.

Table 3: The Relationship Between Research Parameters in Patients with CKD and Their Controls

Parameters	Disease Status	Mean	Std. Deviation	P. Value
Urea	CKD with HBP	22.82	10.99	0.0002**
	CKD without HBP	19.64	10.17	
	Control	4.62	1.4	
Creatinine	CKD with HBP	216.05	114.93	0.0003**
	CKD without HBP	213.46	99.64	
	Control	65.82	11.51	
Urinary Microalbumin	CKD with HBP	114.49	32.98	0.0002**
	CKD without HBP	56.62	31.97	
	Control	23.42	8.68	

-ANOVA and Kruskal-Wallis tests have been utilized to discern statistically significant differences across multiple independent groups.

- The mean difference is significant at the 0.05 level.**

In contrast, the levels of Urinary microalbumin were found to be significantly higher ($p<0.05$) in the group of individuals with chronic kidney disease (CKD) and high blood pressure (HBP) compared to the other groups included in the research. Notably, the control group exhibited the lowest value of these biomarkers.

In the analysis of chronic Kidney disease across various categories, the findings indicated significantly higher differences ($p < 0.05$), as shown in the table above.

8. Discussion

The results of the present study are shown in Table (1), where screening for Urinary Microalbumin is a sensitive and trustworthy way to detect renal disease and mortality in hypertension. Additionally indicative of end-stage renal illness is Urinary Microalbumin.

According to Yang's 2021 results, there was a significant difference ($P<0.05$) in the Urinary microalbumin levels between the groups with hypertension and hypertensive nephropathy and the control group. Comparability was shown by the lack of statistically significant differences in age and gender between the three groups of people ($P>0.05$).

In contrast to ordinary persons, most cases developed mAlb. Overt mAlb was present in more than half of the cases. This demonstrated a correlation between CKD and mAlb, according to the research by Chin'ombe et al. 2013.

Hwang et al. 2000 Compared to women with normotension, hypertensive women had a significantly greater prevalence of mAlb (16% vs. 4%, $P < 0.001$). These findings imply that whereas hypertension is often the primary cause of mAlb, other illnesses may also play a role.

As time went on, more cases of microalbuminuria were discovered, and in women, the condition was once more severe. There is a strong correlation between hypertension and microalbuminuria. It has been reported that mAlb is linked to excess body weight. The prevalence of mAlb in the hypertensive female population was higher than that of the male population. This difference in prevalence may be related to the fact that women have lower muscle mass than men—roughly 15% less—and, therefore, lower levels of creatinuria. A

research by Poudel et al.,2012 indicated obesity to be an independent risk factor for microalbuminuria, which is consistent with the current finding.

The higher level of mAlb and creatinine may be due to subclinical ultra-structural changes in the glomeruli of hypertensive patients. The presence of microalbuminuria in the early stage of hypertension can be taken as a significant independent predictor for the progression of renal disease.

So, mAlb in hypertensive subjects may prove to be a valuable marker in the evaluation of target organ damage and control of risk factors amenable to prevention (regular treatment of HT, weight control, normal lipid levels) may have a favourable effect in preventing, delaying and lessening prevalence of mAlb.
In conclusion, mAlb should be used as a marker of renal dysfunction in CKD. It may also be a marker for other metabolic problems associated with CKD.
The levels of the routine chemistries (creatinine and urea) were consistent with the expected pattern in CKD. They were significantly different from those of the control group, according to Chin'ombe et al. 2013 study. This agrees with our study's findings in Table (2).
A Hanratty et al. 2011 study found that higher treated BP was associated with early kidney function decline (a rise in serum creatinine ≥0.6 mg/dl).
Also, the present study's findings align with Hussein's 2022 study, which also examined renal function tests. The results showed statistically significant differences ($p<0.01$) in serum urea and creatinine across all groups studied.
The present research's findings are consistent with those of a previous study done by Yang, 2021 whereby the results of biochemical index detection indicated that levels of BUN and creatinine were significantly elevated in the hypertensive nephropathy group compared to both the control group and the hypertension group ($P<0.05$). This agrees with our study's findings in Table (3).
Bae et al. 2022 study showed higher hazard ratios for CKD among males younger than 40 years compared to females younger than 40.
Hasan et al.,2013 study found that hypertension is higher among men than women. Age was shown to be a significant associated risk factor for hypertension.
Abd Allah et al. 2021 study observed that age had a positive association with serum urea and also negative with diastolic pressure. Diastolic pressure had a positive correlation with systolic pressure and also a positive correlation with serum urea. No significant differences between serum parameters in the study population were found. The serum creatinine was significantly increased in males than females.
In light of the given information, the findings suggest clinical implications associated with higher urinary albumin levels in the general population. These elevated levels should be considered as potential risk factors for the future onset of hypertension and related complications, such as chronic kidney disease. The timely identification and intervention of hypertension are anticipated to mitigate the advancement of renal impairments and hypertension.

9. Conclusion

1. The study showed that there was a strong relationship between Urinary microalbumin and chronic renal disease and hypertension, and the nature of the relationship was synergistic between them.
2. Creatinine and Urea, routine markers for CKD, were increased in CKD with/without hypertension, while average values were in the control group.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Role of Ultrasound in Diagnosis of Fibrocystic Breast Disease

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Received: 2023/10/25

Accepted: 2023/11/16

Published: 2024/01/04

Keywords :Ultrasound, Breast, BI-RDS, Fibrocystic Changes.

DOI:10.62472/kjps.v14.i23.54-62

Abstract

Background: Fibrocystic breast disease is the most typical benign breast disease, which is seen in women worldwide with features of pain and feeling of nodules. Its diagnosis is based on clinical symptoms, ultrasound, mammography, and in doubt cases, biopsy is indicated. For assessment of the breast, we clinically examined the breast, axilla, and both supra and infraclavicular regions for lymph node assessment, followed by ultrasound examination. Detection of lesions in any breast quadrant assessed by BI-RADs, Breast Imaging Reporting, and Data System grouped in five brackets. Scanning by sonography using B-mode and Doppler study was improved for detecting and characterizing benign and malignant lesions.

Objective: To assess a breast lesion by ultrasound with features suggestive of a benign or malignant nature.

Method: This study included 210 women aged 20 - 45 who visited a clinic from October 2020 to April 2022 and were examined by ultrasound machine Samsung HS50 (KOREA) with an LA3-14AD probe. B-mode images obtained detection of the lesion, and we also used Doppler ultrasound for assessment of the vascularity of the lesion. The BI-RADS system was used for the categorization of all findings.

Result: In 210 women, complaints of lumps were involved in our study. Imaging by B-mode ultrasound using the Doppler study assesses the vascularity of the lesion and provides more characterization of benign and malignant lesions. By B–mode imaging, 99 patients had regular ultrasound study, and 79 patients had simple cysts that appeared well defined, had familiar outlines, and had an anechoic rounded or oval shape with posterior enhancement. Twenty-eight patients with fibroadenoma visualized as round or oval-shaped hypoechoic lesions horizontally oriented to the planes of the breast with lateral shadowing. 2 patients had complex cysts that appear as well-defined, irregular outlines, rounded shape, a thick wall with internal echoes with posterior enhancement. 2 patients have a malignant lesion that appears ill-defined rough outline vertically oriented with breast planes and show posterior shadowing.

Conclusion:

Ultrasound was established as the most suitable imaging modality for the categorization of breast lesions and exclusion of malignancy, which is helpful for evading unnecessary biopsies.

دور الموجات فوق الصوتية في تشخيص مرض الثدي الكيسي الليفي

الخلاصة

مرض الثدي الكيسي الليفي هو مرض الثدي الحميد الأكثر شيوعًا والذي يصيب النساء في جميع أنحاء العالم مع وجود ألم وشعور بالعقيدات. يعتمد تشخيصه على الأعراض السريرية، والموجات فوق الصوتية، والتصوير الشعاعي للثدي، وفي حالات الشك يتم إجراء خزعة. لتقييم الثدي، قمنا بفحص الثدي والإبطين وفوق الترقوة وتحت الترقوة سريريًا لتقييم العقد الليمفاوية يليها فحص بالموجات فوق الصوتية. يتم تقييم الكشف عن الآفة في أي ربع من الثدي بالتوافق مع BI-RADsوتقارير تصوير الثدي ونظام البيانات المجمعة في خمس فئات. تم تحسين المسح باستخدام التصوير بالموجات فوق الصوتية باستخدام الوضع. Bودراسة الدوبلر لاكتشاف وتوصيف الآفات الحميدة والخبيثة

الهدف: تقييم آفة الثدي عن طريق الموجات فوق الصوتية مع ميزات توحي بأنها حميدة أو خبيث

الطريقة: هذه دراسة تشمل ٢١٠ امرأة في الفئة العمرية بين ٢٠-٤٥ عامًا زارن إحدى العيادات في الفترة من أكتوبر ٢٠٢٠ الى ابريل ٢٠٢٢ وتم فحصهن بوسطه جهاز الموجات فوق الصوتيه سامسونغ اش اس ٥٠ صناعه كوريا باستخدام مسبار LA3-14AD.

استخدمنا الموجات فوق الصوتية دوبلر تم الكشف عن الافه التي تم الحصول عليها بواسطه صور الوضع بي

وايضا واستخدمنا الموجات فوق الصوتيه دوبلر لتقييم الاوعيه الدمويه للافه. نظام بايردزلتصنيف جميع النتائج. .

النتيجة: في ٢١٠ امرأة كيف تشتكي من الورم المتضمن في دراستنا. التصوير بالموجات فوق الصوتية من النوع بي.

مع استخدام دراسة الدوبلر لتقييم الأوعية الدموية للآفة وإعطاء مزيد من التوصيف للآفة الحميدة من الآفة الخبيثة من خلال التصوير بالوضع B، 99 مريضًا لديهم دراسة بالموجات فوق الصوتية العادية، و٧٩ مريضًا لديهم كيس بسيط يبدو محددًا جيدًا، ومخططًا منتظمًا، وشكلًا مستديرًا أو بيضاويًا عديم الصدى مع تعزيز خلفي. ٢٨ مريضًا مصابين بالورم الغدي الليفي تم تصويرهم على أنهم آفة ناقصة الصدى مستديرة أو بيضاوية الشكل وموجهة أفقيًا إلى مستويات الثدي مع تظليل جانبي. مريضان يعانيان من كيس معقد يظهر بشكل جيد، مخطط غير منتظم، مستدير الشكل، جدار سميك مع أصداء داخلية مع تعزيز خلفي. يعاني مريضان من آفة خبيثة تبدو مخططًا غير منتظم محدد المعالم وموجهًا عموديًا مع مستويات الثدي ويظهر تظليلًا خلفيًا.:

خاتمة :تم إنشاء الموجات فوق الصوتية باعتبارها طريقة التصوير الأكثر ملاءمة لتصنيف آفات الثدي واستبعاد الأورام الخبيثة وهو أمر مفيد لتجنب الخزعات غير الضرورية.

1. Introduction

Fibrocystic change of the breast is a benign condition that involves changes in the terminal ductal lobular unit of the breast, which may or may not associated with fibrosis. Fibrocystic changes (FCC) are established as the most typical benign disorder of the breast (Malherbe, Khan and Fatima, 2019), mainly seen in premenopausal women between age 20-40 years of age, with about half of women affected throughout their lives. Clinically, they presented with breast pain, which increases in severity during ovulation; ultrasound plays an important role in approaching breast pain (Alsalami and Alattabi 2018). Breast pain is associated with swelling and lumps in one or both breasts; sometimes, pain may cause pain under the arm. The etiology of fibrocystic breast changes is not well known, but there is a correlation with reproductive hormones, especially estrogen, like estrogen stimulation and progesterone deficiency.

The American College of Radiology established a system for describing and imaging breasts called BI-RADs system, Breast Imaging Reporting and Data System, which relates to mammography, ultrasound, and MRI. The fifth edition of BI- the RADS system nowadays is commonly used. In ultrasound, this system depends on the shape, margin, echo pattern, and orientation of the lesion (Kaplan *et al.*, 2022) in the breast and is employed to demonstrate any lesion, either benign (Burgess and O'Neal, 2019), (Stachs *et al*., 2019) or malignant lesion (Kim, Kim and Moon, 2020), (Brem *et al.*, 2015) and classified in five brackets (Eghtedari *et al.*, 2021), (Spak *et al.*, 2017).

To perform breast examination by ultrasound, we commonly use B–mode ultrasound, which is a safe, non-invasive, non-radiative screening method for breast lesions. For better demonstration and characterization of the solid lesion as a benign or a malignant lesion, we used Doppler imaging (Choi, Tsunoda and Moon, 2024), both color Doppler flow and power Doppler imaging, to demonstrate vascularity of the lesion like hypervascular mass, or mass with central vessels, which is suggestive malignant masses (Elverici *et al.*, 2015), (Niu *et al.*, 2019). Color Doppler ultrasound allows us to show the flow direction. A little pressure on breast tissue was applied to better demonstrate slow flows within the mass. Power Doppler ultrasound is excellent for displaying slow flow in small vessels as it is Doppler angle independent but has the disadvantage of the increased number of artifacts (Horvath *et al.*, 2011).

Thus, ultrasound plays a vital role in the demonstration and analysis of breast lesions and is usually utilized as an additive to mammography for screening breast lesions (Harada-Shoji *et al.*, 2021).

2. Methodology

Patients' age, marital state, and breast appearance (swelling, redness, and any indentation in skin) were recorded. B-mode and Doppler ultrasound were done using Samsung HS50 (KOREA) in Kerbala, Iraq, with a linear LA3-14AD probe. The preset conditions were imaging gain at 65%, enhancement at 4, depth at 40 mm, and persistence at 6. The patient is lying down slightly obliquely with an arm over the patient's head. We examine the patient. Images were taken in both transverse and longitudinal planes for all breast quadrants. Ultrasound features involved echotexture (as either homogeneous or heterogeneous), size, shape, margin, calcification, and axillary lymph node. These ultrasound features for each mass were categorized according to the BI-RADS (Horvath *et al.*, 2011). Doppler study was done with both color and power to rule out abnormal vascularity; the color gain was adjusted to enable the recognition of low-velocity flow within the mass with negligible background noise.

3. Results

Two hundred ten ultrasounds of ladies who visited the outpatient clinic were involved in this study from October 2020 to April 2022. The mean age is 30.4 SD± 7.76 (between 20 and 45 years old), displayed in Table [1]. From 210 patients, 126 females (60%) were married, and 84 females (40%) were unmarried, as shown in table [2]. Seventy-six females (36.2%) had painful masses, and 134 females (63.8%) had painless masses, as shown in [fig.1]. One hundred twenty-eight females (61%) have non-palpable mass, and 82 females (39%) have palpable mass, as shown in [fig.2]. The exam was done in both planes transverse and longitudinal planes by B-mode for imagining breasts.

The ultrasound findings in our study are shown in the table and figure [3]; 99 females (47.1%) have regular ultrasound studies with no pathology seen. Simple cysts were visualized as rounded or oval-shaped anechoic, shared borders, and thin walls with posterior enhancement in 79 females (37.6%) [Fig.4, 5].

Fibroadenoma was visualized in 28 females (13.3%) as a hypoechoic oval-shaped lesion lying horizontally with lateral shadowing. Most of the masses are pictured in the outer quadrant of both breasts. Doppler study was performed for visualization of the vascularity of the lesion. Small fibroadenoma appears avascular on color Doppler study. However, large fibroadenomas appear vascular with minimal flow and a high resistive index.

Complex cystic lesion seen in 2 patients (1%) as well defined, rounded shape, irregular outline, thick wall, internal echoes causing posterior enhancement. 2 patients (1%) had a malignant mass which was visualized as irregular ill-defined mass vertically oriented with breast planes and causing posterior shadowing.

4. Statistics

Table 1: Descriptive Statistics of Age

No. 210	
Mean	30.40
Std. deviation	7.763
Minimum	19
Maximum	46

Table 2: Frequency of Marital State

Marital state	Frequency	Percent
Married	126	60.0
Un married	84	40.0
Total	210	100.0

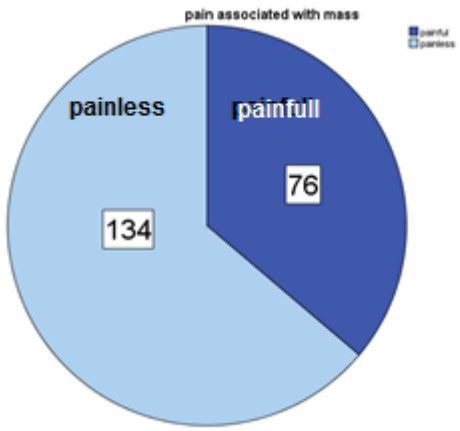

Figure 1: Frequency of Pain Associated with Mass

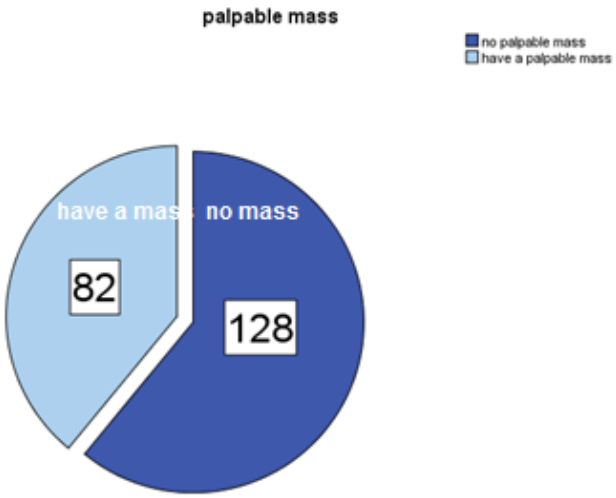

Figure 2: Frequency of Palpable and Non- Palpable Mass

Table 3: Ultrasound Finding

	Frequency	Percent
Normal Breast Tissue	99	47.1
Simple Cyst	79	37.6
Fibroadenoma	28	13.3
Complex Cyst	2	1.0
Solid Mass Suspicious Malignancy	2	1.0
Total	210	100.0

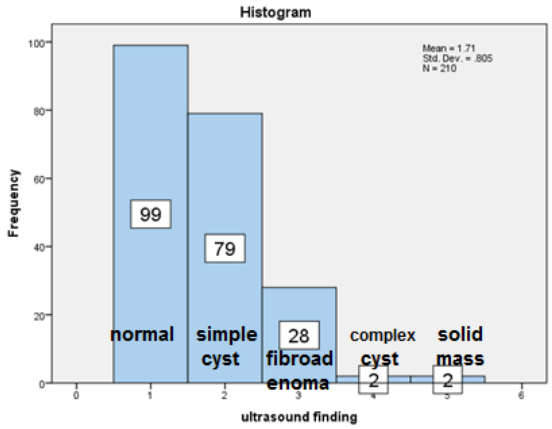

Figure 3: Frequency Ultrasound Finding

5. Discussion

Fibrocystic breast disease is the most typical benign breast disease, which is seen in millions of ladies in the world. It is a broad term that involves a wide spectrum of breast conditions affecting women of childbearing age. Histologically, it is divided into nonproliferative breast lesions and proliferative breast lesions. Nonproliferative breast lesions include simple breast cysts, stromal fibrosis, and apocrine metaplasia. The proliferative type includes ductal epithelial hyperplasia and sclerosing adenosis. The nonproliferative type has a low risk of breast cancer, while a high risk of cancer accompanies the Proliferative lesions with cell atypia.

The patient complains of pain before menstruation bilaterally that may or may not be associated with a palpable mass. Edematous skin and nipple discharge may be seen.

This study was done in an ultrasound clinic with a standard breast with no lesion in 99 females (47.1%). Simple cysts appear with a well-defined anechoic lesion and a thin wall, causing posterior enhancement detected in 79 females (37.6%) (Figure 4, 5). Fibroadenoma has a well-defined oval shape, regular outline, and low-level internal echoes with a transverse diameter more than the anteroposterior diameter seen in 28 females (13.3%). Complex cysts appear in ultrasound as thick wall cystic lesions and lobulated margins, causing posterior shadowing in 2 cases (1%). Suspected malignant lesions were seen in 2 cases (1%). B-mode and Doppler ultrasound are used to characterize lesions, which help differentiate malignant from benign lesions and the precision of making the judgment for biopsy (Lee *et al.*, 2017).

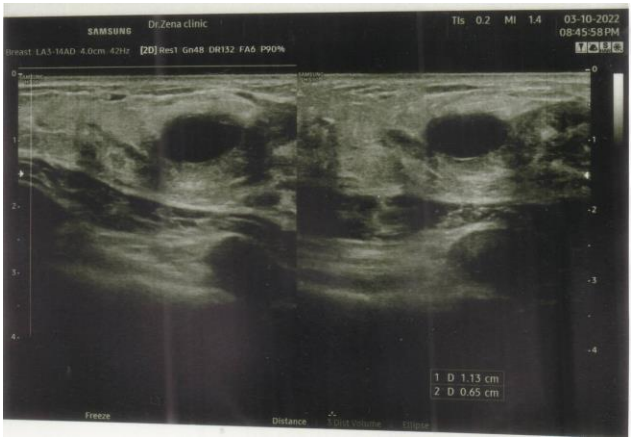

Figure 4: Ultrasound Image of 34 Year Old Female Show Simple Cyst Measure 11x 6.5mm in Left Breast

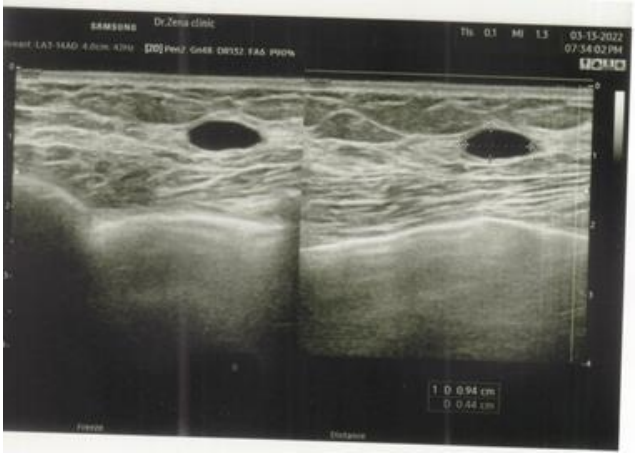

Figure 5: Ultrasound Image of 29 Years Old Female Show Simple Cyst in Right Breast Measure 9.4 x 4.4mm.

Detection of abnormal breast lesions by B-mode ultrasound can be further assessed by additional functions in ultrasound, such as elastography, which helps differentiate lesions. Sonoelastography can determine the relative

elasticity of suspicious masses compared to the surrounding tissue. Strain ratio is used to determine the elasticity of breast lesions compared to adjacent fatty tissue; malignant lesions are stiffer than normal tissue (Gheonea, Stoica and Bondari, 2011), (Ranjkesh *et al.*, 2020). Both color Doppler and elastography can alter how breast lesions are managed, as demonstrated

at screening ultrasound (Sehgal *et al.*, 2006). Both mammography and ultrasound can increase the rate of early detection of cancers even though decreasing interval cancers in females with dense breasts (Hooley *et al.*, 2012), 20. Ultrasound plays a vital role in detecting and differentiating masses, whether benign or malignant, and with the help of elastography, can aid in diagnosing cancer (Sehgal *et al.*, 2006). Ultrasound of the breast is widely used as a screening tool for mass lesions and can differentiate cystic from solid masses. It is also used as an adjunct with mammography for distinguishing solid masses, whether benign or malignant 21.

6. Conclusion

(Kim, Kim and Moon, 2020)Ultrasound is highly

used in breast diseases as it is a simple, non-invasive, non-expensive imaging technique for the detection of breast lesions and also for the characterization of the lesion, which could be either benign or malignant lesions.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Serum ADAM -17 and Interleukin-6 Levels as a Predictors in Type 2 Diabetic Patients with Myocardial Infarction Patients

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Received: 2023/10/12

Accepted: 2023/11/16

Published: 2024/01/04

Keywords: *Disintegrin, Metallop01rotease-17, Interleukin-6, Diabetes Mellitus, Myocardial Infarction, Chronic and Acute Inflammation.*

DOI:10.62472/kjps.v14.i23.63-73

Abstract

The association of heart diseases with type 2 diabetes, especially myocardial infarction, calls for a search for biomarkers that have a relationship between the two. Which facilitates the process of reducing the development of myocardial infarction in patients with type 2 diabetes. The most prominent of these associations is long-term inflammation and its first and largest factor is interleukin 6, and its close association with A disintegrin and metalloprotease 17 (ADAM-17) and its inverse relationship with type 2 diabetic patients with and without myocardial infarction. This study aims to investigate the role of ADAM-17 in the pathogenesis of diabetic type 2 Iraqi men patients with and without MI by comparing them with a apparantely helthy as control group and to see their association with interleukin-6 levels and other biomarkers. The current study was conducted on 90 Iraqi men between Ja., 2023 and Aug. 2023, 60 samples with T2DM with or without MI and the remaining 30 as apparently healthy control. The patients were selected from the visitors of the coronary care unit (CCU) and Al-Hassan Center for Diabetes and Endocrinology in Kerbala, and they were diagnosed clinically and by laboratory investigations. Various biomarkers such as ADAM-17 and IL-6 have been determined by different biochemical techniques. As a result The highest ADAM-17 and IL-6 level in type 2 diabetes without myocardial infarction was seen as comparison serum levels of IL-6, and ADAM-17 for T2DM (with or without myocardial infarction) with the control group. The correlation between IL-6 and ADAM-17 is strong in type-2 diabetes without MI and between IL-6 and electrocardiogram represented by the two types STEM and NSTEMI in type-2 diabetes with MI. In conclusion, The current study found that ADAM-17 and IL-6 have a negative effect on chronic inflammation as in T2DM without MI is more severe than acute inflammation as T2DM with MI , du to elevation of ADAM-17 and IL-6 levels in type 2 diabetic patients without MI than type 2 diabetic patient with MI.

مستويات ADAM -17 وInterleukin-6 ٢ في الدم كمتنبئات لدى مرضى السكري من النوع ومرضى احتشاء عضلة القلب

ماهر عدنان قلاف، فاضل جواد الطعمة، ماهر عبود مخيف، أحمد قاسم الحيدري

الملخص

إن ارتباط أمراض القلب بمرض السكري من النوع الثاني، وخاصة احتشاء عضلة القلب، يدعو إلى البحث عن المؤشرات الحيوية التي لها علاقة بين الاثنين. مما يسهل عملية الحد من تطور احتشاء عضلة القلب لدى مرضى السكري من النوع الثاني. وأبرز هذه الارتباطات هو الالتهاب طويل الأمد وعامله الأول والأكبر هو الإنترلوكين ٦، وارتباطه الوثيق مع البروتين المحلل ١٧ (ADAM-17) وعلاقته العكسية مع مرضى السكري من النوع الثاني المصابين باحتشاء عضلة القلب وبدونه. تهدف هذه الفدراسة الى التحقق من دور ADAM-17 في التسبب في مرض السكري من النوع الثاني لدى المرضى الرجال العراقيين الذين يعانون من احتشاء عضلة القلب أو بدونه من خلال مقارنتهم مع مجموعة الاصحاء كمجموعة ضابطة ورؤية ارتباطه بمستويات الانترلوكين ٦ والمؤشرات الحيوية الأخرى. أجريت الدراسة الحالية على تسعين رجلاً عراقيًا في الفترة ما بين يناير ٢٠٢٣ وأغسطس ٢٠٢٣، ستين عينة مصابة بـ T2DM مع أو بدون MI والـ الثلاثون المتبقية كمجموعة مراقبة صحية على ما يبدو. تم اختيار المرضى من زوار وحدة العناية التاجية ومركز الحسن للسكري والغدد الصماء في كربلاء، وتم تشخيصهم سريرياً وعن طريق الفحوصات المخبرية. تم تحديد المؤشرات الحيوية المختلفة مثل ADAM-17 و IL-6بواسطة تقنيات كيميائية حيوية مختلفة. ونتيجة لذلك، فإن أعلى مستوى لـ ADAM-17 و IL-6في مرض السكري من النوع الثاني دون احتشاء عضلة القلب كان قد وجد عند المقارنة مع المجموعة الضابطة. العلاقة بين IL-6 و ADAM-17 قوية في مرض السكري من النوع ٢ بدون احتشاء عضلة القلب وبين IL-6 ومخطط كهربية القلب المتمثل في النوعين STEM و NSTEMI في مرض السكري من النوع ٢ مع احتشاء عضلة القلب. في الختام وجدت الدراسة الحالية أن ADAM-17 و IL-6 لهما تأثير سلبي على الالتهاب المزمن حيث أن T2DM بدون احتشاء عضلة القلب أكثر شدة من الالتهاب الحاد مثل T2DM مع MI نتيجة لارتفاع ADAM-17 و IL-6 مستويات في مرضى السكري من النوع ٢ دون MI أكثر من مرضى السكري من النوع ٢ مع.MI

1. Introduction

Type 2 diabetes is a disease that comes primarily from insulin resistance (Wondmkun, 2020), caused by long-term inflammation under the auspices of the factors released by ADAM-17, the most important of which is interleukin- 6 (Iemmolo, Ghersi and Bivona, 2023).

A disintegrin and metalloprotease 17 (ADAM-17) has been identified as the sheddase for many memb1rane-bound proteins present in various cell types. It significantly impacts the release of chemokines and cytokines, cell signaling, proliferation, and growth (Iemmolo, Ghersi and Bivona, 2023). There are both positive and negative impacts of ADAM-17. Although it supports healthy liver function, adipocyte differentiation, and embryonic development, it is also linked to the pathophysiology of numerous illnesses, including but not limited to heart disease (MI) and diabetes (Maekawa *et al.*, 2019).

The ADAM-17 activation pathway depends on protein kinase for phosphorylation to switch from the inactive phase to the secretory active phase, which exercises its capabilities in everyday situations, including releasing crucial cytokines and growth hormones (Chen *et al.*, 2023). However, when exposed to cellular activators such as the ROS-dependent p38 membrane-associated protein kinase pathway, ADAM-17 sheds its cells at a higher pace. Reactive oxygen species produced by oxidative stress contribute to the development of atherosclerosis, abnormal blood flow, and arterial wall remodeling (He and Zuo, 2015). Local and systemic inflammation causes an increase in ADAM-17 activation when reactive oxygen species are produced. Additionally, nitric oxide has been shown to activate Adam-17 (Sisto, Ribatti and Lisi, 2021). Nitric oxide (NO) is involved in the physiologic control of circulation and serves a pathogenic function in CAD. The synthesis of oLDLR1, essential for the initiation and progression of athe1rosclerosis, is known to be triggered by the activation of Adam-17 by C-reactive protein (Zhao *et al.*, 2011). As a result, ADAM-17 is a crucial enzyme rather than just one of numerous inflammatory factors. Important mediators at the start and progression of T2DM and CAD are its activators and subsequently shed proteins. ADAM-17 is found on the cell surface as dimers and binds to TIMP3, its inhibitor (Liao *et al.*, 2023). ADAM-17 is released from TIMP3 and changes from a dimeric form to a monomeric structure when the ERK or p38 MAPK pathway is activated (Adu-Amankwaah *et al.*, 2021). ADAM-17 has been linked to the beginning of CAD and its development into acute coronary syndrome (ACS), according to several studies. According to clinical research, patients with AMI had higher plasma levels of IL-6 and TNF than healthy individuals. This suggests that IL-6 and TNF maturation, which depends on ADAM-17, may trigger systemic inflammation and cause plaque rupture(De Queiroz, Lakkappa and Lazartigues, 2020). The white adip1ocytes' activation of ADAM-17 causes the release of inflammatory chemicals, including IL-6. Once a low-grade inflammatory state is created due to this expression, the macrophages are forced to go into (migrate into) adipose tissue, where they cause increased insulin resistance (Ni *et al.*, 2020).

A pleiotropic cytokine called IL-6 is secreted in response to disturbances of homeostasis. When activated, this cytokine exhibits evident pro- and anti-inflammatory characteristics. It's interesting that ADAM-17 can cleave its receptor, IL-6R. The processes involved in IL-6's signaling and stimulation influence its characteristics. As a result, whereas IL-6 is generally beneficial when stimulated acutely, its chronic response results in long-term signaling that induces inflammation and autoimmune infarction. Which are crucial in the development of type 2 diabetes and heart attacks (Scheller *et al.*, 2011), (Adu-Amankwaah *et al.*, 2021). As mentioned above, IL-6 and TNFα maturation, which relies on ADAM-17, may activate systemic inflammation and contribute to plaque rupture.

2. Materials and Methods

This case-control study includes ninety participants, sixty of whom are type 2 diabetes (T2DM) with and without myocardial infarction, thirty patients per group, and another thirty participants are nondiabet1ic persons as a control group. The study was conducted in the coronary care unit (CCU) at Kerbala Centre for Cardiac Diseases and Surgery and Al-Hassan Center for Endocrinology and Diabetes / Kerbala Health Directorate from Jan. 2023 to Aug. 2023. Thirty patients presented with typical chest pain to the coronary care unit (CCU); the diagnosis was based on clinical history, physical examination, ECG, and investigation of a cardiac biomarker. Thirty patients with T2DM without MI attended the Al-Hassan Center for Endocrinology and Diabetes. They were diagnosed according to the analysis of RBS and HbA1c. Thirty nondiabetic persons were selected as a healthy control group. Exclusion criteria involve subjects who are suffering from acute kidney injury (AKI), cancer, infections, or other inflammatory conditions.

The current study analyzed several clinical parameters, including serum or blood tests (cTnI, RBS, HbA1c, lipid profile, and (ADAM- 17 and IL-6), which were measured by the following methods (immune-chromatography with a unique two-site sandwich immunoassay. Hexokinase (HK), colorimetric enzymatic, and Sandwich-ELISA methods, respectively. They include a number that identifies the participants (sample No.), sex, age, smoking status, sedentary

lifestyle, family history regarding MI, and the duration of MI (in the case of a patient). Furthermore, the collected data are also registered. As a mandatory step, this study was approved by the following ethical committees: the University of Kerbala, the College of Medicine, and the Kerbala Centre for Cardiac Diseases and Surgery committee. Al-Hassan center for endocrinology and diabetes, and Kerbala Health Directorate / Kerbala-Iraq.

The Statistical Program for Social Scientists program, version 28.0 (IBM, SPSS, Chicago, Illinois, USA), and the Real Statistical Resources Pack software for Mac (Release 7.2) of the resources pack for Excel 2016 were used to create the data analysis for this project. 2013 until 2020 for copyright. Scale-related variables were displayed as mean 2 SD. Analytical, statistical tests confirmed significant variations in categorical variables between the parameters. The results of all hypothesis tests were deemed statistically significant when their p-values were 0.05 (two-sided). Using Fisher's LSD method, the simultaneous confidence level for each of the confidence intervals was computed. This accompanying confidence level represents the likelihood that the genuine difference is contained within each confidence interval. ANOVA used Fisher's LSD method to generate confidence intervals.

3. Results

Figure 1 compares the serum levels of ADAM-17 and IL-6 between the T2DM and the health control groups. The level of ADAM-17 and IL-6 increased in type 2 diabetic patients, and there was a statistically significant difference between type 2 diabetic patients (with and without MI) compared with the healthy control. The mean ± SD of each ADAM-17 and IL-6 level in type 2 diabetic patients was (3256.4 ± 883.03 pg/dl; 30.04 ± 1.75 pg/dl) respectively, while their mean ± SD level in the healthy control group was (1645.33 ± 41 pg/dl), (23.44 ± 1.46 pg/dl) Table 1.

Table 1: Difference Between Mean Levels for ADAM-17 and IL-6 for Between T2DP with or without MI and the Nondiabetic Control Group

Parameters	**Type 2 Diabetic Patients, N=60** Mean ± SD	**Healthy Control N=30** Mean ± SD	**P value**
IL-6, pg/dl	30.04 ± 1.75	23.44 ± 1.46	**<0.001[S]**
ADA1M-17, pg/dl	3256.4 ± 883.03	1645.33 ± 41	**<0.001[S]**
T-test was used *: significant at p ≤ 0.05 SD: standard deviation; S: significant; NS= Non-significant.			

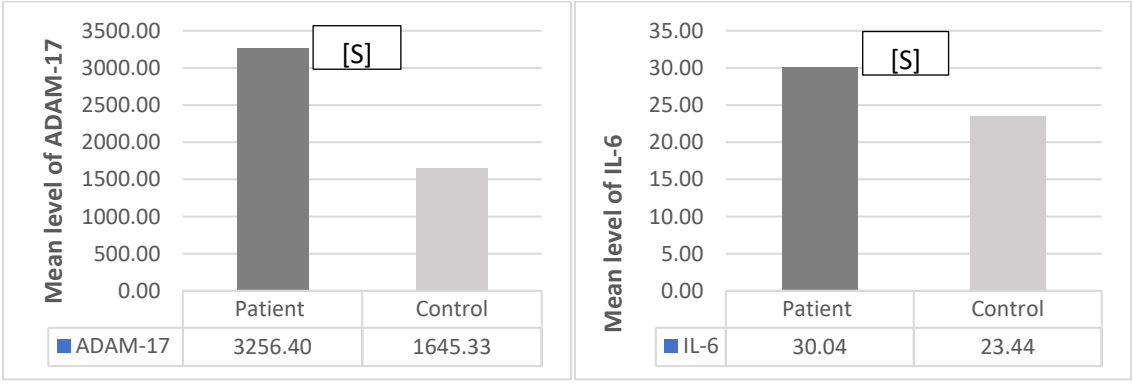

Figure 1: Diffe1rence Between Mean ± SD Levels of IL-6, ADAM-17 in Each T2DM with or Without MI Compared to Healthy Control. (T-Test was used S= Significant at p ≤ 0.05, NS= Nonsignificant)

In Figure 2, a comparison of serum levels of ADAM- 17 and IL-6 among type 2 diabetic patients with MI, type 2 diabetic patients without MI, and the control group was performed. A statistically significant difference in the mean ± SD levels of ADAM-17 and IL-6 was found between DM+MI, DM without MI, and the control group. The highest mean ± SD level was observed in diabetic patients without MI. Their values for ADAM-17 and IL-6 were 3371.05 ± 995.97 pg/ml, 30.20 ± 1.67 pg/dl, while their values for the non-diabetic control group were 1645.33 ± 413.91, 23.43 ± 1.46 pg/dl respectively as shown in Table 2.

Table 2: Difference in ADAM-17 and IL-6 Mean Levels Between T2DM with or without MI and the Nondiabetic Control Group

Biomarkers	**MI+T2DM N=30** Mean ± SD	**Control N=30** Mean ± SD	**T2DM N=30** Mean ± SD	**P value**
IL-6, pg/dl	29.89±1.85	23.44±1.46	30.20±1.67	**<0.001[S]**
ADAM-17, pg/dl1	3141.75±753.16	1645.33±413.91	3371.05±995.97	**0.002[S]**
Two Way ANOVA Test was Used *: Significant at p ≤ 0.05 N: Number of Cases; SD: Standard Deviation; S: Significant; NS= Non-Significant				

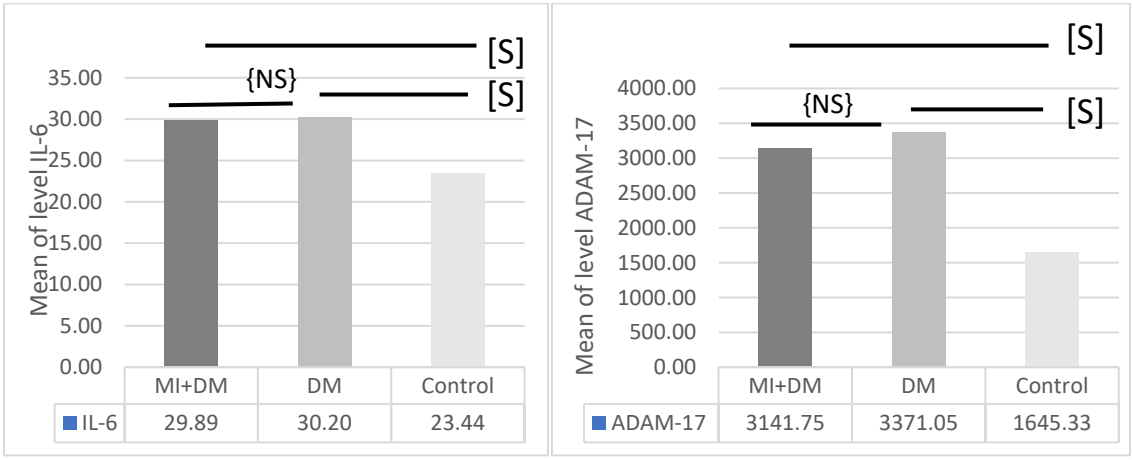

Figure 2: Difference in IL-6 and ADAM-17 mean ± SD levels between T2DM with and without MI and the nondiabetic control group (Two way ANOVA test was used S= significant at p ≤ 0.05, NS= Nonsignificant)

The results showed that there was a very strong relationship and a significant correlation between IL-6 and ADAM-17 in type 2 diabetic patients without MI .((p = 0.001, r=0.9), as shown in Table 3

Table 3 :The Correlation Coefficient between IL-6 and ADAM-17 in T2DM without MI

Biomarkers	**ADAM-17, pg/dl**	
	Correlation coefficient (r)	**P value**
IL-6, pg/dl	0.9	<0.001[S]

Figure 3 and 4 shows a comparison in the serum levels of ADAM-17 and IL-6 between the type 2 diabetic patients groups and the control group according to the BMI. The mean ± SD level of ADAM-17 and IL-6 was increased and a

statistically signifi1cant difference in type 2 diabetic patients as compared to control within the BMI ranges (normal weight), (overweight), and (obesity) respectively. Mean ± SD values of ADAM-17 and IL-6 in the BMI ranges mentioned above was (3130.30 ± 819.98, 3328.19 ± 888.57, 3274.62 ± 940.77), (30.07 ± 1.73, 30.30 ± 2.19, 29.82 ± 1.39 pg/ml) in patients group and mean value (1762.75 ± 382.20, 1629.53 ± 433.44, 1652.58 ± 41948), (22.92 ± 0.14, 23.66 ± 1.81, 23.08 ± 0.20) pg/dl in the control group respectively as shown in table 4.

Table 4: The effect of BMI on serum level of ADAM-17 and IL-6 as compared between Type 2 Diabetic Patients with or without Myocardial Infraction and the control group, BMI ranges were used :((normal weight), (overweight), and (obesity)

		Patient	N=60	Control N=30	N=30	P value
IL-6, pg/d1l	**Normal weight**	30.07±1.73	15	22.92±0.14	2	<0.001[S]
	Overweight	30.30±2.19	20	23.66±1.81	18	<0.001[S]
	Obesity	29.82±1.39	25	23.08±0.20	10	<0.001[S]
ADAM-17, pg/dl	**Normal weight**	3130.30±819.98	15	1762.75±382.20	2	0.038[S]
	Overweight	3328.19±888.57	20	1629.53±433.44	18	<0.001[S]
	Obesity	3274.62±940.77	25	1652.58±419.48	10	<0.001[S]
T-test was used *: significant at p ≤ 0.05 SD: standard deviation; S: significant; NS= Non-significant.						

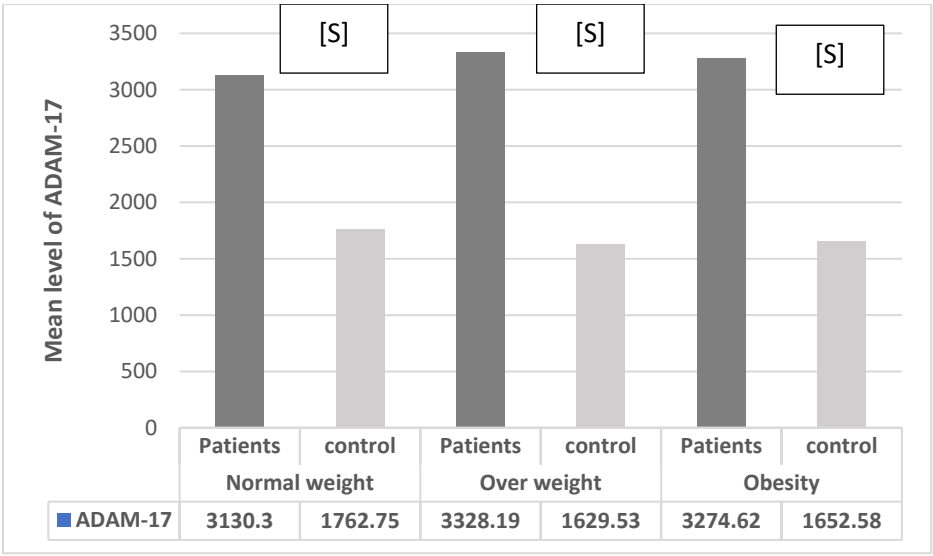

Figure 3: The effect of BMI on serum level of ADAM-17 as compared between T2DP with or without MI and the nondiabetic control group, BMI ranges were used :((normal weight), (overweight) and (obesity), (T-test was used, S= significant at p ≤ 0.05, NS= Nonsignificant).

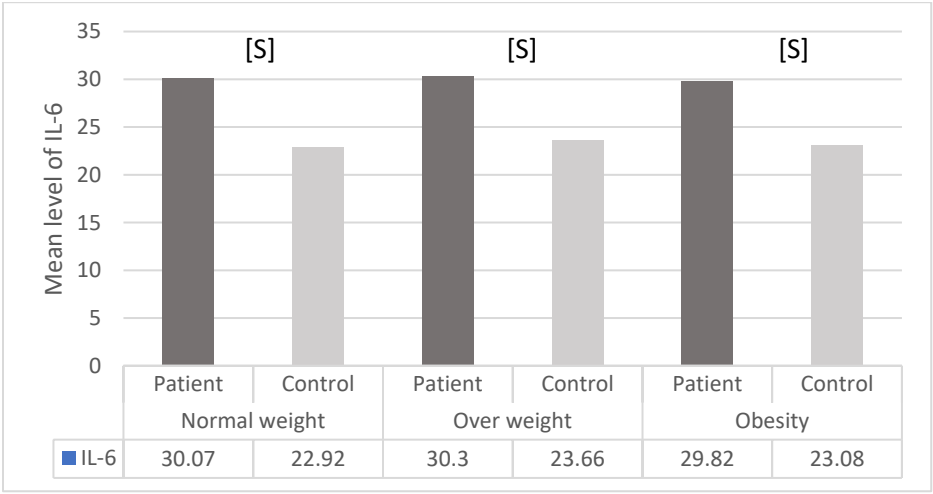

Figure 4: The effect of BMI on serum level of IL-6 as compared between T2DP with or without MI and the nondiabetic control group, BMI ranges were used :((normal weight), (overweight) and (obesity) (T-test was used, S= significant at $p \leq 0.05$, NS= Nonsignificant).

4. Discussion

Several studies explain the elevation of the biomarkers mentioned above for several reasons. ADAM-17 sheds more frequently when exposed to cellular activators such as lipopolysaccharides, which depend on reactive oxygen species (ROS) and the p38 mitogen-activated protein kinases (MAPK) pathway (Chemaly *et al.*, 2017). ADAM-17 exists as dimers on the cell surface and interacts with TIMP3, its inhibitor. ADAM-17 is released from TIMP3 metallopeptidase inhibitor three and changes from a dimeric to a monomeric structure when the ERK or p38 MAPK pathway is activated. The balance between ADAM-17 dimers and monomers can change with activation of the p38 mitogen-activated protein kinase pathway. This change is accompanied by increased ADAM-17 cell surface presentation and decreased TIMP3 interaction (Sikora *et al.*, 2023). Due to the cleavage and activation of several pro-inflamma1tory cytokines and their corresponding receptors, ADAM-17 has become a key regulatory hub in regulating inflammation. The most prominent examples are TNF, IL-6R, and tumor necrosis receptors 1 and 2 [(Ferencova *et al.*, 2023), (Finneran *et al.*, 2023)]. As membrane-bound proteins, these cytokines are abundantly expressed in the heart (Kunnathattil, Rahul and Skaria, 2024). Following the cleavage of ADAM-17, excessive elevations in their soluble forms can set off cascades that lead to acute cardiac inflammation, myocardial lipotoxicity, and poor energy generation (Defer *et al.*, 2007).

Increased plasma levels of IL-6 and TNF were found in patients with AMI in clinical studies by Latini *et al*. and Suzanne *et al*., suggesting that TNF and IL-6 maturation, which depends on ADAM-17, may activate systemic inflammation and contribute to plaque rupture [(Latini *et al.*, 1994), (Engelen *et al.*, 2022)].

It has been suggested that high glucose levels in people with type 2 diabetes may lead to protein misfolding and aggregation in the endoplasmic reticulum, which in turn results in a reduction in the ADAM-17 inhibitor TIMP3 and an increase in ADAM-17 activity (Lorenzon *et al.*, 2021).

An increase in IL-6 levels was observed in MI patients. This increase may be related to macrophages, the predominant cells in vascular atherosclerotic lesions, which abundantly produce and secrete IL-6, along with other cytokines, growth factors, and chemokines. Additionally, co-stimulating1 endothelial cells leads to the stimulation of protein kinase and thus the recruitment of many macrophages, leading to endothelial dysfunction (Souza *et al.*, 2008). IL-6 promotes many mechanisms that contribute to the inflammatory process of atherosclerosis (Zhang *et al.*, 2023). The buildup of atherosclerotic plaques in the coronary artery is one of the causes of MI (Iuchi, Harada and Tanaka, 2018). The development and advancement of plaque and the rupture of the fibrous cap, which would result in local thrombosis and hypoxia-related myocardial damage, are primarily influenced by vascular inflammation. (Xu, Yuan and Wang, 2023). IL-6 signaling is connected to the adverse effects of acute ischemia as well as the development and

destabilization of plaque. Therefore, the data obtained hypothesizes that IL-6 and MI etiology are related (Huang *et al.*, 2015).

The results showed a strong relationship and a significant correlation between IL-6 and ADAM-17 ($p = 0.001$, $r=0.9$) for T2DM without MI. ADAM-17, i1s responsible for sIL-6R shedding in humans (Mahmud-Al-Rafat, 2023). Based on what was previously mentioned about the participation of the two biomarkers in insulin resistance and vascular inflammation, evidence for that is treatment with the ADAM-17 inhibitor improves insulin sensitivity, corrects hyperglycemia, and inhibits vascular inflammation. As a result, ADAM-17 overactivity likely causes the balance of IL-6 signaling to shift toward trans-signaling, resulting in vascular inflammation and diabetes (Federici *et al.*, 2005). Another study highlighted that when ADAM-17 is activated within the white adipocytes, it leads to the expression of inflammatory molecules such as IL-6. This expression then leads to a low-grade inflammatory state that forces the macrophages to migrate into adipose tissue, mediating enhanced insulin resistance and T2DM (Menghini *et al.*, 2013).

The current study noted a significant increase in levels of ADAM17 and IL-6 in both groups under study, T2DM with or without MI, and the reasons for this increase in both are the same, but when compared between the two groups with the control, T2DM without MI showed the most significant rise in the two biomarker levels.

These data are consistent with other studies in which high glucose causes stimulation of ADAM17. This condition is chronic, so the higher the sugar, the more protein misfolding is generated in the endothelial cell, thus reducing the ADAM17 inhibitor and thus increasing the activity of ADAM17, which in turn increases the 1activity of IL-6 by ectodomain shedding, causing a decrease in the insulin receptor alpha subunit (IRα) in all three layers of the vascular wall (Lorenzon *et al.*, 2021).

As mentioned previously, activation of ADAM17 within white adipocytes causes the expression of interleukin 6 (IL-6). A low-grade inflamed condition is then brought on by this process, forcing macrophages to quickly move into inflamed adipose tissue, where they drive increasing insulin -resistance, and T2DM.

5. Conclusion

ADAM 17 and its substrate IL-6 hurt chronic inflammation, as in T2DM, which is more severe than acute inflammation in AMI. This result was supported by what was shown of a higher ADAM 17
and IL-6 level in type 2 diabetic patients without myocardial infarction than in type 2 diabetic patients with MI.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

ORAI1 Genetic Polymorphism and its Correlation with Erythropoietin Resistance, Hemoglobin, and Erythropoietin Serum Level in Iraqi Patients with Chronic Renal Failure on Hemodialysis

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Received: 2023/11/12

Accepted: 2023/11/19

Published: 2024/01/04

Keywords: chronic renal failure, ORAI1, genetic polymorphism, hemoglobin, erythropoietin resistance, hemodialysis.

DOI:10.62472/kjps.v14.i23.74-83

Abstract

Introduction: Chronic renal failure CRF is a widespread, irreversible disease that develops gradually and is classified into five stages depending on the glomerular filtration rate GFR. Anemia is a considerable complication of CRF that becomes serious as GFR decreases, erythropoietin-stimulating agent ESA is used for treating anemia, but the resistance to it stands against its goal in many patients. Studying the genetic polymorphism in one of the erythropoietin-activated pathway store-operated calcium channels SOCs gene is a trial for investigating the cause of resistance.

Method: In this study, 174 subjects were included. Among 112 hemodialysis patients with CRF, 54.5% were non-responders, and the enrolled participants ranged from 20 to 79 years; a single nucleotide polymorphism in the ORAI1 gene was selected. Genotyping was done using an allele-specific polymerase chain reaction technique, and the data was analyzed through the Statistical Package for the Social Sciences.

Results: The results showed that hemoglobin levels differ significantly between the genetic groups, while erythropoietin levels showed no significant variation.

Conclusion: ORAI1 genetic polymorphism has no significant role in erythropoietin resistance in Iraqi hemodialysis patients with CRF, but this genetic variation significantly affects hemoglobin levels, depending on this study.

تأثير تعدد الأشكال الجيني لجينَيORAI1 وSTIM1 على مقاومة الارثروبويتين في المرضى العراقيين الذين يعانون من الفشل الكلوي المزمن و المعتمدين على الديلزة الدموية

الخلاصة

المقدمة: يعد الفشل الكلوي المزمن مرض تقدمي يتطور تدريجياً ويتم تصنيفه إلى خمس مراحل بناءً على معدل الترشيح الكبيبي، فقر الدم هو مضاعفة كبيرة للفشل الكلوي المزمن وتزداد خطورته مع انخفاض معدل الترشيح الكبيبي . تستخدم العوامل المنشطة للإريثروبويتين لعلاج فقر الدم ولكن المقاومة لها تعيق فعاليتها لدى العديد من المرضى. تُعد دراسة تعدد الأشكال الجينية في احد جينات قنوات الكالسيوم المعتمدة على المخازن وهي إحدى مسارات تنشيط الإريثروبويتين محاولة لاستكشاف سبب المقاومة.

الطرق: في هذه الدراسة، تم ضم ١٧٤مشاركًا من بينهم ١١٢مريضًا بغسيل الكلى يعانون من الفشل الكلوي المزمن، وكان ٥٤,٥٪منهم غير مستجيبين، وتراوح عمر المشاركين المسجلين بين ٧٩-٢٠عامًا، تم اختيار تعدد الشكل الجيني المفرد في جين ، ORAI1 تم إجراء تحديد النمط الجيني باستخدام تقنية تفاعل البوليميراز المتسلسل النوعي للأليل وتم تحليل البيانات من خلال الحزمة الإحصائية للعلوم الاجتماعية.

النتائج: أظهرت النتائج اختلافًا ملحوظًا في مستويات الهيموجلوبين بين المجموعات الجينية، بينما لم يظهر أي تباين ملحوظ في مستويات الإريثروبويتين.

الإستنتاج : أوضحت هذه الدراسة أن التعدد الشكلي الجيني في جينORAI1 لا يؤثر بشكل كبير على مقاومة الإريثروبويتين لدى مرضى غسيل الكلى العراقيين المصابين بالفشل الكلوي المزمن ، ولكن هذا التباين الجيني له تأثير ملحوظ على مستويات الهيموجلوبين.

1. Introduction

Chronic renal failure CRF is a widespread, irreversible disease that develops gradually and is classified into five stages depending on the glomerular filtration rate GFR. (Ammirati, 2020) one of the complications of CRF is anemia, which contributes to poor clinical outcomes and Increased mortality rate, and as the GFR decreased, anemia became serious (Shaikh, Hashmi and Aeddula, 2019). The most common types of anemia in CRF are Normocytic, normochromic, and hypo-proliferative (Rosita *et al.*, 2021).

As the renal failure progresses, the kidney mass will reduce, and so will the Erythropoietin. Production will decrease because the kidney is the only source of this hormone in adults. (Hayat, Haria and Salifu, 2008) The Food and Drug Administration (FDA) approved the use of the erythropoietin-stimulating agent ESA in 1989 (Wish, 2021). Still, the resistance to ESA stands against treating anemia in many patients (Hanna, Streja and Kalantar-Zadeh, 2021), which can be caused by Iron deficiency, hyperparathyroidism, inadequate dialysis, malnutrition, and chronic inflammation. (Alves *et al.*, 2015) Even when these causes are treated, some patients still exhibit erythropoietin resistance, which needs more explanation.

In 2003, a Retrospective observational study in London was done to investigate the impact of angiotensin-converting enzyme (ACE) polymorphisms on erythropoietin (EPO) requirement and found that patients with the II genotype require higher doses of ESA. (Varagunam *et al.*, 2003) A study in China to predict the association of genetic polymorphism in the DDAH2 gene and erythropoietin resistance in patients on maintenance hemodialysis suggests that complex genetic variations may influence erythropoietin resistance in those patients. (Wang *et al.*, 2017) An observational cross-sectional study done in Saudi Arabia showed that genetic polymorphisms in ACE may affect the response of hemodialysis patients to ESA. (Hamdan Almaeen and Mostafa-Hedeab, 2021) ORAI1 gene was selected for this study because it is one of the store-operated calcium channels (SOCs) genes, as SOCs have a novel role in erythropoiesis (Lee *et al.*, 2019).

Aim of the study: this study was designated to find the impact of ORAI1 genetic polymorphism on erythropoietin resistance, hemoglobin, and erythropoietin serum levels in patients with CRF on hemodialysis in Iraq.

2. Material and Method

2.1 Patients' Constant and Enrollment

This cross-sectional observational study was performed from 11- 2022 to 4- 2023 in Karbala at Imam Al-Hussain Medical City/ Doctor Adel Al Sabbah Center for Hemodialysis. The study protocol was licensed by the Scientific and Ethical Committee of Pharmacy College / Kerbela University; after explaining the nature and purpose of the study, each subject signed an informed consent form.

One hundred seventy-four subjects were included in the study, 112 patients ranging from 20-79 years of age with CRF treated with ESA at the recommended weekly dose and being on hemodialysis and treatment for more than four months, 62 healthy subjects were enrolled as a reference for biochemical tests. Clinical and demographic data were obtained from the subjects with blood samples at enrollment.

These data included gender, age, weight, medical history, family history, and biochemistry data.

2.2 Molecular Analysis

Genomic DNA was extracted from a blood sample as stated by the protocol of gSYNC for blood genomic DNA extraction kit. DNA concentration and purity were measured using a nano-spectrophotometer nanodrop. The DNA purity was measured at the A260/A280 ratio.

The single nucleotide polymorphism (SNP) rs6486795 of the ORAI1 gene was selected, and Prof DR. Hassan Mahmood Musa designed the primers for rs6486795 Snv allele T>A

Allele-specific PCR technique was used to detect the SNP after several trials of PCR to The optimization of PCR was performed to obtain the best concentration of primers and best annealing temperature. Bioneer PCR Premix was used.

2.3 Statistical Analysis

The present study's data was entered and analyzed through the Statistical Package for the Social Sciences (SPSS version 22). The data were presented as frequencies, percentages, mean and standard deviation in appropriate tables and graphs, or mean differences in others. Chi-square test, one-way ANOVA test, and post hoc analysis were used where applicable to find out the possible association between the related variables of the current study, as LSD was used when equal variances were assumed. In contrast, Dunnett's T3 was used when equal variances were not considered depending on Levene's test for homogeneity of variances. Besides, Hardy Weinberg equilibrium was used to detect the prediction of alleles distribution. The statistical association was considered significant when the p-value was equal to or less than 0.05 (P value ≤ 0.05).

3. Results

One hundred twelve patients were included (66 male and 46 female), 51 responders and 61 non-responders; table 1 shows Descriptive statistics for continuous variables of the study, the data presented by means.

Table 1: Descriptive Statistics for Continuous Variables

Variable	Min.	Max.	Mean	Std. Deviation
Age (Year)	22.00	79.00	50.9464	13.42033
Weight (Kg)	33.00	130.00	66.2768	15.74127
Duration of disease (Months)	4.00	180.00	41.8661	41.18525
Duration of dialysis (Months)	4.00	180.00	30.9643	29.17976
Duration of treatment (Months)	4.00	156.00	32.8661	30.10836
Epo mlU/ml	2.49	29.80	13.8574	4.46875
Hb g/dl	6.10	13.10	9.6125	1.79835
BU mg/dl	37.00	214.00	115.9865	34.36958
S. Cr mg/dl	3.50	15.00	7.7296	2.09955

*** [Epo] erythropoietin serum level, [Hb] hemoglobin level, [BU] blood urea, [S. Cr] serum creatinine, [Min] minimum, [Max] maximum, [Std] standard.**

The distribution of ORAI1 gene polymorphism (rs6486795) in different genotypes in this study showed that the TT genotype and TC genotype were almost similar in frequencies, 39.3% and 38.4%, respectively, and the CC genotype was the lesser 22.3% in the 112 enrolled patients as illustrated in fig1 presented by numbers.

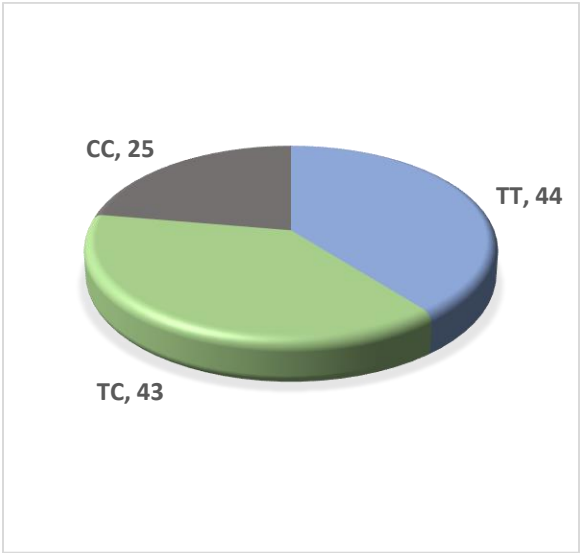

Figure1: Distribution of ORA1 Gene Polymorphism rs6486795 Different Genotypes in Patients

Depending on this study there was no association between gender and genetic variation as shown in Table 2 and no association between the independent variable and response as shown in Table.3

Table 2: Association Between Gender and Genetic Variation

Demographic parameters		Gender	
		Male	Female
Patient Genotype (N=112)	TT N (44)	24	20
	TC N (43)	28	15
	CC N (25)	14	11
P value		0.572	

Hardy Weinberg equilibrium test was done to show the expected frequency of genotype groups, and the expected predominant group will be the hetero TC group based on this study as shown in Fig. 2 which is statistically significant, p-value <0.05.

Table 3: Association Between Independent Variables and Response

Variable		**Responder (No.)**	**Non-Responders (No.)**	**P-value**
Age (Year)	20-39	15	10	0.440 NS
	40-59	29	21	
	60-79	17	20	
Duration of Rx (Months)	4-50	51	39	0.617 NS
	51-90	7	9	
	91-156	3	3	
Duration of Dialysis (Months)	4-60	58	44	0.217 NS
	61-120	2	6	
	121-180	1	1	

[NS]= Non significant

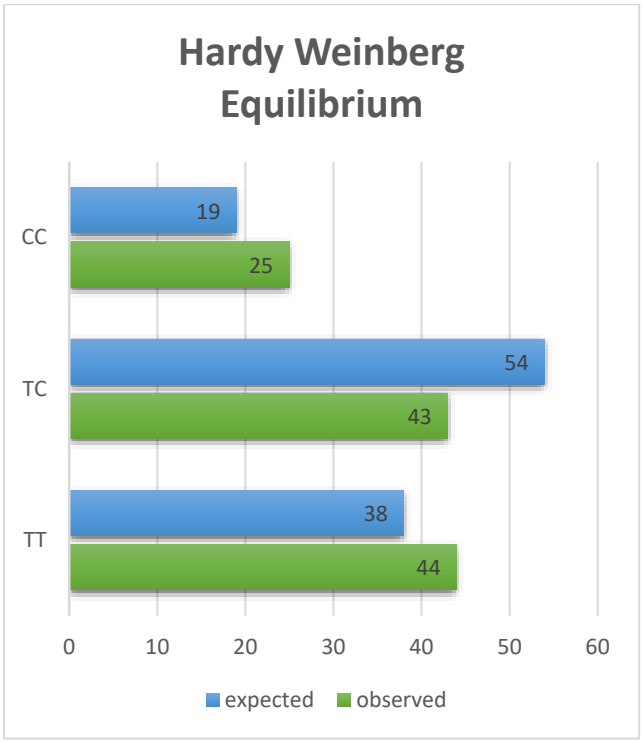

Figure 2: Hardy–Weinberg Equilibrium for ORAI1 Gene (rs6486795) in Patients

Biochemical parameters between genetic groups of patients are summarized in Table.4 presented as mean ± SD showed CC group has a statistically significant rise over TT group in hemoglobin level and also in blood urea, while the control group has a statistically significant difference in hemoglobin level (rise) and serum creatinine (decrease) compared with the genetic groups (p-value <0.05).

Table 4: Mean ± SD of Biochemical Parameters Between Groups of ORAI1 Gene (rs6486795)

Parameters	Groups								P-value
	Control		TT		TC		CC		
	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD		
Epo mlU/ml	13.04	3.10	13.52	4.07	14.30	4.70	14.15	4.77	0.362 NS
Hb g/dl	13.48	1.03	9.13	1,69	9.74	1.83	10.23	1.73	0.001 S 0.005 S
BU mg/dl	25.48	7.79	112.97	34.15	112.74	33.63	126.84	35.15	0.001 S CC
S. Cr mg/dl	0.86	0.16	7.81	2.21	7.78	2.22	7.48	1.69	0.01 S control

[Epo] erythropoietin serum level, [Hb] hemoglobin level, [BU] blood urea, [S. Cr] serum creatinine, [S]= Significant, [NS]= Non significant

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%. (Hwang *et al.*, 2014)

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 have 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. (Kao *et al.*, 2021)

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.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Worse Impact of COVID-19 in Pregnant Women

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Received: 2023/10/02

Accepted: 2023/12/03

Published: 2024/01/04

Keywords: COVID-19, Pregnancy, Infant

DOI:10.62472/kjps.v14.i23.84-91

Abstract

The COVID-19 pandemic has raised concerns about the impact of the virus on pregnant women and their unborn babies. This review aims to provide a comprehensive summary of the outcome of COVID-19 in pregnancy

Studies have shown that the possibility of developing severe illness increased in pregnant women compared with non-pregnant women infected with COVID-19. They have higher rates of ICU admission, mechanical ventilation, and mortality. Additionally, the risk of preterm birth has been increased in pregnant women infected with COVID-19, with several research reporting higher rates of preterm delivery among infected pregnant women.

Cesarean section delivery rates are also higher among pregnant women with COVID-19, possibly due to the need for expedited delivery in cases of severe illness or fetal distress. However, the transmission of the virus from mother to fetus is rare during pregnancy or delivery. The virus is transmitted vertically from mother to fetus, with most infants born to mothers with COVID-19 testing negative for the virus.

While vertical transmission is rare, there is evidence suggesting a potential impact of COVID-19 on fetal development. Some studies have reported cases of fetal growth restriction and abnormal placental findings in pregnant women with COVID-19. However, further research is needed to fully understand the long-term consequences.

Abbreviations: WHO: World Health Organization, SARS: Severe Acute Respiratory Syndrome, MERS: Middle Eastern Respiratory Syndrome, FIGO: International Federation of Gynecology and Obstetrics, ACE2: angiotensin-converting enzyme 2, TMPRSS2: primes the S protein using transmembrane serine protease 2, NK: natural killer, PDCs: plasmacytoid dendritic cells, TLRs: Toll-like receptors, DIC: disseminated vascular coagulopathy, CMV: Cytomegalovirus, HSV: herpes simplex virus, VZV: varicella-zoster virus, ZIKV: zika virus.

Conflict of Interest

The author declared that they have no conflict of interest.

تأثير فيروس كوفيد-١٩ على النساء الحوامل

سارة نجم عابد، وفاء كاظم جاسم، أمال عمران موسى

الملخص

أثارت جائحة كوفيد-١٩ مخاوف حول تأثير الفيروس على النساء الحوامل واجنتهم الذين لم يولدوا بعد. يهدف هذا المراجعة إلى تقديم ملخص شامل لنتائج كوفيد-١٩ في الحمل.

أظهرت الدراسات أن النساء الحوامل المصابات بكوفيد-١٩ يتعرضن لمخاطر أكبر للإصابة بالمرض مقارنةً بالنساء غير الحوامل. لديهن معدلات أعلى للإدخال إلى وحدة العناية المركزة واستخدام اجهزة التنفس ومعدلات الوفيات. بالإضافة إلى ذلك، ارتبطت إصابة كوفيد-١٩ أثناء الحمل بزيادة خطر الولادة المبكرة، حيث أبلغت العديد من الدراسات عن معدلات أعلى للولادة المبكرة بين النساء الحوامل المصابات.

تعد معدلات الولادة القيصرية أيضًا أعلى بين النساء الحوامل المصابات بكوفيد-١٩، ربما بسبب الحاجة إلى إجراء ولادة سريعة في حالات المرض الشديد أو الضيق الجنيني. ومع ذلك، فإن انتقال الفيروس عموديًا من الأم إلى الجنين خلال الحمل أو الولادة نادر، حيث يظهر معظم الأطفال الذين يولدون من أمهات مصابات بكوفيد-١٩ سلبية الاختبار للفيروس.

على الرغم من ندرة انتقال الفيروس عموديًا، هناك أدلة تشير إلى وجود تأثير محتمل لكوفيد-١٩ على تطور الجنين. أفادت بعض الدراسات بحالات تقزم الجنين واضطرابات غير طبيعية في المشيمة لدى النساء الحوامل المصابات بكوفيد-١٩. ومع ذلك، يتطلب الأمر إجراء المزيد من البحوث لفهم العواقب على المدى الطويل بشكل كامل.

في الختام، تشكل إصابة كوفيد-١٩ أثناء الحمل مخاطر كبيرة، بما في ذلك المرض الشديد والولادة المبكرة وزيادة معدلات الولادة القيصرية. على الرغم من ندرة انتقال الفيروس عموديًا، هناك أدلة على وجود تأثير محتمل على تطور الجنين. يتطلب استمرار البحث والمراقبة لفهم تأثير الفيروس.

1. Introduction

The World Health Organization reported that coronavirus was responsible for the coronavirus illness 2019 (COVID-19) in December 2019, which has spread rapidly around the world, and causes severe acute respiratory syndrome coronaviruse2 (SARS-Cov-2). The WHO proclaimed the outbreak to be a pandemic on March 12, 2020. As the COVID-19 pandemic spread, pregnant women were identified as a vulnerable group and advised to take extra precautions. (Lai *et al.*, 2020) Pregnant women are more likely to experience complications and serious illness when infected with other coronaviruses, such as SARS and MERS. The (FIGO) advised eliminating much regular antenatal care and whenever possible, substituting video or phone consultations to reduce the risk of transmission to both pregnant patients and medical workers. (Chen *et al.*, 2020)

1. Pregnancy-Related Physiological Changes and Their Impact on COVID-19

1.1 Biochemical Mechanism

COVID-19 is caused by an RNA virus with a capsule–like capsule–like structure. Like other viral infections, the immune system's ability to fight COVID-19 is reliant on resilience.

A COVID-19 infection can cause a major sickness with a high risk of mortality or a minor illness that is successfully cured by the immune system. Unknown is where exactly pregnant women fall on this spectrum. (Wu and McGoogan, 2020) Pregnancy-related immune responses to infections shift as a result of the immune system's adaptation to the growing fetus. Understanding COVID-19 pathophysiology and molecular mechanisms and researching them in the context of the altered maternal immune response is crucial to understanding the COVID-19 phenotype throughout pregnancy. SARS-CoV-2 enters the body through the nasal cavity, infects pulmonary cells utilizing the angiotensin-converting enzyme 2 (ACE2) and the SARS-CoV receptor, and primes the S protein with transmembrane serine protease 2 (TMPRSS2). It spreads via close human contact, direct contact with insects, and respiratory droplets, and may be produced aerosols. (Hoffmann *et al.*, 2020)

Pregnancy-related changes to the immune system may influence how the body reacts to infections, particularly viruses. According to the alternative idea, a shift in the thCD+4 T cell population toward the Th2 phenotype rather than the Th1 phenotype – a response that prioritizes humoral over cellular immunological responses is responsible for at least some of the inflammatory response to viruses during pregnancy. (Piccinni and Romagnani, 1996) The body's natural killer (NK) cell count declines during pregnancy. Given that viruses are necessary for this process, it may make it more difficult for the innate immune system to get rid of viruses. (Brandstadter and Yang, 2011) A decline in the quantity of plasmacytoid dendritic cells (pDCs), which are essential for producing type 1 interferon, a virus-fighting protein, in the blood. The steroid hormone progesterone has the power to affect the immune system. ((Reizis, 2019), (Druckmann and Druckmann, 2005)) High progesterone levels during pregnancy may help with recovering from viral lung infections because they can enhance lung health. The pattern-recognition Toll-like receptors (TLRs), in particular, are altered by pregnancy in the innate immune system. (Amirchaghmaghi *et al.*, 2013)

1.2. A breathing Reaction

In addition to the systemic immunological changes brought on by pregnancy, the respiratory system exhibits structural changes that may impair lung function. The gravid uterus causes physiological alterations in the diaphragmatic splinting curvature, which affects respiratory function. Despite an increase in the tidal volume of 30-40%, end–expiratory volumes, early pregnancy residual volumes, and functional residual capacity are all decreased by the chest volume reduction. Because their ability to expel secretion is more difficult and their lung capacity is decreased overall, pregnant women may be more vulnerable to severe respiratory infections (Rajewska *et al.*, 2020)

1.3 Inflammation and Thrombosis

According to research including 184 critically sick patients (24% female), COVID-19 is linked to greater risks of thromboembolic events in the general population. 31 percent of them experienced thrombotic events. This is a result of active coagulation pathways, which can eventually lead to diffused vascular coagulopathy (DIC) and fibrinolysis, both of which result in dynamic hypercoagulation and thrombocytopenia. (Rajewska *et al.*, 2020) Excessive bleeding occurs during pregnancy. A deadly thromboembolic event is more likely to occur in pregnant women. Therefore, thrombosis risk factors for pregnant women who carry the COVID-19 virus may be combined or synergistic. A case report of a woman with COVID-19 dying at 29 weeks gestation as a result of a major basilar artery embolism and pulmonary embolism lends support to this theory. (Di Renzo and Giardina, 2020)

2. Placental Responses to SARS-CoV-2

The placenta typically serves as a robust barrier to stop the vertical transmission of maternal diseases to the fetus. It is common knowledge that some diseases can pass through this barrier and cause serious harm to the growing embryo. Each of the syndrome-causing congenital viruses (ZIKV), (VZV), (HSV), and (CMV)—has a distinct rate of transmission and degree of severity that changes depending on when an infection first appears during pregnancy. (Burton and Jauniaux, 2015)

The chorionic villi in the human placenta is in direct contact with the mother's blood because the human placenta is hemochorial. Specifically designed cells called trophoblasts, which are formed from fetal tissue, make up the majority of the placenta. Terminally developed, multinuclear syncytiotrophoblast cells. Syncytiotrophoblasts are produced by progenitor villous cytotrophoblast cells. Extravillous trophoblast cells that enter the uterus and alter its vasculature link the chorionic villi to it. (Brett *et al.*, 2014)

Several case reports have looked into the placentas of women infected with the COVID-19 virus. SARS-CoV-2 expression has been found in mid-trimester placenta samples, but it is unclear whether this was due to a primary infection or because placental damage from other illnesses made the virus more contagious. SARS-CoV-2, which was identified on RT-PCR of swabs and biopsies following spontaneous fetal mortality at 19 weeks of gestation. In samples of the placenta and umbilical cord obtained after a pregnancy that was aborted at 22 weeks gestation, SARS-CoV-2 was also shown to be strongly expressed. (Whittaker *et al.*, 2020)

Severe maternal hypertension, thrombocytopenia, Placental abruption, and coagulopathy all contributed to the pregnancy's termination. Virus-like particles appeared to be present in the cytoplasm of the placental cells. Placental histology revealed macrophage infiltrates and fibrin deposits, which the authors believed were most likely related to a viral infection. ((Vivanti *et al.*, 2020), (Baud *et al.*, 2020))

3. SARS-CoV-2 Vertical Transmission

It's not always the case that diseases or injury to the fetus result from viral infections of the placental cells. The findings of infant SARSCoV2 tests have been reported in 15 studies thus far, with positive results being infrequent. Even in the presence of SARS-CoV2, severe newborn respiratory infections seem to be uncommon. Based on PCR results from SARSCOV-2 testing it is unclear if the infection occur in utero, during labor or delivery, or whether transmission occur from the infected mother or asymptomatic hospital staff in the first days following birth. (Ferrazzi *et al.*, 2020), (Khan *et al.*, 2020)).

However, additional proof that vertical transmission might be a possibility has been presented through antibody testing. Some infants delivered to moms who have COVID-19 have greater IgM and IgG levels for SARS-CoV-2. IgM, which has a higher molecular weight, cannot passively transmit from mother to fetus during pregnancy. Even though at birth, all of the infants in reports so far have been asymptomatic and tested negative for SARS-CoV-2 viral RNA. The finding of circulating SARS-CoV-2 IgM in the newborn indicates vertical transmission of the virus. We still don't know how viruses get into the placenta. (Zeng *et al.*, 2020)

When SARS-CoV-2 enters lung cells via the ACE2 receptor, the serine protease TMPRSS2 is thought to be responsible for cleaving the spike glycoprotein to allow fusion. To ascertain whether ACE2 +/TMPRSS2 expression is found in placental cells, single-cell RNA sequencing data analysis was incorporated into three experiments. (Choudhary *et al.*, 2021) Combined data from an earlier investigation employing samples of two-term placentas with secondary analysis of single-cell transcriptase profiles from decidua and placenta samples at 6–12 weeks of gestation that were openly accessible. The ACE2 gene was discovered to be expressed in decidua stromal, perivascular, cell of syncytiotrophoblast and villous cytotrophoblast types were seen in samples taken during the first trimester and term however the expression of ACE2 was commonly shown to be very low in placental and decide cell types in a 2nd investigation that used the same first trimester date set. ((M. Li *et al.*, 2020), (Pique-Regi *et al.*, 2020))

SARS-CoV-2 most likely accesses the tissues of the placenta through a separate pathway because ACE2 and TMPRSS2 are not expressed in the placenta. Other proteases have been linked to this as well. The placenta's high levels of DPP4 and CD147 expression during gestation may have a role in cell entrance. (Jaimes, Millet and Whittaker, 2020). To stop cell invasion, tranexamic acid is a possible therapeutic target for plasmin, which may also leave this site. RNA from the severe acute respiratory syndrome coronavirus2 was found in a sample of amniotic fluid in circumstances where there have been reports of significant maternal infections, even though neonatal positive following birth has been infrequent. (Zamaniyan *et al.*, 2020)

4. The Effect of COVID-19 on Pregnancy

4.1. Early Pregnancy and SARS-CoV-2

There is little information about COVID-19's potential effects in the early stages of pregnancy (up to 12 weeks gestation). Pregnant women who had previously contracted SARS-CoV-2 did not vary from those who had not in terms of the thickness of the nuchal translucency at the first-trimester scan. Additionally, miscarriage rates among women who received SARS-CoV-2 in the first trimester have not increased. (la Cour Freiesleben *et al.*, 2021)

4.2. SARS-CoV-2 and Late Pregnancy

Based on the impact of other viruses, COVID-19 infection in late pregnancy (more than 24 weeks gestation) is likely to result in the following symptoms. The less favorable pregnancy outcomes include reduced fetal growth, premature birth, and perinatal mortality. (Dorélien, 2019) Pregnant women do not have a larger risk of developing severe COVID-19 than the general population, according to the bulk of studies done so far. The requirement for artificial respiration and the chance of hospitalization were both markedly raised by pregnancy. (Govind *et al.*, 2020) Because hospital and critical care unit admission and breathing requirements are not defined, it is difficult to apply the research's findings to a larger population. Given that there was no rise in mortality, it is also possible that these results are a reflection of the healthcare system rather than the clinical condition of the women. (Blitz *et al.*, 2020) The same risk factors that affect the general population apply to COVID-19 disease, such as being overweight or obese, having concomitant conditions like diabetes, asthma, or hypertension, and belonging to a racial or ethnic minority. (Knight *et al.*, 2021)

4.3. Postpartum and SARS-CoV-2

4.3.1 Newborns' Results

Neonates born to SARS-CoV2-positive women have not shown any substantial negative effects in the great majority of research that reports on neonatal outcomes. There were differences in the frequency of poor neonatal outcomes among pregnant women with established COVID-19 disease and sick pregnant women not infected with SARA-CoV-2 only three investigations found incidences of SARSCoV-2 positivity after 13 research studies screened newborns for the virus. Newborn SARS-CoV-2 carriers frequently had minimal or transitory symptoms. Three studies found evidence of newborn fatalities. Two of them still had no identified cause. (N. Li *et al.*, 2020) However, no study that revealed higher-than-average preterm birth rates included a denominator group for comparison. Various variables contributed to the premature birth, all of which were iatrogenic due to the mother's deteriorating health. Even though the origin of the COVID-19 pandemic is uncertain, Denmark and Ireland's observational results show that level rates of population preterm birth have dramatically decreased over this time. It is not yet known if COVID-19 infection acts as a sole risk factor for preterm birth. ((Ferrazzi *et al.*, 2020), (Marín Gabriel *et al.*, 2020))

4.3.2. Breastfeeding

SARS-CoV-2 was discovered in breast milk four times in one case investigation. Nine mothers' breast milk samples were evaluated in a different trial, but none of the samples tested positive for the SARS-CoV-2 virus. Current recommendations urge moms to nurse their babies during the postpartum period and even if they test positive during childbirth. (Groß *et al.*, 2020)

Women who have COVID-19 should regularly wash their hands and follow basic hygiene precautions, such as using a surgical mask during feeding if one is available. The advantages of nursing may outweigh any potential transmission risk because newborn diseases are frequently mild and asymptomatic. (Vassilopoulou *et al.*, 2021)

5. Conclusion

It is difficult to say for sure whether pregnant women are more prone to have severe COVID-19 effects based on the available evidence. Most women will only experience mild, transient, or asymptomatic sickness. It seems remarkable that most babies appear to be unaffected by vertical transmission. There are still many questions that need to be answered, including whether COVID-19 is a risk factor for preterm birth on its own, whether infection during pregnancy is almost certain to have long-term negative effects on the fetus, and whether these effects vary depending on gestational age at the time of infection.COVID-19 infection during pregnancy poses significant risks, including severe illness, preterm birth, and increased cesarean section rates. Although vertical transmission is rare, there is a need for continued monitoring and research to better understand the impact of the virus on fetal development and long-term outcomes.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Evaluation of Lipid Profile in Patients with Ischemic Heart Disease

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Received: 2023/11/13

Accepted: 2023/12/03

Published: 2024/01/04

Keywords: *Triglycerides, HDL-c, ischemic heart disease, coronary artery disease, atherogenesis, dyslipidemia, risk prediction, personalized medicine*

DOI:10.62472/kjps.v14.i23.92-105

Abstract

Ischemic heart diseases (IHDs), like coronary artery disease, remain a significant global health burden. While established risk factors such as low-density lipoprotein cholesterol (LDL) and hypertension are well-recognized, the role of triglyceride (T.G.) and high-density lipoprotein cholesterol (HDL-c) remains complex and multifaceted. This work was designed to examine the role of the T.G./HDL-c ratio in the IHD group and their effect on the effect of age, gender, and biomass index (BMI).

Method: A total of 90 samples, including 60 samples (30 male, 30 female) for the IHD patients group with an age range of 40-85 years, and 30 samples (15 male, 15 female) for the control group with matching age ranges, were randomly selected. Lipid profile parameters (HDL, LDL, VLDL, T.G., Cholesterol) were estimated according to Kit Shenzhen Mindray Bio—Medical Electronics—Germany's manual procedure. Then, the ratio TG/HDL-C was calculated.

Results: IHD patients illustrated a significant increase in the mean levels of all lipid profile panels, including [total cholesterol (T.C.), triglyceride (T.G.), LDL, and the ratio of T.G./HDL-c], except HDL, which was lower in patients group compared to the healthy control. The level of T.G./HDL-c was increased in IHD patients with increasing BMI associated with the healthy participants; it was also elevated in male IHD patients than in IHD female patients.

Conclusion: The ratio of T.G./HDL-c represents a valuable indicator for valuing IHD risk and personalized therapeutic approaches. Future research should delve deeper into the underlying mechanisms and refine strategies for effectively managing T.G./HDL-c for improved IHD prevention and management.

Key points:•Elevated T.G./HDL-c ratio, rather than individual T.G. or HDL-c levels alone, emerges as a stronger predictor of IHD risk. This ratio reflects increased atherogenic lipoprotein burden (high T.G.) and impaired cholesterol efflux capacity (low HDL-c).

•Mechanisms underlying the adverse effects of high T.G./HDL-c involve:

•Increased inflammation and oxidative stress

•Remnant lipoprotein formation and plaque instability

•Impaired endothelial function and vascular relaxation

•Increased postprandial lipemia and triglyceride-rich lipoprotein accumulation

•Heterogeneity exists within both T.G. and HDL-c, further influencing IHD risk. Particle size and apolipoprotein composition of these lipoproteins play crucial roles.

•Therapeutic strategies targeting T.G./HDL-c should consider diverse mechanisms and individual patient profiles. Lifestyle modifications, lipid-lowering medications, and novel HDL-raising therapies hold potential.

تقدير مستويات الدهون في مرضى نقص تروية القلب

الملخص

ان أمراض القلب الإقفارية(IHDs) ، مثل مرض الشريان التاجي، عبئًا عالميًا كبيرًا على الصحة. في حين أن عوامل الخطر المعروفة مثل الكوليسترول الدهني منخفض الكثافة (LDL) وارتفاع ضغط الدم معروفة جيدًا، فإن دور الدهون الثلاثية (TG) والكوليسترول البروتيني عالي الكثافة (HDL-c) لا يزال معقدًا ومتعدد الأوجه. صمم هذا العمل لفحص دور نسبة TG/HDL-c في مجموعة IHD وتأثرها بالعمر والجنس ومؤشر كتلة الجسم .(BMI) شملت الدراسة ٩٠ عينة ٦٠: عينة(٣٠رجال,٣٠ نساء) كمجموعة مرضى IHD تتراوح أعمارهم بين ٤٠ و ٨٥ عامًا، و ٣٠ عينة (١٥ رجال ,١٥ نساء) تم اختيارها عشوائيًا كمجموعة تحكم بنفس الفئة العمرية. تم تقدير معايير ملف الدهونات(HDL ، LDL، VLDL، TG، الكوليسترول(وفقًا للإجراء اليدوي لمجموعة .Shenzhen Mindray Bio- Medical Electronics- Germany ثم تم حساب نسبة, .TG/HDL-C أظهر مرضى IHDزيادة ملحوظة في متوسط مستويات جميع لوحة ملف الدهونات بما في ذلك]الكوليسترول الكلي(TC) ، الدهون الثلاثية(TG) ، LDLونسبة] TG/HDL-cباستثناء HDL الذي تبين أنه أقل في مجموعة المرضى مقارنة بالمجموعة الضابطة الصحية. زادت نسبة TG/HDL-c في مجموعة IHDمع زيادة مؤشر كتلة الجسم مقارنة بالمجموعة الضابطة الصحية، كما زادت أيضًا لدى الذكور من مرضى IHD مقارنة بمرضى IHD الإناث. نستنتج من ذلك ان نسبة TG/HDL-c علامة قيمة لتقييم مخاطر IHD والنهج العلاجية الشخصية. ينبغي أن تتعمق الأبحاث المستقبلية في الآليات الأساسية وصقل الاستراتيجيات لإدارة TG/HDL-c بشكل فعال لتحسين الوقاية من IHD وإدارتها .

1. Introduction

Cardiovascular disease is the chief cause of death worldwide, and ischemic heart disease is the top one. However, risk factors for Ischemic heart diseases are adaptable and avoidable; it is not well explored in the local context. (Brodmann *et al.*, 2020) . The most significant risk factors were adaptable and avoidable, among which nutritional and interactive risk factors were central (Organization, 2009). Lipids and lipoproteins have a crucial influence on the origination and development of Cardiac Vascular Disease (CVD) by revenues of their cellular synthesis, transportation, assemblage, degradation, oxidation, and plasma concentrations (Yu *et al.*, 2019). Many studies confirmed the associations of lipid profiles with the risk of significant adverse cardiovascular outcomes in patients with heart disease (Zhao, Wang and Qin, 2021)

The blood cholesterol level was well-known as the first straight relation between flowing lipids and cardiac diseases. Amplified cholesterol levels are linked with an enlarged 10-year risk of cardiovascular death from 3.8% to nearly 19.6% in men with a pre-existing cardiac disease. Patients with atherosclerotic plaques show a relationship with 45% higher plasma oxidized LDL concentrations as matched with control participants. Additionally, patients with elevated total LDL have a 3.7 times higher risk of coronary artery calcification than those with lower LDL (Prado, Shugg and Backstrand, 2011). Epidemiological researches also connect the proportions of other lipids with cardiac disease risk, representing that enlarged plasma T.G. levels are related to a 14% increase in cardiac disease risk in men and a 37% increase in cardiac disease risk in women, individually, which is credited to a greater frequency of myocardial infarction, stroke, and total mortality. Patients with a TG/HDL ratio >3.5 have an unadjusted hazard risk of cardiac disease mortality (Vega *et al.*, 2014). The correlation of the TG/HDL proportion and cardiovascular risk likened to serum HDL levels is more precise than individual values because it relates to the negative impression of T.G.s and the positive impression of HDL on cardiac diseases. (De Backer *et al.*, 2019) Chronic inflammatory progressions can raise the risk of CVDs. Current indication proposes that arrhythmogenic diseases comprising atrial fibrillation, arrhythmogenic cardiomyopathy, and cardiac sarcoidosis can have an inflammatory constituent. Autoimmune and inflammatory-mediated cytokines, including IL-1, IL-6, and TNFα control the expression of ion channels by acting on cardiomyocytes, causing a reduction of Potassium (K+) and a rise of calcium (Lazzerini *et al.*, 2018)

IHD is a prolonged inflammatory disease of the arterial wall and has been revealed to be TNF-driven. A probable positive link of TNF with ischaemic stroke (Shah *et al.*, 2018)

The fundamental mechanism for TNF-driven atherothrombosis might be through a diversity of suggested mechanisms, including favorable effects on circul1ating lipids, insulin resistance, endothelial dysfunction, leucocyte staffing, oxidative stress, vasodilation, or coagulation (Nair *et al.*, 2022). Tumor necrosis factor-α (TNF-α) is a multifunctional flowing cytokine derived from endothelial and smooth muscle cells and macrophages linked with coronary atheroma. Initially recognized as a factor that stimulated hemorrhagic necrosis in transplanted tumors, TNF-α is involved in several cardiovascular processes (Yuan *et al.*, 2020)

Recent studies in literature showed a significant association between elevated TG/HDL-C ratio levels and various clinical conditions. The authors reported a significant correlation between TG/HDL-C ratio and arterial stiffness (Chen and Dai, 2018). In another study, authors concluded that elevated TG/HDL-C ratio could predict the development of type 2 Diabetes mellitus in the Korean population (Lim, Lee and Lee, 2020). The TG/HDL-C ratio indicates the severity of CHD. In 2014, research done by Yunke et al. reported that the harshness of coronary heart disease accompanied the TG/HDL-C ratio and may cause new-onset heart failure incidents (Yunke, Guoping and Zhenyue, 2014). Moreover, Chen et al. documented that elevated TG/HDL-C ratio levels were associated with greater cardiovascular event risk in Chinese people. All these conditions are related to metabolic, inflammatory, and vascular disturbances such as H.T. (Chen *et al.*, 2020).

Amplified levels of triglycerides in the blood result in blood viscosity, which is augmented, which leads to a disturbance of blood flow.' The blood vessels so the heart works harder to pump' blood and raise blood pressure (Neama and Shwaikh, 2024). Hypercholesterolemia can also result in the accumulation of cholesterol in the arterial lumen, denoted as atherosclerosis, and causes artery tightening, inurement, and stiffness; this is what leads to raised peripheral vascular resistance and increased pressure (Kaidah *et al.*, 2020)

Enlarged plasma triglyceride levels are related to the build-up of large, triglyceride-rich VLDL-VLDL1(Adiels *et al.*, 2008). The liver can vary the number of lipids overloaded onto the rising lipoprotein particle in the endoplasmic reticulum, and depending on triglyceride obtainability, it can collect and secrete particles that range in size from VLDL1 to LDL (Sundaram and Yao, 2010).

Kinematic surveys have established that the metabolic destiny of flowing VLDL particles is a function of their size and lipid and apoprotein arrangement, especially their apoE and apoCIII content (Sacks, 2015). LDL subfraction

dissemination is also obviously affected by plasma triglyceride levels. A number of investigators have revealed that the concentration of small, dense LDL (LDL-III) rises meaningfully when triglyceride increases above about 1.5 mmol/l, and there is a mutual drop in LDL-II, the most abundant subfraction in regular participants (Krauss, 2010) (Boren *et al.*, 2020).

Conversely, apoCIII is the primary controller of plasma lipid concentrations. Its role in triglyceride transportation and as a latent interference target has been studied lately (Taskinen and Borén, 2016). Overproduction of apoCIII is related to elevated triglyceride levels. There are several ways in which apo CIII can affect triglyceride metabolism. It has been described as improving VLDL assemblage and exudation in the liver, preventing the action of lipoprotein lipase, henceforward slowing VLDL lipolysis, and perhaps delaying the straight clearance of VLDL fragments by receptors (Adiels *et al.*, 2019).

2. Materials and Methods

The samples were collected in Karbala (Imam AL Hussein Medical City and Imam Al-Hassan Al-Mujtaba Hospital); a total of 90 samples, including 60 samples from the IHD patients group with an age range of 40-85 years, and 30 samples were randomly selected as the control group with matching age rang. The Ethical Committee reviewed and approved the study's protocol, and all participants or their relatives gave written informed consent. A sample of 5 ml blood was withdrawn, allowed to clot for 1 hour, centrifuged at 3000 xg, then serum was separated after 15 min of the centrifugation and finally stored at - 80 °C until the biochemical assessment was performed.

Lipid profile parameters (HDL, LDL, VLDL, T.G., Cholesterol) were estimated according to Kit Shenzhen Mindray Bio-Medical Electronics- Germany's manual procedure. Then, the ratio TG/HDL-C was calculated.

Statistical analysis: All subjects' survey data were entered into a data sheet and given a serial identifier number. Numerous entrance was used to evade errors. The association between the analyzed parameters was assessed using Pearson regression, and a 95% Confidence Interval Range was planned by a non-conditional logistic regression. Significant differences in categorical variables among the parameters were established through analytical statistical tests. Results of all hypothesis tests with p-values <0.05 (two-sided) were considered statistically significant.

3. Results

A total of (60) participants were included in this study. The clinical demographic characteristics and laboratory parameters of the study groups were summarized in Table (1). The mean age of the IHD cases was 60.4± 13.7 years. The BMI mean of patients was (25.7± 3.6) while in the control group was (26.8± 3.6); as a risk factor, both groups were categorized using the body mass index (BMI) according to the traditional World Health Organization as shown in Figure (1). Results also demonstrated the Medical History, Type of medications, and Blood Pressure as Systolic / Diastolic (mm Hg), which was divided into subgroups based on the latest guidelines of the American College of Cardiology, American Heart Association, and the European Society of Cardiology, European Society of Hypertension blood pressure (Brodmann *et al.*, 2020)

Table 3: Demographic and Clinical Characteristics of the Study Population

Variables		Patients	Control
Age (Years)		60.4± 13.7	53.2± 9.6
Sex (Male/ Female) No.(%)		30/ 30	15/15
BMI (Kg/m^2)		25.7± 3.6	26.8± 3.6
Medical History	**Family History (Yes/ No)**	5/ 55	1/ 29
	Gastric intestinal disorder (Yes/ No)	7/ 53	2/ 28
	Smoking status (Yes/ No)	18/ 42	1/29
Type of Treatment	Tenormin	11	/
	Diostar	16	/
	Concor	6	/
	Capoten	7	/
	Tansartan	6	/
	Atacand	1	/
	No Treatment	13	/
Blood Pressure Systolic / Diastolic (mm Hg)	Normal Blood Pressure	12	11
	Elevated Blood Pressure	6	/
	Hypertension, stage 1	9	7
	Hypertension, stage 2	33	12

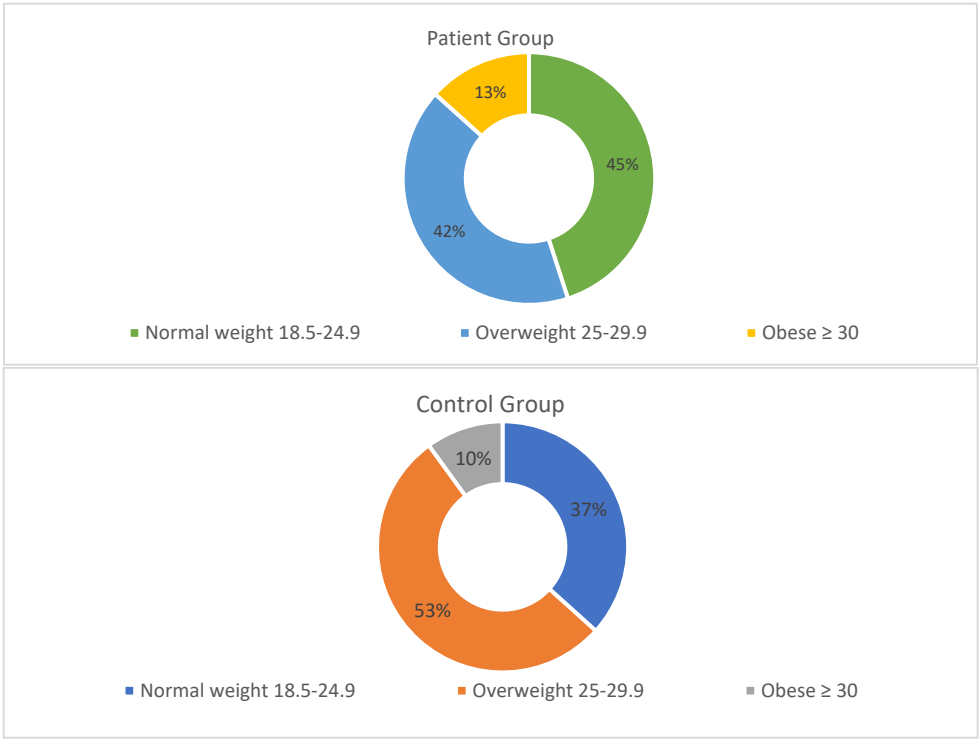

Figure 1: Subgroups of Body Mass Index (BMI) According to the Traditional World Health Organization (WHO)

A box plot was used to screen data dissemination by exhibiting the data quartiles and averages. Box plots show the five-number summary of a data set, including the least score, first (lower) quartile, median, third (upper) quartile, and maximum score. Concerning the valuation of biomarkers levels in IHD patient groups matched to the healthy control, Figures (2 & 3) demonstrated a significant accumulative in the mean levels of all lipid profile panels comprising [total cholesterol (T.C.), triglyceride (T.G.), LDL, and T.G./HDL-c ratio] except HDL which was found to be lesser in patients group compared to the healthy control. The mean levels of T.C., T.G., and LDL in IHD patients were (202.7, 137.48, and 33.98)
respectively, while in the control group were (175.6, 123.27, and 26.9) mg/dl.

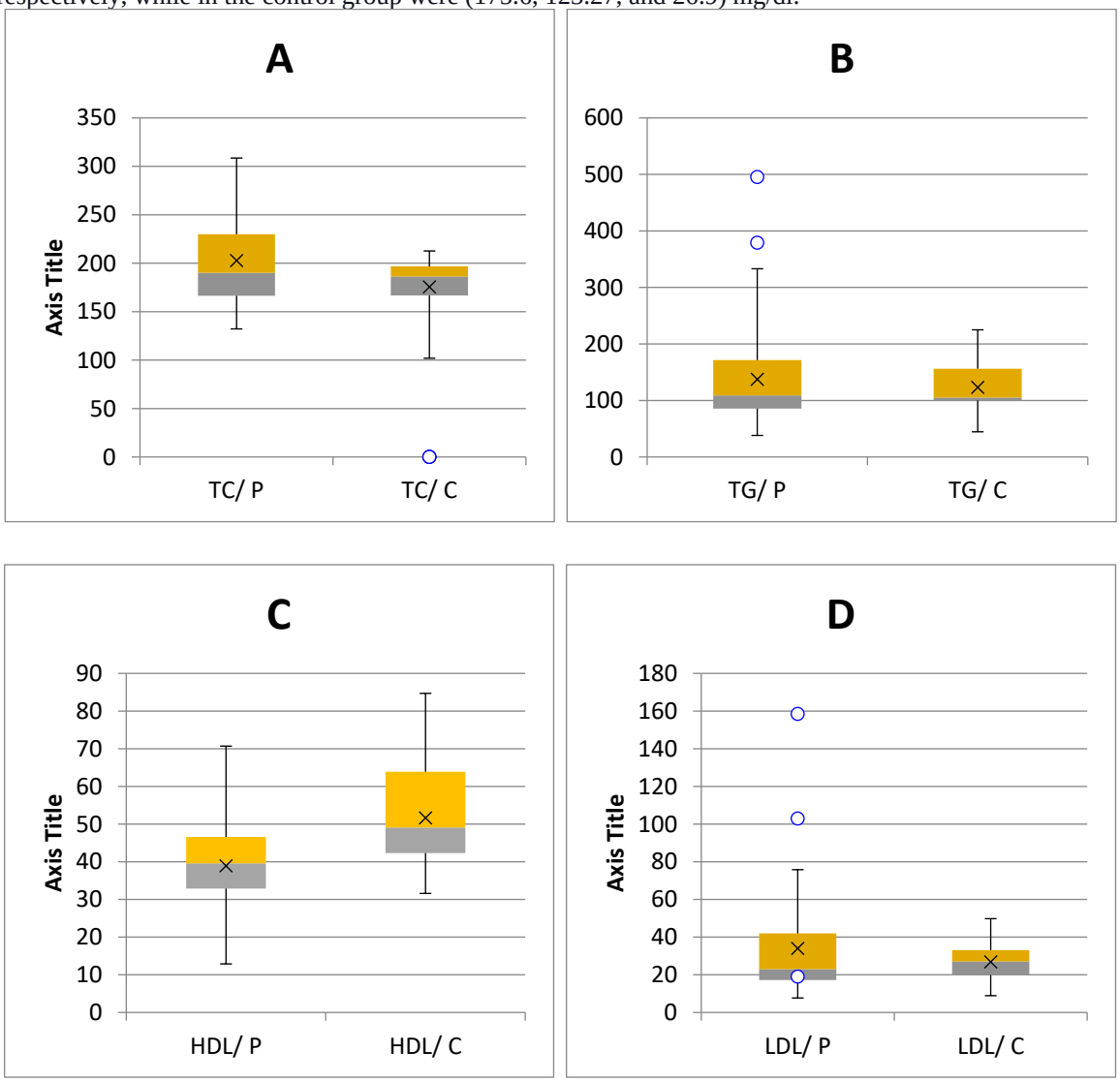

Figure 2: Boxplot for the Dispersal of Lipid Profile Board (A) Total Cholesterol (B) Triglyceride (C) High-Density Lipoprotein (D) Low-Density Lipoprotein in IHD Patients and Control Groups

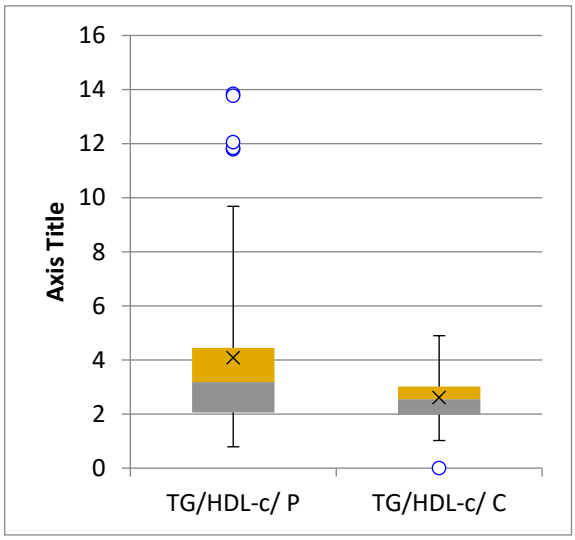

Figure 3: Boxplot for the Distribution of T.G./HDL-c Ratio in IHD Patients and Control Groups

The effect of BMI groups was also examined using the T.G./HDL-c ratio. Results illustrated that the serum level of T.G./HDL-c was enlarged in IHD patients with increasing BMI compared to the healthy control, as shown in Figure (4). In obese participants, the mean T.G./HDL-c ratio was (3.66) in IHD cases compared to (2.58) in the control group. This was agreed with another study that reported that lipid metabolism and the following dyslipidemia contribute to the formation of plaques in cardiovascular disease. (Kosmas *et al*., 2023)

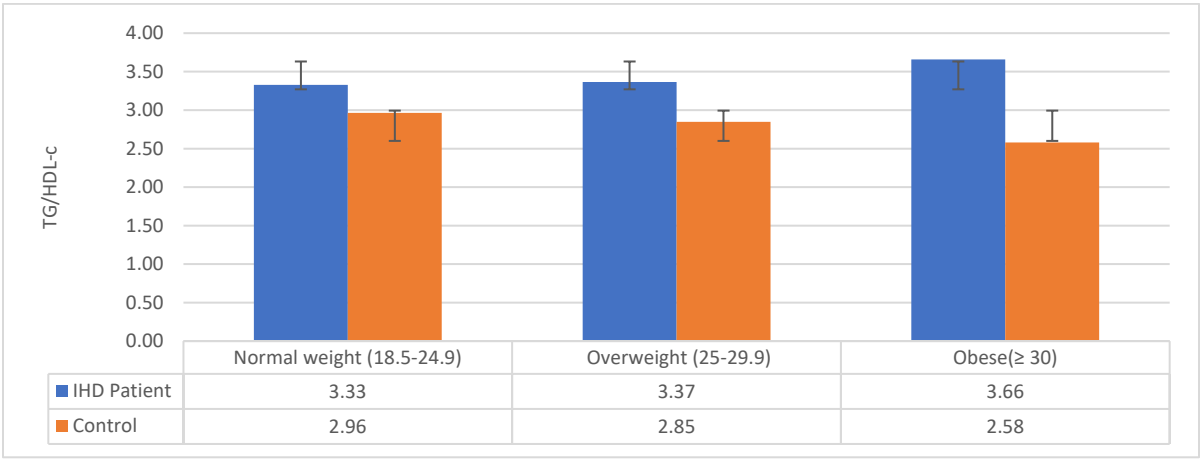

Figure 4: Mean Level of Serum T.G./HDL-c Ratio Based on the Body Mass Index in IHD Patients Compared to the Control Group

In both study groups, the participants' sex was subdivided into Male and Female groups. The effect of sex groups was examined with the biomarkers. Results illustrated that the T.G./HDL-c ratio serum level increased more in male IHD patients than in female IHD patients, as presented in Figure (5). The subclinical problem of coronary atherosclerosis accompanies a greater TG/HDL-C radionecrosis; sex differences in these relationships remain uncertain. (Patil *et al.*, 2020)

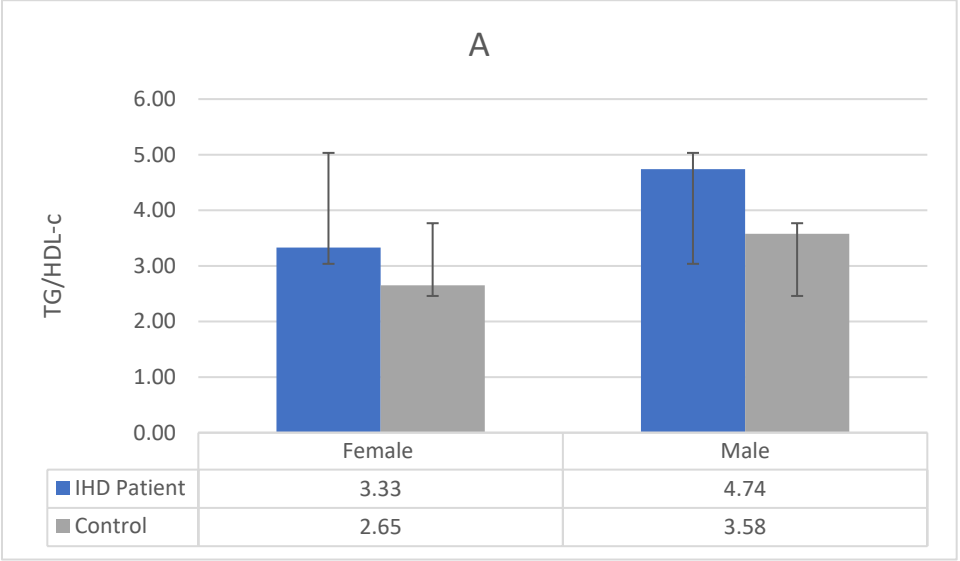

Figure 5: Mean Level of Serum T.G./HDL-c Ratio Based on the Sex in IHD Patients Compared to the Control Group

4. Discussion

Adipokines are highly deregulated by obesity and may control cardiovascular homeostasis (Boulet *et al.*, 2015). Adipose tissue can release free fatty acids (FFA) in the nearness and around the coronary arteries, moderating vascular receptiveness to vasoactive agents (Henrichot *et al.*, 2005) and revolving into an opposing lipotoxic, pro-thrombotic, and pro-inflammatory factor (IFNγ) to overexpress chemotactic cytokines. In addition, fatty tissue can discharge FFA into the bloodstream, alarming vascular homeostasis and endothelial dysfunction, which leads to an amplified risk of heart disease (González *et al.*, 2017).

Second, raised blood pressure leads to systemic arteriole spasm by increasing the penetrability of the vascular endothelium, delaying the interaction time of lipoproteins with the vascular wall, and decreasing endothelium-dependent vasodilation. Systemic arteriole spasm was proposed to raise the risk of heart disease (Yuan and Braun, 2017).

Third, blood pressure regulation is placed on endothelial function, which is controlled by the interaction of the renin–angiotensin–aldosterone system, adrenergic receptors, and metabolic reactions; these endothelial function-related mechanisms are also faithfully related to adipose tissue (Mu *et al.*, 2018). Also, obesity-related FFA constrains the sodium/potassium exchange pump and sodium-ATP pump, which increases smooth muscle tone, peripheral resistance, and blood pressure (Bell and Rahmouni, 2016).

In this study, the T.G./HDL-c ratio was amplified enormously in the IHD group compared to the control; these results were consistent with another finding. Since abnormal lipid panel (HDL-C <40 mg/dl in men and <50 mg/dl in women and T.G.≥150 mg/dl) was considered as a central define of metabolic syndrome (Belete *et al.*, 2021) and felt a brilliant predictor of chief hostile cardiovascular proceedings (Qiao-Yu *et al.*, 2022).

For the previous decade, widespread research has been showing concerning the interlink between T.G.s and other lipoproteins, yet it has not been entirely productive. (Nur Zati Iwani *et al.*, 2022). Patients in the maximum quartile of TG/HDL-C ratio had the uppermost rate of opposing cardiovascular proceedings with excellent TG/HDL-C ratio might raise the risk of ISR via amplified insulin resistance, endothelial dysfunction and atherosclerosis, oxidative stress, pro-inflammatory status, and proliferation of vascular smooth muscle cells (Kundi *et al.*, 2017)

The mechanisms fundamental to the correlation between TG/HDL-C ratios and IHD patients remain unidentified. An earlier study presented significant relations between TG/HDL ratio rises and fractional esterification rates of cholesterol in plasma exhausted of apoB lipoproteins. Many factors have been established to raise the serum concentrations of T.G. and HDL-C, and the TG/HDL-C ratio was involved in poor glycemic control (Hermans, Ahn and Rousseau, 2010)

The logarithm of the ratio TG/HDL-C associates well with HDL particles' size and the fractional esterification rate of cholesterol by lecithin: cholesterol acyltransferase (LCAT) in plasma (Dobiášová *et al.*, 2011). The fractional

esterification level of cholesterol in plasma exhausted of apoB-containing lipoproteins reveals the reactivity of HDL to LCAT. It has been well-known that the molar ratio of the concentration of TG/HDL-C is meaningfully amplified in patients who have experienced a myocardial infarction, matched with age- and sex-paralleled control subjects (Dobiasˇova, 2004). It can be relieved by calculating the atherogenic index of plasma (AIP). It has been recurrently revealed that the AIP value (log[TG/HDL-C]) is strongly associated with the size of the lipoprotein particles. Therefore, the AIP value precisely indicates the attendance of atherogenic LDL particles, and it is also a sensitive predictor of coronary atherosclerosis and cardiovascular risk (Soška *et al*., 2012)

The influence of BMI was also established in the TG/HDL ratio; the ratio was amplified with cumulative BMI. These results were totally consistent with other research that showed that the TG/HDL-C ratio has been established to rise along with the increase of the BMI and waist circumference, and this ratio has elevated up to three times in obese persons. Numerous studies have defined the TG/HDL-C ratio as an insulin resistance indicator (Özkaya, Bavunoglu and Tunçkale, 2014). The ratio of triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) can reveal the body's complete level of lipid metabolism, and it has been established to be a risk factor for numerous cardiovascular diseases (Chen *et al.*, 2020).

The mechanism may be associated with low levels of HDL-C that are unfavorable to regulating extracellular matrix rebuilding, cell differentiation, and proliferation (Prasad *et al.*, 2019). Its pathophysiological meaning may be connected to the amplified serum T.G. level irritating the reduced endothelial vasodilation function of aortic vessels. All at once, the study established that T.G. was meaningfully positively linked with the risk of cardiovascular proceedings and all-cause mortality. With the rise of T.G. levels, the occurrence of ischemic heart disease and ischemic stroke in the research subjects presented a significant upward drift (Bittner *et al.*, 2009).

Nevertheless, matched with the single blood lipid pointers of T.G. and HDL-C, it cannot ultimately reveal the overall level of blood lipids. TG/HDL-C ratio, as a joint blood lipid index, can be used as a simple, available, and dependable hematological index for expecting cardiovascular risk. The TG/HDL ratio has been revealed to be a strong predictor of total mortality, coronary heart disease occurrence, and cardiovascular mortality. It was not associated with critical predictive variables, including age, civilization, smoking, hypertension, diabetes, and severity of coronary heart disease (Drexel *et al.*, 2005). Caselli et al. (Caselli *et al.*, 2021) establish that low HDL-C levels and excellent TG/HDL-C ratios were risk factors for cardiovascular proceedings in patients with coronary heart disease. The ratio of TG/HDL-C can more precisely reflect the complete level of lipid metabolism in patients than the single blood lipid measurement results. So, using the ratio of TG/HDL-C as a predictor of cardiovascular proceedings in coronary heart disease can more precisely predict the remaining cardiovascular risk in patients with coronary heart disease. Studies have found that high levels of TG/HDL-C can lead to the development of abdominal aortic aneurysm, and it is positively correlated with the severity of the patient's disease (Ma *et al.*, 2019).

Many types of research have revealed that hypertriglyceridemia is an independent analyst of IHD and may be a more potent risk factor among gender (Sarwar *et al.*, 2007). Atherogenic dyslipidemia, the combined incidence of high triglycerides (T.G.) and low HDL-C, is linked to an intensely prognostic of CHD (Grundy *et al.*, 2005). The ratio of TG/HDL-C has been suggested as a simply available atherogenic indicator. Documents on the predictive value of the TG/ HDL-C ratio are restricted. The ratio intensely expected risk of myocardial infarction. Others have connected a high TG/HDL-C ratio to coronary atherosclerosis, reduced heart rate, and IHD incidence (Collaboration, 2005).

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Exploring Interleukin 6 as a Promising Marker for The Diagnosis of Gestational Diabetes Mellitus

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Received: 2023/11/04

Accepted: 2023/12/03

Published: 2024/01/04

Keywords: *Gestational Diabetes Mellitus (GDM), Interleukin-6 IL-6, Insulin Resistance*

DOI:10.62472/kjps.v14.i23.106-115

Abstract

Gestational diabetes mellitus (GDM) is a condition in which the ability of the mother's pancreatic β-cells to function properly is compromised. This leads to inadequate insulin production and, therefore, poor glucose regulation throughout pregnancy. In recent years, there has been a significant surge in interest in determining the impact of inflammation on the progression of GDM.

Inflammatory factors may function as antagonists to insulin and induce insulin resistance. Interleukin -6 (IL-6), a kind of cytokine, significantly impacts the development of glucose intolerance and may be used as a possible indicator in the blood for early detection of glucose intolerance. This study aims to assess the potential role of IL6 as a prospective diagnostic marker for Gestational Diabetes mellitus.

Our study is a case-control study started from September 2022 to June 2023 and enrolled 200 pregnant women aged between 15 and 45 years; cases included 100 patients selectively collected with a confirmed diagnosis of gestational diabetes mellitus in Kerbala obstetrics and gynecology hospital, and the control group included 100 healthy pregnant women also gathered from the obstetrics and gynecology hospital in Kerbala governorate. Results show that the mean of Interleukin 6 was significantly higher in pregnant women with GDM, with p-values of 0.05 and 0.001. these results suggest that Interleukin6 (IL6) can be used as a prospective diagnostic marker for GDM.

استكشاف الإنترلوكين ٦ كعلامة واعدة لتشخيص داء سكري الحمل

ديمه ضياء عزيز, سامي الخطيب, نور ضياء عزيز

الملخص

يتم تعريف داء سكري الحمل (GDM) على أنه حالة تضعف فيها قدرة خلايا بيتا البنكرياسية لدى الأم على العمل بشكل صحيح، مما يؤدي إلى عدم كفاية إنتاج الأنسولين، وبالتالي ضعف تنظيم مستويات الجلوكوز طوال فترة الحمل. هناك زيادة كبيرة في الاهتمام بتحديد تأثير الالتهاب على حدوث سكري الحمل في السنوات الأخيرة. قد تعمل العوامل الالتهابية كمضادات للأنسولين وتحفز مقاومة الأنسولين مثل الانترلوكين-٦(IL-6)) ، وهو نوع من السيتوكين، له تأثير كبير على تطور عدم تحمل الجلوكوز ويمكن استخدامه كمؤشر محتمل في الدم للكشف المبكر عن تحمل الجلوكوز. تهدف هذه الدراسة إلى تقييم الدور المحتمل لـلانترلوكين -٦ كعلامة تشخيصية محتملة لمرض سكري الحمل.

دراستنا هي دراسة مراقبة الحالة بدأت من سبتمبر ٢٠٢٢ إلى يونيو ٢٠٢٣ حيث شملت ٢٠٠ امرأة حامل تتراوح أعمارهن بين ١٥-٤٥ سنة، وشملت الحالات ١٠٠ مريضة تم جمعها بشكل انتقائي مع تشخيص مؤكد لمرض سكري الحمل في مستشفى كربلاء للأمراض النسائية والتوليد وتضمنت المجموعة الضابطة ١٠٠ امرأة حامل سليمة ايضا تم جمع النساء الحوامل الأصحاء من مستشفى النسائية والتوليد في محافظة كربلاء. أظهرت النتائج أن متوسط إنترلوكين ٦ كان أعلى بشكل ملحوظ في النساء الحوامل المصابات بـ سكر الحمل مع قيم p 0.05 و ٠,٠٠١. تشير هذه النتائج إلى أنه يمكن استخدالانترلوكين -٦ كعلامة تشخيصية محتملة لسكري الحمل.

1. Introduction

Gestational Diabetes Mellitus (GDM) is a prevalent disorder during pregnancy, characterized by impaired glucose tolerance. It affects around 9-25% of pregnancies globally. However, the percentages may vary depending on the demographic and diagnostic criteria. Gestational diabetes mellitus (GDM) is defined as a condition where the ability of the mother's pancreatic β-cells to function correctly is compromised, leading to inadequate insulin production and, therefore, poor regulation of glucose levels throughout pregnancy [(Mechchate *et al.*, 2021), (Choudhury and Rajeswari, 2021)]

During pregnancy, the placenta secretes a hormone called human placental lactogen. It induces significant metabolic changes during pregnancy and has a structure comparable to the growth hormone figure (1). to ensure the fetus receives enough nutrients. This hormone can induce changes and modifications in the insulin receptors. The following molecular alterations seem to be associated with a reduction in glucose absorption by peripheral tissues:

1) The beta-subunit of the insulin receptor undergoes molecular changes. 2) The phosphorylation of tyrosine kinase is reduced. 3) The insulin receptor substrate-1 and phosphatidylinositol 3-kinase undergo remodeling. Elevated maternal glucose levels pass via the placenta and increase the fetus's blood sugar levels. The fetal pancreas is activated in response to high blood sugar levels. The anabolic characteristics of insulin stimulate accelerated development in embryonic tissues [(Spaight *et al.*, 2016), (Moyce and Dolinsky, 2018)].

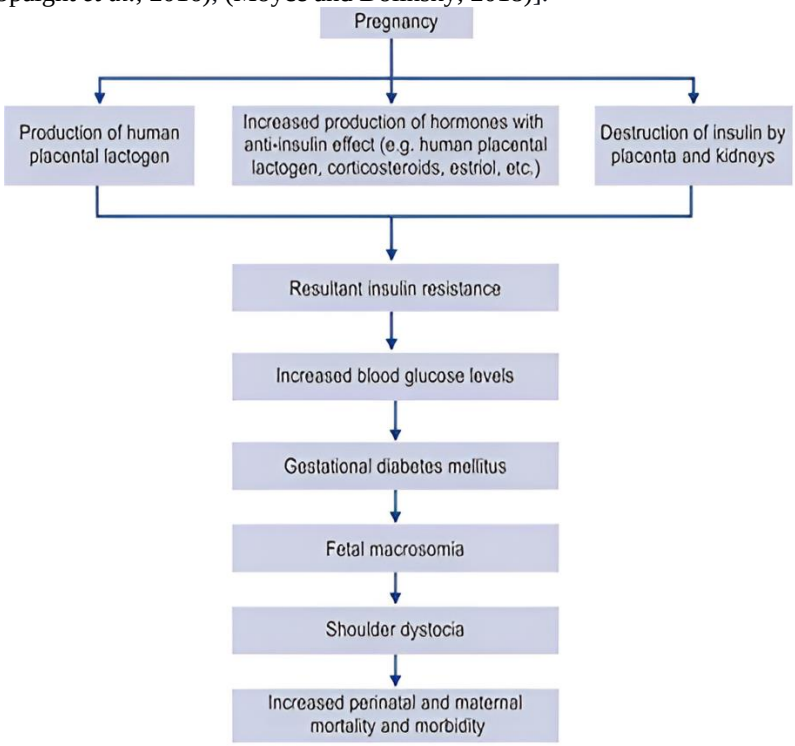

Figure 1: Pathophysiology of Gestational Diabetes Mellitus (Bedside Obstetric & Gynecology 2014).

The International Association of Diabetes and Pregnancy Study Groups (IADSPG) standards are the primary basis for diagnosing gestational diabetes mellitus (GDM). These protocols include doing an oral glucose tolerance test (OGTT) between the 24th and 28th week of pregnancy. The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) research, conducted in 2010, set out revised criteria for determining Oral Glucose Tolerance Test (OGTT) thresholds from the 24th to 28th weeks of pregnancy. The fasting plasma glucose level is 5.1 mmol/L, the one-hour plasma glucose level is ten mmol/L, and the two-hour plasma glucose level is 8.5 mmol/L (Kopylov *et al.*, 2020).

Early and precise identification of women with a high susceptibility to developing GDM offers a chance to implement effective prenatal care strategies and future treatments aimed at mitigating the advancement of gestational diabetes. This, in turn, may help lower the related healthcare costs and adverse consequences. Nevertheless, there is ongoing

controversy over the diagnostic criteria for GDM. A prior investigation revealed a direct correlation between maternal blood glucose levels and unfavorable perinatal outcomes.
In recent years, there has been a significant surge in interest in determining the impact of inflammation on the progression of GDM.
Inflammatory factors may function as antagonists to insulin and induce insulin resistance. Interleukin-6 (IL-6), a kind of cytokine, has a significant impact on the development of glucose intolerance IL-6 causes insulin resistance by impairing the phosphorylation of insulin receptor and insulin receptor substrate-1 and may be used as a possible indicator in the blood for early detection of glucose intolerance (Farrar *et al.*, 2017).

In individuals with type 2 diabetes, inflammatory cytokines may lead to insulin resistance by inhibiting several pathways in the tissues essential for effective insulin signaling. Insulin resistance is linked to the atypical release of proinflammatory cytokines, such as IL-6 (Murthy *et al.*, 2018). During pregnancy, the placenta secretes IL-6, which is essential for normal placenta development and successful pregnancy; it may cause a long-lasting inflammatory response in fat tissue and aid in the incidence of insulin resistance during pregnancy.

2. Materials and Methods

2.1 Ethical Statement

The study was conducted with the endorsement of the Scientific and Ethical Committees at the Faculty of Medicine, University of Kufa, Iraq.

2.2 Patient Selection and Studying Design

The present study is a case-control study started from September 2022 to June 2023 and enrolled 200 pregnant women aged between 15 and 45 years (childbearing age); cases included 100 patients selectively collected with a confirmed diagnosis of gestational diabetes mellitus in Kerbala Obstetrics and Gynecology Hospital.
The (control group) included 100 healthy pregnant women also collected from the obstetrics and gynecology hospital in Kerbala governorate.
Included patients: All pregnant women in the second and the third trimester (because GDM occurs in these trimesters) who have a confirmed diagnosis of gestational diabetes mellitus.
Excluded patients: pregnant women who have diabetes (both types I and II), pregnant women in the first trimester with diabetes, pregnant women with a baby with congenital anomalies, obese pregnant women, and pregnant women who smoke.

2.3 Research Methods

After skin sterilization, five milliliters of blood were aspirated from the anti-cubital vein and split into two halves. The first two milliliters of blood were used to detect the blood group type and measure HbA1c.
Allow the remaining three milliliters of blood to clot for 10-20 min. At room temperature, gel tubes to obtain serums by centrifuging at (2000-3000) r.p.m. for 20 minutes. After that, the obtained serum is stored at -15 C to perform the biochemical tests for Interleukin 6 (IL6) measurement.

2.3.1 Measurement of IL6 by Enzymes Linked Immunosorbents Assay (ELISA-Kits, Elab-science Cat.No. : E-EL-H6156

Principle of the Assay

We used the Sandwiches-ELISA principles with this ELISA kit. These kit's (micro) ELISA plates already have an antibody specific to human IL-6. Before combining the specified antibody with the samples or standards, they added it to the wells of the (micro) ELISA plates. The next step is to incubate every microplate well after adding the detection biotinylated antibodies specified to humans IL-6 and avidins-horseradish peroxidases (HRP). Wash to remove the waste product. Each one is filled with the substrate solutions. Only wells containing Human IL-6 will appear in blue. The components include a detection antibody that has been biotinylated, as well as an Avidin-HRP conjugate. Using a stop solution halts the enzymatic-substrate interaction, resulting in a yellow color change.
The spectrophotometric measurement of (OD) optical density is obtained at 450 nm wavelength with a tolerance of ± 2 nm. The concentration of Human IL-6 is directly proportional to the OD value. By comparing the samples' optical density (OD) with the standard curve, it is possible to ascertain the concentration of human IL-6 in the samples.

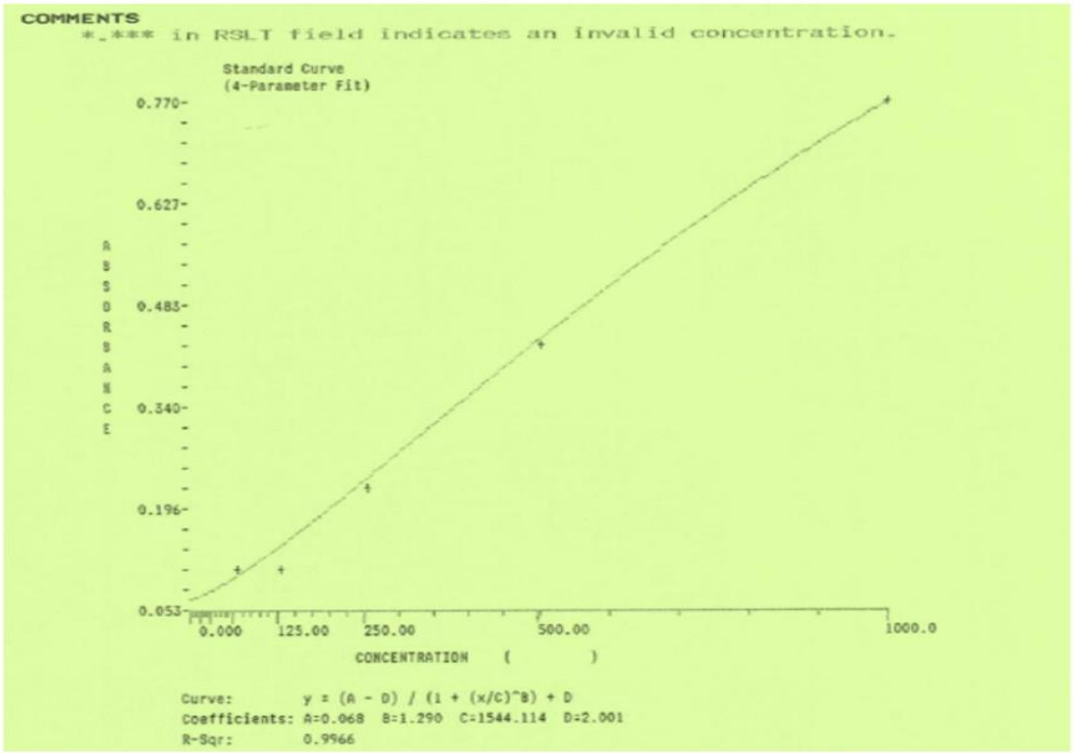

Figure 2: IL6 Standard Curve

2.3.2 Measurement of Glycosylated Hemoglobin (HbA1c) by HbA1c HPLC Kit , Eagle Biosciences Catalog Number: A1C31-H100

Principle of the assay :

First, the blood cells are lysed to determine HbA1c. An incubation step is performed on the samples at 37 °C to remove the unstable aldimine format. We inject the centrifuged liquid, the supernatant, into the HPLC equipment. It takes 5 minutes for the gradient separation to complete at 30°C using HPLC. A UV detector records the chromatograms.
We can measure concentrations using the supplied blood calibrator by integrating the corresponding peak heights and regions.

2.4 Statistical Analysis

Data was analyzed using SPSS version 26, a statistical tool for the social sciences. Presenting descriptive statistics in the form of frequency tables, The representation of continuous variables includes portraying them as the mean value offset by the standard deviation and utilizing the Student T test to identify associations between categories of data and continuous variables. Statistical analysis by Receiver operative characteristic curve (ROC) was used to evaluate the performance of IL6 in detecting GDM—a P-value of 0.05 or less determined statistical significance.

3. Results

3.1 level of Interleukin 6 in the two groups :

The mean interleukin 6 was significantly higher in pregnant women with GDM (p-values 0.05 and 0.001, table (1)) than in healthy pregnant women.

Table 1: The Level of Interleukin 6 in Patients and Controls

Variable	Studied group				P value
	Pregnant with GDM		Control pregnant		
	Mean± SD	Range	Mean± SD	Range	
IL6	41.7±23.9 pg/ml	10-98.1	4± 3.2 pg/ml	0.1-20.6	<0.001*

***Student T test, Significant ≤0.05.**

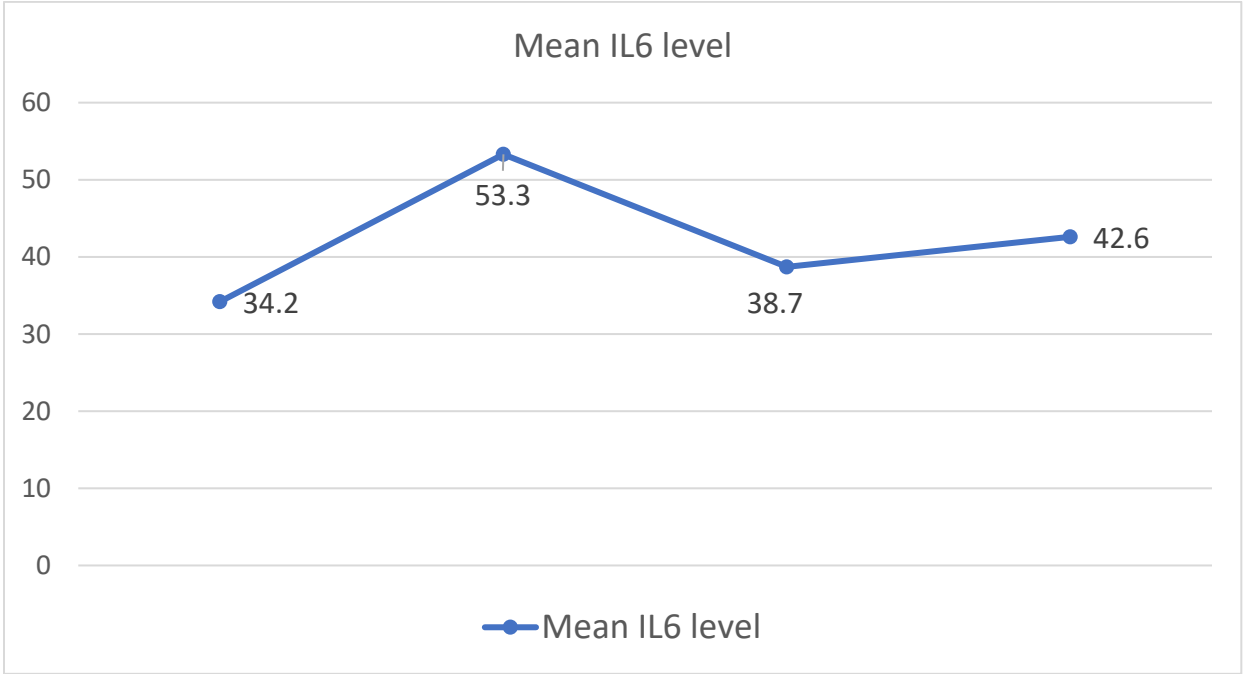

Figure 3: Mean Level of IL6

3.2 Evaluation of Interleukin 6 for diagnostic purposes using a Receiver Operating Characteristic (ROC) profile.

The ROC curve for IL6 was shown in Figure (4), obtained from the values of OGTT and IL6 tests with an area under the curve 0.992 and p-value <0.001 and with the cut of the value of 10.1 had a sensitivity of 99% and specificity of 10%.

4. Discussion

Gestational diabetes is an inflammatory disorder triggered by generating inflammatory cytokines such as interleukin-6 (HAMZA and MAJEED, 2021). Siddiqui et al. mentioned a valuable (case-control study) to detect the probable correlation between IL-6 and GDM in Indian women (Siddiqui *et al.*, 2019). The study's findings indicated that GDM women exhibited substantially elevated serum levels of IL-6 compared to control women. Additionally, our research revealed that pregnant GDM patients had a considerably higher mean IL6 level than pregnant healthy women. In addition, levels of IL-6 were connected with weight before pregnancy, blood sugar levels during fasting, and blood sugar levels after eating.

Furthermore, intestinal flora imbalances with higher inflammatory markers like IL6 and TNFα were likely to occur in GDM patients. These imbalances damaged the patients' immune systems and may have played a significant part in the development of diabetes. These results are based on a study by Yu et al. that examined how patients with GDM's gut flora and other inflammatory markers changed over time. Serum levels of proinflammatory factors, such as IL-6, were considerably higher in the patients than in the controls (Yu, Liu and Dong, 2018).

Zhao et al. study, which looked into the possible relationship between inflammatory markers and Chinese women's glucose intolerance and GDM, found that ladies who are expecting a child and have gestational diabetes or glucose intolerance had IL-6 levels significantly higher than those of the group of healthy individuals and that inflammatory cytokines were positively correlated with body mass index (BMI) and hemoglobin A1c (HbA1c) (Zhao *et al.*, 2018).

Zhang et al. found a significant difference in the serum and placental levels of inflammatory and placental indicators, such as IL-6, in women with gestational DM compared with women whose pregnancies were normal and healthy (Zhang *et al.*, 2017). This finding helped to clarify the relationship between inflammatory and metabolic biomarkers in women with GDM in Mongolia.

In recent years, much attention has been focused on how inflammation contributes to the onset of GDM. Inflammatory substances acting as antagonists to insulin can cause insulin resistance. As a cytokine, IL-6 is involved in the pathophysiology of glucose intolerance and may be used as a serum marker for early detection of glucose intolerance (Kleiblova *et al.*, 2010).

The inflammation of pancreatic macrophages and adipocytes, which increases IL-6 production, explains the link between IL-6 and gestational diabetes; thus, reduced insulin production brought on by the death of pancreatic β-cells results in elevated blood glucose levels (Tutar *et al.*, 2022).

The gold standard test, OGTT testing with 75 grams of oral glucose (O'Malley *et al.*, 2020), is conducted nearly late in pregnancy and necessitates an overnight fast. Furthermore, taking glucose during pregnancy is unpleasant for the expectant mother and requires the drawing of blood three times. On the other hand, measuring IL-6 levels is simple, inexpensive, and comfortable for a pregnant lady, and it does not present the difficulties mentioned above. As a result, IL-6 can be utilized as a marker to determine a pregnant woman's risk of GDM.

An appropriate measure like IL-6 is desperately needed, given the rising incidence of gestational diabetes globally and the importance of prompt identification and treatment of GDM to minimize harmful consequences for both the mother and the fetus.

The Receiver Operating Characteristic ROC curve is a graph that may be used to analyze how well a binary diagnostic classification system is performing. Using the ROC curve, another way to assess a test's diagnostic efficacy is to compare its findings to those of other tests.

The ROC curve offers several benefits. Initially, the whole range of test results can be distinguished between normal and abnormal using the detailed depiction provided by the ROC curve. Sorting the data in a histogram format is not essential to constraining the ROC curve; it displays the sensitivity and specificity for all possible cutoff values using the test data.

Finally, because the ROC curve depends only on sensitivity and specificity, samples can be obtained irrespective of the presence of a disease in the community because it is not impacted by prevalence (Joo, Cho and Kim, 2020).

Understanding the concept of sensitivity and specificity, which are used to assess a diagnostic test's performance, is a prerequisite to comprehending the ROC curve. The percentage of people who test +ve for a target disease who genuinely have it is known as sensitivity. In contrast, the percentage of people who try -ve for the disease is known as specificity (Jung, Lee and Park, 2019).

Area under the curve (AUC) is a commonly used tool to assess diagnostic tests' accuracy. Near the top left corner of the graph, where the sensitivity =1 and false positive rate =0 (specificity =1), the test's accuracy rises as the ROC curve moves closer to that area.

AUC = 1.0 is the perfect ROC curve. Nonetheless, when there is a one-to-one correspondence between the x- and y-axes and the actual positive rate is equal to the false positive rate (AUC=0.5), it would be like tossing a coin or using any other haphazard method. It would be inappropriate to ascertain the existence or absence of illness.

Thus, the AUC needs to be more than 0.5 for any diagnostic method to be relevant, and it should generally be more than 0.8 to be deemed appropriate. Furthermore, the ROC curve with the most excellent AUC was thought to perform the best diagnostics when comparing the results of two or more diagnostic tests (Hajian-Tilaki, 2013).

Our investigation revealed that the ROC curve for IL6 was displayed in Figure (4). The area under the curve analysis was 0.992, with a sensitivity of 99%, a specificity of 10%, and a cutoff value of 10.1. Since more information

is needed regarding the IL6 ROC curve for GDM, our work may be considered one of the first in this area. The considerable AUC value shows a high accuracy of the IL6 test, meaning it can be used to predict GDM in women.

5. Conclusion

The current study's results indicate that Interleukin6 (IL6) can be used as a prospective diagnostic marker for GDM.

Acknowledgments

The authors would like to thank all the women participating in this study.

Conflict of Interest

The authors declare no conflict of interest.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Evaluation of Immunological Levels of IL-37, IL-38, and IL-17A in Iraqi Patients with Diabetic Foot Ulcers

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Received: 2023/11/05

Accepted: 2023/12/03

Published: 2024/01/04

Keywords*: *Diabetic foot ulcer, DM, IL-37, IL-38, IL-17 A, ELIZA.

DOI:10.62472/kjps.v14.i23.116-124

Abstract

Diabetes mellitus, a significant cause of mortality around the globe, can result in several secondary complications, including diabetic foot syndrome, which is brought on by diabetic neuropathy and ischemia. Approximately 15% of diabetic patients suffer from diabetic foot complications, which results in high rates of morbidity and mortality of people with Diabetes mellitus. The study's objective is to measure serum levels of IL-37, IL-38 & IL-17A in diabetic mellitus (type 1 & 2) patients with and without diabetic foot ulcer complications and in the control group as well as the possibility of using them as early biomarkers for diagnosis of diabetic mellitus complications & future prevention. Overall, of 193 participants included in this case-control study, they were divided into three groups: the first one contains patients with type 1 diabetes mellitus (29 with diabetic foot ulcer DFU+ 35 non-diabetic foot), the second group includes type 2 diabetes mellitus patients (41 with DFU + 38 Non). The third group includes (50) as apparently healthy controls. Serological techniques of sandwich ELISA did laboratory tests for specific serum human IL-37, IL-38, and IL-17A. The study revealed that IL-37 and IL-17A levels were significantly high ($P<0.01$) in all diabetic groups compared to the control healthy group. The results of IL-38 show substantially higher levels of T1DM and T2DM ($P<0.05$) compared to the control group. In addition, the DFU group of T2DM illustrated higher levels of IL-37, IL-38, and IL-17A compared with other diabetic groups.

In conclusion, Iraqi DM subjects with and without complications had higher values of interleukins, IL-37, IL-38, and IL-17A, than healthy controls, which suggests an inflammatory state in these patients. In addition, the DFU of T2DM patients expressed higher levels of interleukins than other diabetic groups. This can be focused on using them as novel therapeutic targets for preventing and treating DM complications. As well as the possibility of using them as markers of inflammation and progression for complications in DM patients.

تقييم مستويات المناعة لـ IL-37 و IL-38 و IL-17A في المرضى العراقيين الذين يعانون من قرح القدم السكرية

الملخص

يمكن أن يؤدي داء السكري، وهو أحد الأسباب الرئيسية للوفيات في جميع أنحاء العالم، إلى العديد من المضاعفات الثانوية، بما في ذلك متلازمة القدم السكرية، والتي تنتج عن الاعتلال العصبي السكري ونقص التروية. يعاني حوالي ١٥٪ من مرضى السكري من مضاعفات القدم السكرية، ويؤدي ذلك إلى ارتفاع معدلات الإصابة بالمرض والوفيات بين الأشخاص المصابين بداء السكري .

الهدف من الدراسة هو قياس مستويات IL-37 و IL-38 و IL-17A في مصل الدم لمرضى السكري من النوع الأول والثاني الذين يعانون من مضاعفات قرحة القدم السكرية او بدونها وفي المجموعة الضابطة وكذلك إمكانية استخدام هذه الحركيات كمؤشرات حيوية مبكرة لتشخيص مضاعفات مرض السكري والوقاية منها في المستقبل.

إجمالي ١٩٣ مشاركًا ادرجوا في هذه الدراسة؛ تم تقسيمهم إلى ثلاث مجموعات: المجموعة الأولى تضم مرضى السكري من النوع الاول ٢٩ مصابين بقرحة القدم السكرية و ٣٥ غير مصابين بقرحة القدم السكري، والمجموعة الثانية تضم مرضى السكري من النوع الثاني ٤١ مصابين بقرحة القدم السكرية و ٣٨ غير مصابين، أما المجموعة الثالثة فقد ضمت (٥٠) مشارك وهي مجموعة السيطرة.

تم إجراء الاختبارات المعملية بواسطة التقنيات المصلية لELISA ، وتم اختبار مصل الدم المحدد IL-37 ، IL-38و IL-17A. أظهرت نتائج الدراسة أن مستويات IL-37 و IL-17A كانت ذات دلالة عالية ($P<0.01$) في جميع مجموعات مرضى السكري مقارنة بمجموعة السيطرة. بينما أظهرت نتائج IL-38زيادة معنوية مع النوع الأول والثاني للسكري مقارنة بمجموعة السيطرة. بالإضافة إلى ذلك، أظهرت مجموعة المصابين بالقدم السكري للنوع الثاني مستويات أعلى من IL-37 و IL-38 و IL-17A بالمقارنة مع مجموعات مرضى السكري الأخرى.

في الاستنتاج؛ كان لدى الأشخاص العراقيين الذين يعانون من مرض السكري مع أو بدون مضاعفات قيم أعلى للحركيات؛ IL-37، وIL-38، و-IL 17A، مقارنة بمجموعة السيطرة التي تشير إلى وجود حالة التهابية لدى هؤلاء المرضى. بالإضافة إلى ذلك، أظهرت مجموعة المصابين بالقدم السكري من النوع الثاني مستويات أعلى من الحركيات مقارنة بمجموعات مرضى السكري الأخرى، يمكن أن يركز هذا على استخدامها كأهداف علاجية جديدة للوقاية من مضاعفات مرض السكري وعلاجها. وكذلك إمكانية استخدامها كعلامات للالتهاب وتطور المضاعفات لدى مرضى السكري.

1. Introduction

Diabetes mellitus (DM) is a significant health problem worldwide. High blood glucose levels indicate this metabolic disease due to insufficient insulin production or action. The immune response to high blood glucose levels and the presence of inflammatory mediators produced by adipocytes and macrophages in fat tissue causes an inflammatory response. A low and chronic state of inflammation damages pancreatic beta cells, resulting in insufficient insulin production and hyperglycemia. Diabetes hyperglycemia is thought to cause immune response dysfunction, which fails to control the spread of invading pathogens in diabetic subjects. As a result, diabetic patients are known to be more susceptible to infections ((AL-Sahi, AL-Hasnawi and Ali, no date), (Soto-Chávez *et al.*, 2022)).

The most commonly encountered micro-vascular complication of diabetes is diabetic neuropathy, with a prevalence of 50-60%; neuropathy may cause decreased nerve functions and nerve blood perfusion with persistent nerve damage. Diabetic peripheral neuropathy increases the development of foot ulceration risk (Barua *et al.*, 2022).

Interleukin IL-37 is also known as IL-1 family member 7 (IL-1F7). It is a novel anti-inflammatory cytokine with immunomodulatory effects in three ways, i.e., by reducing the synthesis of pro-inflammatory cytokines, lowering the expression of transcriptional cytokines, and inhibiting the activation of kinase signaling (Tan *et al.*, 2022). IL-37 is widely expressed in multiple human tissues and organs, including the skin, heart, kidney, gut, lymph node, thymus, bone marrow, lung, testis, placenta, and uterus (Tian *et al.*, 2022).

However, the expression of distinct subtypes differs according to the specific tissues and organs involved. Under physiological conditions, IL-37a is mainly found in the lymph nodes, thymus, bone marrow, placenta, colon, lung, testicles, and brain, whereas IL-37b is primarily found in the peripheral blood, lymph nodes, placenta, colon, lung, testicles, and kidney. IL-37c is mainly expressed in the lymph nodes, placenta, colon, lung, testis, and heart, whereas IL-37d is predominantly expressed in the testis, bone marrow, blood system, umbilical cord tissue, and adipose tissue mesenchymal stem cells ((Țiburcă *et al.*, 2022), (Tille, 2015), (Tirichen *et al.*, 2021)).

Interleukin (IL)-38 is a recently discovered, novel anti-inflammatory cytokine that belongs to the IL-1β family and inhibits subsequent signaling pathways, thereby regulating the differentiation and function of T cells, peripheral blood mononuclear cells, macrophages, and dendritic cells (Tola, Regassa and Ayele, 2021). Generally, IL-38 is expressed in the human heart, thymus, etc., but not in T cells in the tonsil. Additionally, IL-38 binds to the receptors via nuclear factor kappa-B (NF-κB), activating protein-1 (AP-1) and c-Jun N-terminal kinase (JNK) signaling pathways to regulate the inflammatory cytokines generation ((Turns, 2011), (Ubeid, 2020)). This data indicated that IL-38 might be related to autoimmune diseases. Furthermore, IL-38 may affect the mechanism of autoimmune diseases in regulating the balance of anti-inflammatory and pro-inflammatory. A study by Aravindhan et al. (2022) showed significantly decreased serum levels of IL-38 in diabetes subjects (Aravindhan *et al.*, 2022).

IL-17 secreted by Th17 cells initiates the secretion of pro-inflammatory factors and further amplifies the inflammatory response in inflammatory and autoimmune diseases. Therefore, Th17 and IL-17 might be involved in the pathogenesis of DM (Parhi *et al.*, 2019). The pathogenicity of IL-17 has been well-recognized in several diseases, including psoriasis, rheumatoid arthritis, multiple sclerosis, cancer, and diabetes. Studies (Ma *et al.*, 2022) showed elevated plasma IL-17 levels compared to healthy individuals in patients with diabetes (Țiburcă *et al.*, 2022).

The goal of the present study is to compare the levels of IL-37, IL-38, and IL-17A in diabetic mellitus patients without diabetic foot ulcers (DFUs), diabetic patients with DFUs, and healthy people using (a control group) using the ELIZA technique. To assess any immunological association between IL-37, IL-38 & Il-17A serum levels and diabetic foot ulcer. In addition, it is possible to use them as early biomarkers for diagnosis of DFUs & future prevention.

2. Materials and Methods

2.1 Ethical Approval

Patients involved in this study were informed about the detailed aim of the study, and verbal agreement was obtained from each one before samples were collected. This study was approved by the Scientific Council of Karbala Medical College with reference no. 64.

Experimental design

A case-control study was conducted for six months, from August 2022 to January 2023. All patients enrolled in the study were from Imam AL-Hussain Medical City.

The total number of participants was 193 subjects; they were divided into three groups: the first one includes patients with type 1 diabetes mellitus (29 with diabetic foot ulcer + 35 Non-diabetic foot), the second group includes type 2

diabetes mellitus patients (41 with diabetic foot ulcer + 38 Non-diabetic foot ulcer), and the third group includes (50) as apparently healthy control.
Serological techniques of sandwich ELISA were used to do laboratory tests for specific serum human IL-37, IL-38, and IL-17A. Cat. No. (E-EL-H2571, E3276Hu and E-EL-H0105, respectively).
Inclusion Criteria: The study included any case with a clinical diagnosis of type 1 and type 2 diabetes, with and without Diabetic foot ulcers (DFUs) complications and their duration.
Exclusion criteria include: if a different diagnosis was documented, Pregnancy, patients with multiple autoimmune diseases and a history of current inflammation and infection, patients with cardiovascular disease (CVD), patients taking immunosuppressive therapy, renal failure patients, Liver failure patients, and patients with an age group below 18 years.
Blood Sample collection: Five milliliters of venous blood were taken from each participant. The blood sample was immediately transformed into a gel tube and left to clot for 15 minutes at room temperature (20-25) °C. Then, after collecting, it was centrifuged at (3000 rpm) for approximately (15) minutes to obtain serum. The isolated serum of samples was distributed into three aliquots (0.5ml) in tightly closed Eppendorf tubes. Then, the tubes were stored at -20 C until ELIZA assayed them for immunological markers testing.

3. Results

3.1. IL-37 Level in Diabetes Groups

Comparison of IL-37 among the studied groups revealed a highly significant difference (P<0.001) in the mean level of IL-37 across the studied groups of T1DM and T2DM, as in Table (1.1).

Table 1: IL-37 Levels Among Different Groups

Parameter	**Group**				**Control (N=50)**	
	T1DM (N=64)		**T2DM (N=79)**			**P. value**
	DFU(N=29)	**Non(N=35)**	**DFU(N=41)**	**Non(N=38)**		
IL-37 pg/mL	**Mean± SD**	**Mean± SD**	**Mean± SD**	**Mean± SD**	**Mean± SD**	
	28.20±8.05	23.29±7.06	30.94±9.95	25.02±7.74	16.09±4.10	**<0.01 Sig.**
Multiple pairwise comparisons./ least significant difference (LSD) post hoc test						

Subgroups	**P. value**		
	DFU	**Non**	**DFU vs Non**
T1DM			**<0.05**
T2DM			**<0.05**
T1DM vs. T2DM	**<0.01**	**<0.01**	
T1DM vs. controls	**<0.01**	**<0.01**	
T2DM vs. controls	**<0.01**	**<0.01**	

SD; Standard Deviation of mean, sig: significant**.** P. value (≤ 0.05), (≤ 0.01).

*** SD; Standard Deviation of mean, sig: significant. P. value (≤ 0.05), (≤ 0.01)**

The mean IL-37 was 28.20 and 23.29 in the DFU and non-DFU groups of T1DM, respectively. On the other hand, T2DM had 30.94 and 25.02 mean in the DFU and non-DFU groups of T2DM patients, while the mean of the control group was 16.09. Generally, in all comparisons, the results illustrated a higher level of IL-37 in the T1DM and T2DM patient groups compared with the control (Table 1.1).

As shown in the table above, there were many significant differences when compared with study groups. There are significant differences (p <0.05) when compared between DFU and Nongroups in T1DM and T2DM. In addition, there are highly significant differences (p <0.01) when comparing comparing T1DM and T2DM with the control group. As well as, there are highly significant differences (p <0.01) when compared among DFU and Nongroups T1DM and T2DM diabetic groups.

3.2. IL-38 Level in Diabetic and Healthy Control Groups

A comparison of IL-38 among the studied groups revealed that all groups of T1DM and T2DM had significantly higher levels of IL-38 than control groups. The DFU group of T2DM patients had a higher mean level of IL-38 (3.57) than the DFU group of T1DM (3.05); on the other hand, there was a significant difference (P <0.05) in IL-38 between the studied groups Table 2.

Table 2: IL-38 Levels in Studied Groups

Parameter	**Group**				**Control (N=50)**	
	T1DM (N=64)		**T2DM (N=79)**			**P. value**
	DFU(N=29)	**Non(N=35)**	**DFU(N=41)**	**Non(N=38)**		
	Mean± SD	**Mean± SD**	**Mean± SD**	**Mean± SD**		
IL-38 ng/L	3.05±1.90	2.13±1.87	3.57±1.66	2.55±1.75	1.13±0.40	**<0.05**
Multiple pairwise comparisons./ least significant difference (LSD) post hoc test						
Subgroups	**P. value**					
	DFU	**Non**	**DFU vs Non**			
T1DM			**<0.01**			
T2DM			**<0.01**			
T1DM vs. T2DM	**0.13 ns**	**0.24 ns**				
T1DM vs. controls	**<0.01**	**<0.01**				
T2DM vs. controls	**<0.01**	**<0.01**				
SD; Standard Deviation of mean, sig: significant**.** P. value (≤ 0.05), (≤ 0.01). NS: not significant.						

3.3. IL-17A Level in Studied Groups

The results summarized in Table (3.6) indicated a statistically significant difference (P<0.01) in levels of IL-17A in all diabetes case groups compared to a healthy control group. On the other hand, the level of IL 17 was highest in the group with chronic complication (DFU) with a mean value (37.44), followed by
DFU of T1DM with a mean level (of 25.19), then the Non-group of T2DM with a mean level (of 23.87), and lastly Non-group of T1DM (21.05). While the mean level of apparent control is (6.12). However, there are significantly higher differences (P<0.01) when compared between studied diabetic groups (Table 1.3).

Table 3: Mean Levels of IL-17A in Different Groups

Parameter	Group				Control	P. value
	T1DM (N=64)		T2DM (N=79)			
	DFU(N=29)	Non(N=35)	DFU(N=41)	Non(N=38)		
	Mean± SD	Mean± SD	Mean± SD	Mean± SD		
IL-17A pg/mL	25.19±10.84	21.05±8.19	37.44±10.90	23.87±11.24	6.12±3.80	**<0.01**
Multiple pairwise comparisons. / Least significant difference (LSD) post hoc test						
Subgroups	**P. value**					
	DFU	**Non**	**DFU vs Non**			
T1DM			**<0.05**			
T2DM			**<0.01**			
T1DM vs. T2DM	**<0.01**	**<0.05**				
T1DM vs. Controls	**<0.01**	**<0.01**				
T2DM vs. Controls	**<0.01**	**<0.01**				
SD; Standard Deviation of mean, sig: significant**.** P. value (≤ 0.05), (≤ 0.01).						

4. Discussion

The IL 37 is of the IL 1 family. It has anti inflammatory properties. It appears widely. IL 37 is usually expressed in granule cells and T cells, with the best degree of statement in regulatory T cells (Treg cells) (Alhayali, Yücel and Ashoor, 2021). In this study, the comparison of İL 37 level between different experiment groups, IL-37 was significantly higher (P<0.01) in patients with T1DM and T2DM when compared with the control group. This finding is similar to the findings of Alhayali et al., who found that the mean of IL-37 was higher in patients with DM than in control healthy (Alhayali, Yücel and Ashoor, 2021). In our study, significant high differences were found between the study groups examined in terms of serum IL 37 levels (P < 0.01). Serum IL 37 levels were highest in DFU of T2DM patients and lowest in the healthy control group; the reason for this can be explained by a study in the literature showing that IL 37 administration, at least by reducing local and systemic inflammation, correct the established metabolic disorders caused by diabetic and thus contributes to improved systemic insulin sensitivity. In addition, IL 37 causes a decrease in proinflammatory cytokines. In addition, in diabetics, the production of pro-inflammatory cytokines and chemokines increases, which can attract and activate macrophages and other immune cells; this causes chronic low grade inflammation and promotes diabetic complications (Jia, Liu and Han, 2018).

In the present study, comparing IL-38 among the studied groups revealed that T1DM and T2DM patients had higher levels of IL-38 than controls. Likewise, the level of IL-38 was higher in DFU of T2DM compared with other diabetic groups. These results are consistent with a study (Gurău *et al.*, 2021), which found that plasma IL-38 was more elevated in T2DM patients.

On the other hand, this study's findings contrast with those reported by (Zhao *et al.*, 2020). These findings illustrated that Serum IL-38 levels in T2DM patients were significantly lower than those in controls.

Another study by (Yu *et al.*, 2017) in studied on gestational diabetes showed that IL-38 was increased 3.3, 2.6, or 2.6 fold in chorionic villi (P<0.01), umbilical artery (P<0.05), umbilical vein (P<0.05) from GDM women, respectively, compared to that from non-GDM women and herein, IL-38 produced in the chorionic villi and umbilical

cords may be a response to local inflammation during the development of GDM. Thus, such a dysregulated micro-environment may contribute to the development of GDM via an immune-mediated mechanism.

IL-17, a pro-inflammatory cytokine, has been studied in the development of diabetes. The present data revealed a highly significant increase in IL17A serum levels in all diabetic group patients compared with the healthy group. It is believed that IL17A had a crucial role in the development of diabetic mellitus and its complications, such as DFU. The DFU group of T2DM also showed higher mean levels. These findings are consistent with the studies of Parhi et al., which showed an increase in the level of IL-17 in newly diagnosed diabetes than the healthy controls (Parhi *et al.*, 2019), and one of the possible mechanisms for that is the binding of IL17A with its receptor may enhance activation of metalloproteinase, hypertensive and vascular dysfunction. Another mechanism is the activation of the JAK/STAT pathway that leads to hepatic insulin resistance, beta and liver cell apoptosis, and downregulation of gluconeogenesis-related molecules (Yousefidaredor *et al.*, 2014).

Likewise, the level of IL 17A was even higher in the group of patients with diabetic complications (DFU); this finding is in corroboration with Yousefidaredor et al., who found that IL 17 plays a vital role in the development of T2DM and its complications via the up-regulation of several inflammatory molecules including angiotensin II type I receptor and JAK 2 STAT 3 pathway related molecules ((Yang and Jiang, 2022), (Yuan *et al.*, 2022)). Thus, higher IL17A levels in patients may cause inflammation in the ulceration, deterioration of skin integrity, and various types of bacteria causing infection (Kaleli *et al.*, 2019). These results indicate that IL17A is a contributory factor to the inflammatory process in T2DM and its complications (Kaminski *et al.*, 2019).

5. Conclusion

Iraqi DM subjects with and without complications had higher values of interleukins, IL-37, IL-38, and IL-17A, than healthy controls, which suggests an inflammatory state in these patients. Also, the DFU of T2DM patients illustrated higher levels of interleukins than other diabetic groups, so they might be using them as novel therapeutic targets for preventing and treating DM and preventing organ damage. However, these novel markers (IL-37, IL-38, and IL-17A) might be associated with the progression of diabetes mellitus and may be used as markers of inflammation, progression, and complications in patients with DM.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

The Role of Lepidium Sativum as Free Radical Scavenger in Laboratory Mice

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Received: 2023/11/12

Accepted: 2023/12/22

Published: 2024/01/04

Keywords*: *Garden Cress Seeds, Lepidium Sativum, Free Radical Scavenger

DOI:10.62472/kjps.v14.i23.125-131

Abstract

Background: Lepidium sativum is a by-product remaining after the oil is extracted from Garden cress seeds. This herb is considered one of the popular medicinal herbs used in Arabian countries and has traditionally been used to control many clinical problems. The present study aims to uncover these seeds' free radical-scavenging properties and their Role in improving the testicular tissues and seminal properties.

Material and Methods: Thirty-two sexually mature mice were used, and the treated groups were divided into three groups; the first one (G1) was treated with 0.1 ml of GC seed extraction in a dose of 5mg/kg BW, the second one(G2) was treated with 0.1 ml sodium nitrate as oxidant, and the last treated group (G3) was treated with 0.1 ml of GC seed extraction after one hour of injection of 0.1 ml sodium nitrate.

Results: The results showed improvement of the testicular tissue and a significant elevation in sperm viability, in addition to significant enhancements of RBC count and Hb concentration. By its Role as a free radical scavenger, sodium nitrate removed the negative effect on blood parameter values.

دور بذور الرشاد ككاسح للجذور الحرة في فئران المختبر

الخلاصة

الخلفية: الليبيديوم ساتيفوم هو منتج ثانوي يبقى بعد استخلاص الزيت من بذور حب الرشاد. تعتبر هذه العشبة من الأعشاب الطبية الشعبية المستخدمة في الدول العربية وقد تم استخدامها تقليديا للسيطرة على العديد من المشاكل السريرية. تهدف الدراسة الحالية إلى الكشف عن خصائص هذه البذور في مكافحة الجذور الحرة ودورها في تحسين أنسجة الخصية وخواص الحيوانات المنوية.

المواد والطرق: تم استخدام اثنين وثلاثين فأراً ناضجاً جنسياً، وتم تقسيم المجموعات المعالجة إلى ثلاث مجموعات؛ عولجت المجموعة الاولى ب ٠,١ مل من مستخلص بذور الرشاد بجرعة ٥ ملغم /كغم من وزن الجسم اما المجموعة الثانية عوملت ب ٠,١ مل من نترات الصوديوم كمادة مؤكسدة، بينما المجموعة الثالثة فعوملت ب ٠,١ مل من مستخلص بذور الرشاد بعد ساعة واحدة من حقنها ب ٠,١ مل من نترات الصوديوم

النتائج: أظهرت النتائج تحسنا ملحوظا في أنسجة الخصية وارتفاعا ملحوظا في حيوية الحيوانات المنوية، بالإضافة إلى تحسن كبير في عدد كرات الدم الحمراء وتركيز الهيموجلوبين. من خلال دورها ككاسح للجذور الحرة، أزالت نترات الصوديوم التأثير السلبي لنترات الصوديوم على قيم معاملات الدم.

1. Introduction

Lepidium sativum is locally known as 'garden cress' (GC). The plant and its seeds are considered popular medicinal herbs used in many Arabic countries as a good mediator for bone fracture healing in the human skeleton (Dixit Jr *et al.*, 2020).

Several recent studies pointed out the traditional uses of Lepidium sativum seed extract in controlling many clinical problems. They were used as anti-asthmatics, antiscorbutics, aperients, diuretics, galactagogues, poultices, and stimulants. The leaves are antiscorbutic, diuretic and stimulant (Ejigu and Endalifer, 2023).

Lepidium sativum has been studied pharmacologically for its laxative (3), antibacterial (4), bronchodilatory (5), contraceptive effects (6), and inflammatory bowel disease ( ). The seedlings contain a significant amount of protein, fat, iron, calcium, folic acid, and vitamins A and C. (Doke and Guha, 2014)

The present investigation was undertaken to screen these seeds' free radical scavenging activity and their Role in activating epididymal sperms.

2. Material and Methods

The Lepidium sativum seeds were purchased from local Mark T. They were cleaned manually to remove dirt, dust, and extraneous mats and were ground with electrical bland r. Oily extracted preparation using hot continuous Soxhlet extraction meth d. ( ). The extract was stored in a dark container in a refrigerator until needed.

Thirty-two sexually mature mice aged 6-7 weeks were used (16 male and 16 female) divided as follows:

1. Control group: 4 males and four females were injected intraperitoneally with normal saline and served as a control group.
2. G1: 4 males and four females were injected intraperitoneally with 0.1 ml of GC seed extraction in a 5mg/kg BW dose.
3. G2: 4 males and four females were injected intraperitoneally with 0.1 ml sodium nitrate as an oxidant.
4. G3: 4 males and four females were injected intraperitoneally with 0.1 ml of GC seed extraction in a dose of 5mg/kg BW after one hour of injecting 0.1 ml sodium nitrate.

The experiment lasted for 21 days, and at the end of the treatment period, the specimens were collected from the mice, including blood for measuring the blood values, epididymis for seminal analysis, and tests for studying the histological changes.

Statistical analysis: experimental data were analyzed using one-way ANOVA

The P-values less than 0.05 were considered statistically significant.

3. Results

The injection of GC seed oil extraction significantly elevated RBC count and Hb concentration but had no effect on WBC compared with the control group Table 1.

Table 1: Effect of Garden Cress Seeds Extract on Blood Parameters in Mice

Groups	RBC count Cell / cmm*10^6	WBC count Cell / cmm*10^3	Hb g / dl
G1 Control group	3.16 ±0.02 B	6.56 ± 0.11 A	12.2 ± 0,89 B
G2 Treated with GCS extract	3.34 ± 0.16 A	6.52 ± 0.22 A	13.9 ± 1.04 A

***Different Letters Represent Significant Differences at (P≤0.05)**

The blood parameters values (RBC, Hb, and WBC) were suppressed significantly ($p \leq 0.05$) by injection of sodium nitrate as an oxidant. The blood parameters return to their average values by the effect of GC seed oil extraction when injected one hour after sodium.

Nitrate administration Table 2.

Table 2: Effect of Garden Cress Seed Extraction as ROS Scavenger

Groups	RBC count cell / cmm*10^6	WBC count cell / cmm*10^3	Hb g / dl
Control group	6.33 ±0.18 A	3.96 ± 0.29 A	13.42 ± 0.43 A
G3 Treated with sodium nitrate	5.2 ± 0.10 B	2.62 ± 0.1 B	7.1 ± 0.04 B
G4 Treated with GCS extract after 1h of sodium nitrate injection	6.65 ± 0.03 A	3.2 ±0.24 A	11.45 ± 0.63 A

***Different letters represent significant differences at (p≤0.05)**

It seems that the injection of GC seeds oil extraction caused significant ($p \leq 0.05$) elevation in testes weight and sperm count, whereas the dead and deformed sperms number significantly ($p \leq 0.05$) decreased compared to that of normal animals, but it wasn't any effect neither on the viability of the sperms nor the individual and massive movement of the sperms (table 3)

Table 3: Seminal Analysis of Male Mice Treated with GC Seed Oil Extraction

Groups	Testes Weight(G)	Viable Sperms	Dead Sperms	Deformed Sperms	Sperms Count	Individual Movement	Massive Movement
Control Group	0.3 ± 0.07 B	91.24 ± 9.1 A	11.5 ± 29.2 A	13.5 ± 8.1 A	194.8 ± 0.54 B	90 ± 0.00 A	90 ± 0.00 A
Treated Group	0.62 ± 0.08 A	92.75 ± 1.2 A	8.5 ± 11.1 B	5.5 ± 11.1 B	344.5 ± 57.16 A	90 ± 0.00 A	90 ± 0.00 A

***Different letters represent significant differences at (p≤0.05)**

4. Histological Changes

Injecting 5 mg/kg BW of G C seed oil extraction into male mice showed improvement in the testicular tissue, represented by narrowing of the testicular lumen by increasing spermatogenesis processes and, therefore, an increase in sperm numbers (Figure 2), as compared with the testicular tissue of control male mice Figure 1.

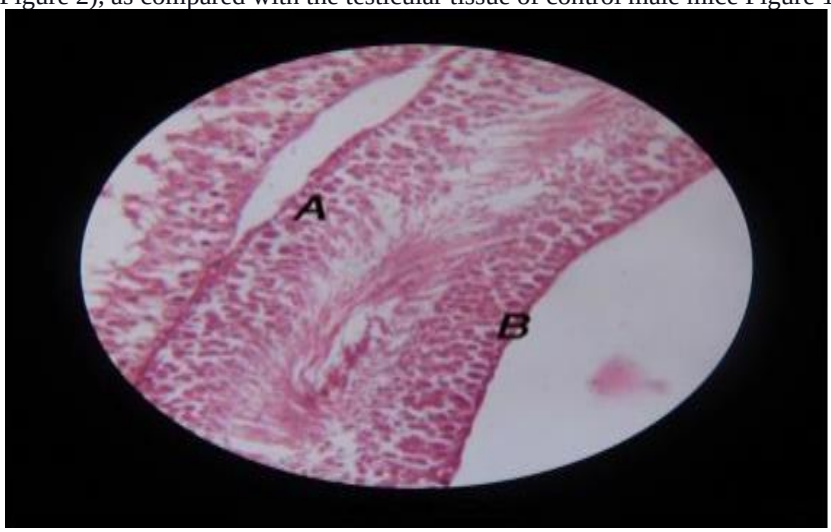

Figure 1: Histological Changes of Testes from The Control Group Show Normal Sperms (A) and Normal Connective Tissue (B. H&E, 40x)

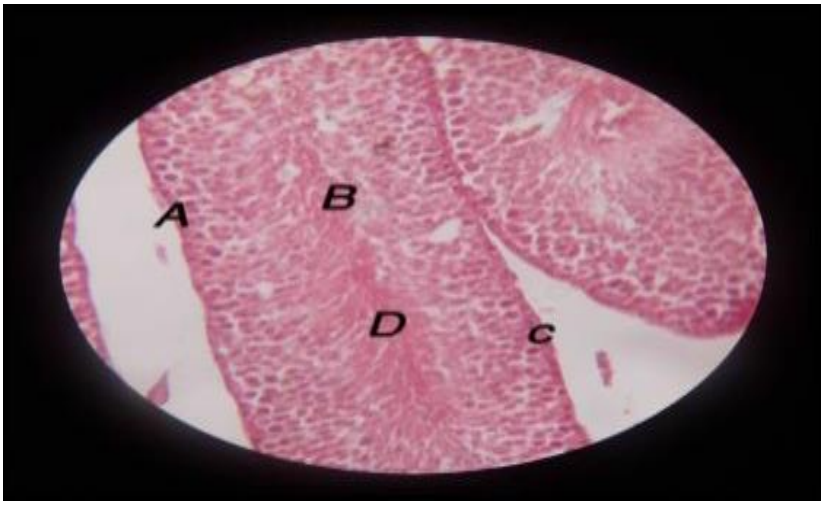

Figure 2: Histological Changes in Testes from Male Mice Treated with GC Seed Extract 5mg/Kg B W Show Normal Connective Tissue (A), Increase in The Number of Sperms (B), Increase in Spermatogenesis (C), And Narrowing of The Testicular Lumen (D). H&E, 40x

5. Discussion

The results showed a significant increase in RBC count and Hb concentration when the GC seed oil extraction was administered to the animals. The blood parameter values were reduced significantly due to sodium nitrate injection. It seems that sodium nitrate increases the production of reactive oxygen species (ROS) (Halliwell *et al.*, 1997) that attack living cells and tissues, including the RBC and WBC (Sikka, 2004).

Increased generation of ROS causes lipid oxidation of the cell membrane of spermatozoa. Still, antioxidant vitamins play an essential role in protecting the cell from being damaged by ROS (Agarwal and Sekhon, 2010) and thus affect reproductive efficiency (Al-Aubody and Al-Diwan, 2014). The GC seed oil extraction contained flavonoids and phenols, considered potent antioxidants (Kasabe *et al.*, 2012). This explains the reason behind the improvements of the blood parameters values after significantly reduced by sodium nitrate due to the ability of the flavonoids to scavenge the ROS (Braun and Cohen, 2015)and may interfere with free radical formation. (Braun and Cohen, 2015)The improvements of the testicular tissue (figure 2) and the significant elevation in sperm count were due to the GC seed oil extraction containing the number of antioxidant vitamins A, E, and C (8, 17, and 1). It has been known that vitamin E improves semen viability and testicular tissue repair (Verma and Kanwar, 1999).

The combination of flavonoids and vitamin C increases both compounds' effectiveness (Mathiesen *et al.*, 1996). They delay the onset of lipid peroxidation (de Whalley *et al.*, 1990). Vitamin C is a major chain-breaking antioxidant that neutralizes hydroxyl, superoxide, and hydrogen peroxide radicals, preventing sperm agglutination (Agarwal and Sekhon, 2010). In addition, it also helps recycle vitamin E (Sies, Stahl and Sundquist, 1992) and also has a role in stimulating spermatogenesis (Acharya *et al.*, 2008)

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Kerbala Journal of Pharmaceutical Sciences

DOI: 1032.25345.v.12-23 / **ISSN**: 2221-7207

Nurse's Knowledge Regarding Active Management of The Third Stage of Labor to Control Postpartum Hemorrhage

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Received: 2023/12/01

Accepted: 2023/12/22

Published: 2024/01/04

Keywords: *Nurses Knowledge, Active Management of Third Stage of Labor, Active Third Stage of Labor, Postpartum Hemorrhage*

DOI:10.62472/kjps.v14.i23.132-140

Abstract

Background: Postpartum hemorrhage is a leading cause of maternal mortality and morbidity globally. The third stage of labor begins after the baby is born and ends with the delivery of the placenta. During this stage, the uterus needs to contract effectively to prevent excessive bleeding. Active Management of the Third Stage of Labor was introduced as a strategy to reduce the risk of postpartum hemorrhage. The study aimed to investigate nurses' knowledge of active management of the third stage of labor to control postpartum hemorrhage and investigate associated factors.

Results: The study examined participant characteristics, revealing an average age of 35.93 (SD=8.52). Around 65.0% of nurses in the study had graduated from preparatory nursing. Most nurses (56.7%) had 5-10 years of hospital experience. A majority (65.0%) worked in postpartum areas. Over half (58.3%) had 4-6 years of experience without training. The study evaluated nurses' knowledge of active management of the third stage of labor, with responses on a scale ranging from 37-64. The overall expertise indicates that 75.0% of nurses needed a better understanding. The results highlighted that factors like nurses' education level (p= .024) and training courses (p= .041) predicted their knowledge of active management of the third stage of labor.

Conclusions: The study found that nurses' knowledge of active management of the third stage of labor varied, overall indicating a poor understanding of nurses. The results highlighted significant predictors of this knowledge, including education level and participation in training courses. To enhance nurses' proficiency in active management of the third stage, it is recommended to focus on targeted educational programs and regular training opportunities.

معرفة الممرضات فيما يتعلق بالإدارة الفعالة للمرحلة الثالثة من المخاض للسيطرة على نزيف ما بعد الولادة

نور علي محمد، ساجدة سعدون عليوي

الملخص

الخلفية

النزيف ما بعد الولادة هو أحد الأسباب الرئيسية لوفيات ومراضة الأمهات على مستوى العالم. يبدأ المرحلة الثالثة من الولادة بعد ولادة الطفل وتنتهي مع ولادة المشيمة. خلال هذه المرحلة، يحتاج الرحم إلى الانقباض بفعالية لمنع النزيف المفرط. تم تقديم الإدارة النشطة للمرحلة الثالثة من الولادة كاستراتيجية للحد من خطر النزيف ما بعد الولادة. هدفت هذه الدراسة إلى التحقيق في معرفة الممرضات بالإدارة النشطة للمرحلة الثالثة من الولادة للتحكم في النزيف ما بعد الولادة ودراسة العوامل المرتبطة بذلك.

النتائج

فحصت الدراسة خصائص المشاركين، وكشفت عن متوسط عمر يبلغ ٣٥,٩٣ عامًا (الانحراف المعياري=٨,٥٢). حوالي ٦٥,٠٪ من الممرضات في الدراسة تخرجن من برامج التمريض التحضيرية. معظم الممرضات (٥٦,٧٪) لديهن خبرة في المستشفى تتراوح بين ٥-١٠ سنوات، و٦٥,٠٪ منهن يعملن في مناطق ما بعد الولادة. أكثر من نصف الممرضات (٥٨,٣٪) لديهن خبرة تتراوح بين ٤-٦ سنوات بدون تدريب إضافي. تم تقييم معرفة الممرضات بالإدارة النشطة للمرحلة الثالثة من الولادة على مقياس يتراوح بين ٣٧-٦٤. أشارت النتائج الإجمالية إلى أن ٧٥,٠٪ من الممرضات لديهن فهم غير كافٍ للإدارة النشطة للمرحلة الثالثة من الولادة. أظهرت النتائج أن العوامل مثل مستوى تعليم الممرضات (p = .024)والدورات التدريبية (p = .041) كانت تتنبأ بمعرفتهن بالإدارة النشطة للمرحلة الثالثة من الولادة.

الاستنتاجات

وجدت الدراسة أن معرفة الممرضات بالإدارة النشطة للمرحلة الثالثة من الولادة متباينة، مما يشير بشكل عام إلى فهم ضعيف لدى الممرضات. أبرزت النتائج العوامل الهامة التي تتنبأ بهذه المعرفة، بما في ذلك مستوى التعليم والمشاركة في الدورات التدريبية. لتحسين كفاءة الممرضات في الإدارة النشطة للمرحلة الثالثة، يُوصى بالتركيز على البرامج التعليمية الموجهة وفرص التدريب المنتظمة.

1.Introduction

The active management of the third stage of labor (AMTSL) is a critical obstetric intervention aimed at preventing postpartum hemorrhage (PPH), which is a leading cause of maternal mortality and morbidity worldwide (Bishanga *et al.*, 2018). Nurses play a vital role in implementing and overseeing AMTSL to effectively control PPH and ensure the well-being of both the mother and the newborn (Ramesh *et al.*, 2018). The third stage of labor begins after the baby's delivery and concludes with the delivery of the placenta and its associated membranes (Essa and Ismail, 2015). AMTSL involves a set of coordinated interventions designed to expedite the delivery of the placenta, minimize blood loss, and reduce the risk of PPH. This proactive approach includes administering uterotonic medications, controlled cord traction, and uterine massage(Jonas *et al.*, 2017).

Nurses at the forefront of patient care during childbirth possess a crucial understanding of AMTSL. They are well-versed in administering uterotonic agents, such as oxytocin or misoprostol, which stimulate uterine contractions, thereby aiding in the prompt separation and expulsion of the placenta(Altraigey *et al.*, 2019). Nurses are skilled in monitoring the mother's vital signs and uterine contractions to gauge the effectiveness of these medications and ensure the prevention of excessive bleeding (Ramesh *et al.*, 2018). Additionally, nurses are knowledgeable about controlled cord traction, a technique employed to facilitate the controlled removal of the placenta. This technique reduces the risk of uterine atony and retained placental fragments, which can contribute to PPH. By employing controlled cord traction, nurses can assist in delivering the placenta while ensuring minimal blood loss(Ramavhoya, 2018). Uterine massage is another component of AMTSL that nurses are well-informed about. After the placenta is delivered, nurses gently massage the uterus to enhance uterine contraction, thereby reducing the risk of postpartum bleeding. This massage aids in promoting uterine tone and preventing atony, a primary cause of PPH(Ramavhoya, 2018). Nurses' knowledge of AMTSL extends beyond the procedural aspects. They understand the importance of clear communication with the mother, involving her in decision-making, and providing reassurance throughout the process. Nurses can identify potential complications or deviations from the norm, enabling timely interventions to prevent or manage excessive bleeding(Abisogun, 2019). Nurses are a cornerstone of successful AMTSL implementation and are pivotal in preventing postpartum hemorrhage. Their comprehensive understanding of the procedure, medications, techniques, and patient communication ensures that childbirth is a safe and positive experience for both the mother and her newborn. By effectively applying their knowledge of active management of the third stage of labor, nurses contribute significantly to maternal well-being and reduce the incidence of postpartum hemorrhage.

2. Methods

2.1 Study Design

To fulfill the objectives of this study, a quasi-experimental research design was employed. This design incorporated an adopted pre- and post-test methodology for both the study and control groups. The study was conducted from December 1st, 2021, to July 3rd, 2022.

2.2 Study Instruments

The questionnaire is valuable for gathering essential data to achieve the study's anticipated outcomes. In pursuit of this, the researcher has meticulously crafted this questionnaire to elucidate the study's aims and significance. This is achieved by systematically obtaining responses to the core inquiries posed by the study.

The questionnaire comprises two distinct sections, each tailored to gather data from participants involved in the study efficiently. The initial section captures socio-demographic variables such as age, educational attainment, years of experience, familiarity with labor wards, and participation in training sessions.

Concurrently, the subsequent section delves into assessing the participants' comprehension of the Active Management of the Third Stage of Labor for the Control of Postpartum Hemorrhage. The researcher meticulously constructed this segment after an exhaustive review of pertinent literature. The section encompasses 35 multiple-choice questions designed to gauge participants' knowledge. Stringent adherence to established guidelines in questionnaire design was maintained to ensure the richness and comprehensiveness of the information obtained. The researcher placed particular emphasis on crafting precise questions devoid of ambiguity, thus enabling trustworthy and insightful responses.

The questions were structured in a closed format to facilitate clarity and ease of response. Respondents are prompted to answer while referencing relevant and appropriate information. This approach ensures that the data collected is reliable and relevant, facilitating a robust analysis of the research problem.

2.3 Study Sitting

The study was undertaken at the Obstetrics and Gynecology Teaching Hospital in Karbala Province Holy, which was strategically selected as the primary site for data gathering to ensure the acquisition of accurate and exhaustive data.

2.3 Validity

Although the scales are valid, the questionnaire was forwarded to 9 experts in problem-related specializations from several colleges in Iraq using a content validity approach to make it more valid.

2.4 Pilot Study

This pilot study was undertaken to evaluate the research tool's reliability, clarity, effectiveness, and consistency, all of which were confirmed. Additionally, it aimed to estimate the average time required for data collection per subject through interview processes and identify potential difficulties.

3. Reliability of the Questionnaire:

Reliability of study tools refers to their ability to yield consistent results when used repeatedly with the same individuals over time. A random sample of 10 nurses participated in a test-retest reliability assessment without awareness of their role as a stability gauge for the tool. Notably, these individuals were excluded from the primary study sample. As demonstrated below, the Cronbach's Alpha coefficient was utilized to calculate the confidence level.

4. Sample of the Study

A purposive non-probability sampling method was employed to select a cohort of 60 nurses, ensuring representative and precise data acquisition.

4.1 Criteria for Sample Selection

4.2 Inclusion criteria

Nurses who have scored below 60% in the assessment are eligible.

4.3 Exclusion Criteria

Nurses who are selected for the pilot study

4.4 Data Collection Methods

The implementation phase extended from January 5th, 2022, to April 29th, 2023.

5. Statistical Data Analysis:

5.1 Data Analysis

The data were analyzed and interpreted using the application of Statistical Package for Social Sciences (SPSS), version 26.

6. Results

Table 1: Distribution Of Study Sample by Their Socio-Demographic Variables (SDVS)

SDVs	Classification	No.	%
Age	20-29 years old	26	43.3
	30-39 years old	18	30.0
	40-49 years old	13	21.7
	50 and older	3	5.0
	M ± SD	**35.93 ± 8.52**	
Education Level	School nursing	4	6.7
	Preparatory nursing	39	65.0
	Diploma nursing	16	26.7
	BSc. Nursing	1	1.7
Experience in Hospital	<5 years	15	25.0
	5-10 years	34	56.7
	>10 years	11	18.3
Workplace	Maternity	21	35.0
	Postpartum	39	65.0
Experience in Workplace	1-3 year	20	33.3
	4-6 years	35	58.3
	>6 years	5	8.3
Training Courses	Yes	25	41.7
	No	35	58.3

***No.= Number; %= Percentage**

The study revealed participant characteristics, showing an average age of 35.93 (SD=8.52). Regarding education, 65.0% of the study group nurses had graduated from preparatory nursing. Experience in the hospital ranged from 5-10 years for 56.7% of nurses. A majority of nurses, 65.0%, worked in postpartum areas. Regarding workplace experience, over half 58.3 of nurses had 4-6 years of experience without training.

Table 2: Nurses' Knowledge Regarding Active Management of The Third Stage of Labor

Scale	Min.	Max	M	SD	Score	No.	%
Knowledge Scale (35 Q)	37	64	41.43	9.37	Poor (35-46.66)	45	75.0
					Moderate (46.67-58.33)	12	20.0
					Good (58.34-70)	3	5.0
					Total	60	100.0

***Min.: Minimum; Max.: Maximum, M: Mean for Total Score, SD=Standard Deviation for Total Score**

Results indicate that the nurse's responses on knowledge of active management of the third stage scale ranged from 37-64 by the overall responses at a total mean score equal to 41.43 (SD=9.37), and according to the study criteria, this indicated that the (75.0%) of nurses indicate a poor level.

Table 3: Simple Linear Regression Among the Study Variables in Predicting the Nurse's Knowledge

Variables	Unstandardized Coefficients		Standardized Coefficients	t	*Sig.*
	B	Std. Error	Beta		
Age	.162	.104	.265	1.548	.128
Education Level	.305	.131	.322	2.321	.024
Experience in Hospital	-.051-	.160	-.061-	-.322-	.749
Workplace	.085	.148	.073	.577	.566
Workplace Experience	.026	.132	.027	.194	.847
Training Courses	.228	.146	.224	1.189	.041

Dependent Variable: Nurses Knowledge

The results have substantiated that factors such as the nurses' education level (β=2.321; p= .024) and training courses (β = 1.189; p= .041) are predictors for their knowledge of active management of the third stage of labor.

7. Discussion

The study focuses on nurses' knowledge regarding the active management of the third stage of labor to control postpartum hemorrhage and the factors associated with this knowledge. The study sheds light on various participant characteristics that could influence their understanding and application of active management techniques.

The average age of the participating nurses was 35.93 years, with a standard deviation of 8.52. This indicates that the participants had a relatively diverse age range, which might contribute to varying levels of experience and knowledge. 65.0% of the nurses in the study had graduated from preparatory nursing programs. This suggests that a significant proportion of the nurses might have received a foundational nursing education, which could impact their familiarity with advanced practices like active management of the third stage of labor. 56.7% of the nurses had 5-10 years of experience in the hospital. This indicates that many participants had a moderate experience level, which could influence their exposure to different clinical scenarios and practices. The majority (65.0%) of the nurses worked in postpartum areas. This suggests that the study focused on a group of nurses who were likely to be directly involved in maternal care, which makes their knowledge of active management of the third stage of labor particularly relevant. A significant proportion (58.3%) of nurses had 4-6 years of experience without specific training. This could mean that many nurses have accumulated a fair amount of general experience but may have yet to receive specialized training in active management techniques.

The study's findings could be interpreted as nurses with a more extended experience might better understand various clinical practices, including active management. Nurses working in postpartum areas might have more exposure to scenarios where active management is relevant, potentially leading to better knowledge. Those who have yet to receive specific training despite having some experience might exhibit gaps in their understanding, especially regarding advanced practices.

Overall, the study provides insights into nurses' characteristics and potential influence on their knowledge of active management of the third stage of labor. This information can help healthcare institutions design targeted interventions and training programs to improve nurses' understanding and application of these crucial techniques to prevent postpartum hemorrhage.

The analysis of the collected data revealed that the nurses' responses on the knowledge of active management of the third stage scale ranged from 37 to 64. The overall reactions had a mean score of 41.43 with a standard deviation (SD) of 9.37. According to the study criteria, this mean score indicated that approximately 75.0% of the nurses needed better knowledge regarding active management of the third stage. This study's findings highlight the nurses' knowledge levels regarding active management of the third stage of labor. The mean score of 41.43 suggests that a significant portion of the nurses surveyed have inadequate knowledge in this critical area of obstetric care. This has potential implications for patient safety and outcomes, as improper management of the third stage can lead to postpartum hemorrhage, a leading cause of maternal morbidity and mortality. Several factors could contribute to the observed poor knowledge levels among nurses. These factors might include insufficient training and education on active management techniques, lack of exposure to updated guidelines and protocols, and limited opportunities for continuous professional development in maternity care. Addressing these factors through targeted educational interventions and training programs could improve knowledge and patient care [(Bhutia, Shadap and Pangambam, 2018)- (Angelina, Stephen and Ipyana, 2021)].

Nurses' Education Level (β=2.321; p=0.024): This part of the results suggests that nurses with a higher education level tend to have higher knowledge scores regarding the active management of the third stage of labor. The coefficient (β) of 2.321 indicates that for every unit increase in the nurses' education level, their knowledge score is predicted to increase by 2.321 units. The p-value of 0.024 suggests that this relationship is statistically significant, meaning it's unlikely to have occurred by chance. Training Courses (β=1.189; p=0.041): This part of the results suggests that nurses who have undergone training courses related to the active management of the third stage of labor tend to have higher knowledge scores. The coefficient (β) of 1.189 indicates that for every unit increase in the extent of training course completion, the nurses' knowledge score is predicted to increase by 1.189 units. The p-value of 0.041 suggests that this relationship is statistically significant as well. In both cases, the p-values below the conventional significance level of 0.05 indicate that these relationships are likely not due to random chance, and there is evidence to support that education level and training courses are associated with nurses' knowledge about active management of the third stage of labor.

These findings suggest that improving nurses' education levels and providing relevant training courses can enhance their knowledge and competence in actively managing the third stage of labor. However, it's important to remember that these results are based on the data and statistical analysis provided, and it's always a good practice to consider the context and limitations of the study before drawing firm conclusions.

Previous studies have delved into similar associations between healthcare professionals' characteristics and knowledge or performance. For instance, the impact of education levels on nurses' knowledge of pain management techniques. The results aligned with the current study, showing that higher education levels were positively correlated with increased knowledge and competence in pain management practices(Angelina, Stephen and Ipyana, 2021). Likewise, the influence of training programs on healthcare professionals' proficiency in patient communication. This study found that participation in communication-focused training courses improved healthcare providers' communication skills, leading to more effective patient interactions. The results align with the present research, highlighting the positive impact of training courses on nurses' knowledge of active management during the third stage of labor(McCutcheon *et al.*, 2015).

The current study contributes to the growing body of literature recognizing the significance of nurses' education level and training courses in predicting their knowledge of active management during the third stage of labor. These findings underline the importance of continuous professional development and tailored training initiatives to ensure that healthcare providers are well-equipped to provide high-quality care during childbirth.

8. Conclusion

The study found that nurses' knowledge of active management of the third stage of labor varied, overall indicating poor understanding. The results highlighted significant predictors of this knowledge, including education level and participation in training courses. To enhance nurses' proficiency in active management of the third stage, focusing on targeted educational programs and regular training opportunities is recommended.

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Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

Medical Comorbidities and Risk Of COVID-19 Severity

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Received: 2023/11/13

Accepted: 2023/12/22

Published: 2024/01/04

Keywords: *COVID-19, SARS-2, Comorbidities, Severity*

DOI:10.62472/kjps.v14.i23.141-150

Abstract

Background: COVID-19 is a disease caused by SARS-CoV-2 that can cause respiratory infections. It may affect the sinuses, the upper airways of the nose and throat, or the lower airways of the trachea and lungs. Infections range from mild to deadly. Older people and people of all ages with medical comorbidities such as hypertension and diabetes may have a poorer prognosis and be at greater risk for hospital and intensive care unit admissions.

Objectives: The present study aimed to determine the effects of pre-COVID-19 comorbidities on the progression of disease severity and outcome in Iraqi patients.

Method: This descriptive cross-sectional study was carried out on COVID-19 patients using an online electronic questionnaire in Google form all over Iraq from September 2021 to July 2023. The total number of participants was 789, of whom approximately 282 were excluded, and the net was 507; they received an online questionnaire,

The questionnaire literature review was completed after debuting in Arabic after a thor. This self-administered questionnaire consisted of 15 closed-ended questions and six open questions.

Result: < 40 was the highest group affected by COVID-19, and those> 60 years were the most minor group involved. Females are affected more than males. Most of the cases were from the middle region of Iraq. 87.8% of participants have no smoking history. Fever, headache & fatigue were the most prevalent symptoms of patients. Past medical history of hypertension presented in 11.8% of patients, while diabetes mellitus represented 9%, asthma 5.5%, and cardiovascular diseases 4.9%. Other comorbidities were present in lower percentages. The history of hospitalization was positive in 37 patients with past medical comorbidities. Of those patients who were admitted to the hospital, 18 required Intensive care unit admission. Among them, 7 had hypertension, 4 had asthma & 3 had diabetes mellitus. Steroid treatment was given to 9% of patients with COVID-19.

Conclusion: The need for hospitalization and Intensive care unit admission was higher among COVID-19 patients with comorbidities, especially hypertension, followed by diabetes, then asthma, and cardiovascular diseases.

الأمراض الطبية المتزامنة ومخاطر خطورة كوفيد-١٩ سوسن محمد جبار الحسناوي , ضياء هادي جواد

الملخص

الخلفية: كوفيد-١٩ هو مرض يسببه SARS-CoV-2 ويمكن أن يسبب التهابات الجهاز التنفسي. وقد يؤثر على الجيوب الأنفية، أو الممرات الهوائية العلوية للأنف والحنجرة، أو الممرات الهوائية السفلية للقصبة الهوائية والرئتين. وتتراوح حالات العدوى من خفيفة إلى مميتة. قد يكون لدى كبار السن والأشخاص من جميع الأعمار الذين يعانون من أمراض مصاحبة طبية مثل ارتفاع ضغط الدم والسكري تشخيصًا تكهنيا سيئًا ويكونون أكثر عرضة لدخول المستشفى ووحدة العناية المركزة.

الأهداف: هدفت الدراسة الحالية إلى تحديد آثار الأمراض المصاحبة لما قبل فيروس كورونا (COVID-19) على تطور شدة المرض ونتائجه لدى المرضى العراقيين.

الطريقة: هذه دراسة مقطعية وصفية تم إجراؤها على مرضى كوفيد-١٩ باستخدام استبيان إلكتروني عبر الإنترنت في نموذج جوجل في جميع أنحاء العراق من سبتمبر ٢٠٢١ إلى يوليو ٢٠٢٣. وكان العدد الإجمالي للمشاركين ٧٨٩، منهم حوالي ٢٨٢ تم استبعادهم، وبلغ صافيهم ٥٠٧، تلقوا استبياناً إلكترونياً، وقد تم الاستبيان بعد مراجعة شاملة للأدبيات وتم توزيعه باللغة العربية على المشاركين. يتألف هذا الاستبيان الذاتي من ١٥ سؤالاً مغلقًا و٦ أسئلة مفتوحة.

النتيجة: كانت الفئة العمرية أقل من ٤٠ عامًا هي الفئة الأكثر تأثراً بـ COVID-19وكانت الفئة العمرية الاكبر من ٦٠ عامًا هي المجموعة الأقل تأثراً. وتتأثر الإناث أكثر من الذكور. وكانت معظم الحالات من المنطقة الوسطى من العراق. ٨٧,٨٪ من المشاركين ليس لديهم تاريخ للتدخين. وكانت الحمى والصداع والتعب من أكثر الأعراض شيوعا لدى المرضى. التاريخ الطبي السابق لارتفاع ضغط الدم يظهر في ١١,٨٪ من المرضى بينما يمثل داء السكري ٩٪، والربو ٥,٥٪، وأمراض القلب والأوعية الدموية ٤,٩٪. وكانت الأمراض المصاحبة الأخرى موجودة في نسب أقل. كان تاريخ الاستشفاء إيجابيا في ٣٧ مريضا يعانون من أمراض مصاحبة طبية سابقة. ومن بين هؤلاء المرضى الذين تم إدخالهم إلى المستشفى، احتاج ١٨ مريضًا إلى دخول وحدة العناية المركزة، بينهم ٧ مصابون بارتفاع ضغط الدم، و٤ مصابون بالربو، و٣ مصابون بداء السكري. تم إعطاء العلاج الستيرويدي لـ ٩٪ من مرضى كوفيد-١٩.

الاستنتاج: كانت الحاجة إلى دخول المستشفى ووحدة العناية المركزة أعلى بين مرضى كوفيد-١٩ الذين يعانون من أمراض مصاحبة، وخاصة ارتفاع ضغط الدم يليه مرض السكري، ثم الربو وأمراض القلب والأوعية الدموية.

1. Introduction

COVID-19 is a disease caused by SARS-CoV-2 that can cause respiratory infections. It may affect the sinuses, the nose and throat's upper airways, or the trachea's and lungs' lower airways. In 2020, after the outbreak in China in December 2019, the World Health Organization specified SARS-CoV-2 as a new type of coronavirus. Like other coronaviruses, it is spread primarily through personal contact. Infections range from mild to fatal (Organization, 2022).

Seven human coronaviruses (HCoV) have been identified, four of which are common. It is
low-risk and usually causes only mild respiratory illness in healthy adults. However, they cause one-third of the common cold and can cause long-term, life-threatening diseases in at-risk people with weakened immune systems. They can cause long-term, life-threatening illness.

The other three (causing cases of MERS, SARS, and COVID-19) are known to cause more severe illness. COVID-19 disease tends to be milder than SARS and MERS but more severe than the diseases caused by the four common coronaviruses (Harrison *et al.*, 2023).

The most common symptoms of COVID-19 include fever or chills, dry cough and shortness of breath, extreme fatigue, Muscle and body aches, headaches, loss of taste and smell, sore throat, nausea and vomiting, and diarrhea. These symptoms may appear 2 to 14 days after being infected with the virus (Wu *et al.*, 2022).

There are many risk factors for COVID-19, such as older age, sex, weak immunity, and past medical comorbidities (Singh *et al.*, 2021). Older adults may have vulnerable immune systems (Landstra and de Koning, 2021).

The risk of hospitalization may increase up to 75% in patients with comorbidities such as hypertension, diabetes, cardiovascular diseases (CVD), and obesity (Statsenko *et al.*, 2022).

Certain drugs used for comorbidity treatment could interfere with angiotensin-converting enzyme 2 (ACE2) receptor expression and increase the risk of infection (Landstra and de Koning, 2021). Females have a lower risk for COVID-19 infection than males. This could be due to the effect of sex hormones, as estrogen can enhance the immune system by impacting Vitamin D. On the other hand, the activity of male sex hormones increases ACE-2 receptor expression (Singh *et al.*, 2021).

The current study aimed to assess the association between medical comorbidities and these variables among Iraqi patients to understand the role of these medical comorbidities in COVID-19 infection, whether there is more risk for disease, or effects on severity of illness and hospitalization.

2. Material and methods

2.1 Study design & Settings

This descriptive cross-sectional study was carried out on COVID-19 patients using an online electronic questionnaire in Google form throughout Iraq from September 2021 to July 2023.

2.2 Data collection and questionnaire:

The total number of participants was 789, of whom approximately 282 were excluded, and the net was 507. They received an online questionnaire. The questionnaire was prepared after a thorough literature review and distributed to the participants in Arabic. This self-administered questionnaire consisted of 15 closed-ended questions and six open questions.

When the electronic questionnaire was published on social networking sites, especially Telegram, Instagram, and Facebook, a note regarding inclusion criteria was added: Only the people for whom the PCR scan was conducted answered the questionnaire. Some people from whom we received responses and answered the questionnaire did not do the PCR, but they were excluded from the study.

2.3 Ethical Consideration

In the beginning, approval from the Department of Family and Community Medicine was obtained. The Ethical Committee of Kerbala College of Medicine/ University of Kerbala provided ethical approval for the study with referral number 71. Also, written approval from each participant was taken before answering the questionnaire. Confidentiality of the information was assured, and the privacy of respondents was maintained at a safe level.

3. Statistical Analysis

Patients' information was extracted into an Excel worksheet, and then the SPSS .chi-square test was used to compare variables. A p-value < 0.05 was considered statistically significant. The data is placed as symbols that represent it. The steps were encoding the data, putting data in the program, choosing the appropriate form, testing and analyzing the data, defining the variable data to be explored, and achieving the statistics process.

4. Result

The age group of < 40 was the highest group affected by COVID-19 (83.33%), and those> 60 years were the most minor group involved (5.1%). Females were affected more than males (71%). Most of the cases were from the middle region of Iraq (81.1%). 87.8% of participants have no smoking history. Fever, headache & fatigue were the most prevalent symptoms of patients Table 1

Table 1: Demographic and Clinical Characteristics of Patients

Variable		**No.**	**%**
Age Groups	< 40	422	83.3%
	40-49	31	6.1%
	50-59	28	5.5%
	60 & >	26	5.1%
Gender	Male	147	29%
	Female	360	71%
Region	Middle Iraq	411	81.1%
	South of Iraq East	62 16	12.2% 3.2%
	North of Iraq West	8 10	1.5% 2%
History of Smoking	Yes	62	12.2%
	No	445	87.8%
Symptoms	Fever	398	78.5%
	Cough	262	51.7%
	SOB	29	5.7%
	Rhinorrhea	12	2.4%
	Sore Throat	6	1.1%
	Headache	398	78.5%
	Red Eye	7	1.4%
	Fatigue	338	66.7%
	Diarrhea	114	22.5%
	N & V	139	27.4%
	Abdominal Pain	9	1.8%
	Constipation	4	0.8%
	Loss of Smell & Taste	322	63.5%
	Palpitation	9	1.8%
	Syncope	3	0.6%
	Vertigo	9	1.8%
	Insomnia, Mode Changes & Loss of Concentration	8	1.6%
	Skin Rash	3	0.6%

For medical comorbidities, a Past medical history of hypertension was present in 11.8% of patients, while diabetes mellitus represented 9%, asthma 5.5%, and CVD 4.9%. Other comorbidities were present in lower percentages **Table 2**.

Table 2: Medical Comorbidities Distribution Among the Study Group

Variable	**No.**	**%**
Hypertension	60	11.8%
Cardiovascular Diseases	25	4.9%
Diabetes Mellitus	46	9%
Atopy & Asthma	28	5.5%
Chronic Pulmonary Diseases	2	0.4%
Renal Diseases	5	1%
AIDS	7	1.4%

The history of hospitalization was positive in 37 patients with past medical comorbidities, which is a highly significant statistical result compared to patients with no comorbidities Table 3.

Table 3: Association of Medical Comorbidities and Hospitalization

Variables	**Hospitalized No. (%)**	**Non hospitalized No. (%)**		**Total**	***p-value***
Positive History of Medical Comorbidities No. (%)	37(25)	111(75)		**148**	**< 0.001**
Negative History of Medical Comorbidities No.	24(7.1)	314(92.9)		338	
Total	61	425		486*	

Of those patients admitted to the hospital, 18 required ICU admission. Among them, 7 had hypertension, 4 had asthma & 3 had D.M. Table 4.

Table 4: Medical Comorbidities and Hospitalization History

Variable	No.	%	ICU requirement	No.
Hypertension	60	11.8%	Yes	7
			No	53
Cardiovascular Diseases	25	4.9%	Yes	2
			No	23
Diabetes Mellitus	46	9%	Yes	3
			No	43
Atopy & Asthma	28	5.5%	Yes	4
			No	24
Chronic Pulmonary Diseases	2	0.4%	Yes	0
			No	2
Renal Diseases	5	1%	Yes	1
			No	4
AIDS	7	1.4%	Yes	1
			No	6

All ICU patients (18) required C-PAP, while 69 other hospitalized patients (with or without comorbidities) Wore Masks. Steroid Treatment Was Given to 9% of Patients with COVID-19 Table 5.

Table 5: Oxygen & Steroid Treatment

Variables		No.	%
Oxygen Requirement	Mask	69	13.6%
	C-PAP	18	3.6%
Steroid Treatment	Yes	46	9%
	No	461	91%

5. Discussion

In the current study, the Age group of < 40 was the highest group affected by COVID-19 (83.3%), while the group > 60 years was the least affected (5.1%). These findings were similar to a previous study in India, where population groups of 20-49 years were highly vulnerable to infection. (Jakhmola, Baral and Jha, 2021) This could be explained as younger people may be in the streets and workplaces more often than older people, making them more vulnerable to the virus.

Present data showed that females were affected more than males, 71%. This may be because X chromosomes increase the expression of essential immune functions, and women have two X chromosomes, not just one X chromosome, like men. Another possibility is that the female sex hormones estrogen and progesterone can promote an immune response and reduce inflammation. (Lamptey, 2021) This result is contrary to a past study in Nigeria that showed that males were affected more than females. Most cases were male (65.8%), and the median age was 43. (Osibogun *et al.*, 2021) Similarly, Iraqi studies in Dyala and Thiqar reported that the female gender was affected more, with 61% of survey respondents female and 39% male. Gender distribution showed females (57.2%) were higher in Thiqar than males (42.8%) (10,11). However, in studies conducted in Thailand, Singapore, and China, men were more affected than women. (Htun *et al.*, 2021) This could be due to the difference in respondents, as more females answered the electronic questionnaire than males. Another explanation is that women in Iraq are not vaccinated with the Corona vaccine

because of the common myths that it causes sterility, death, etc., and the fear and anxiety that women have from the virus reduces immunity. One of the most critical obstacles for our study was the presence of a large percentage of participating women, which led to a difference in the research statistics concerning the number of people affected by COVID-19.

Most of the cases in the present study were from the middle region of Iraq (81.1%). Baghdad is considered one of the most densely populated governorates, followed by the south of Iraq, especially in Basra, and at least one north of Iraq. This is strengthened by a previous study done in Iraq. In comparison, Anbar, Ninewa, Diwaniya, and Salah-Aldeen reported the fewest confirmed cases of COVID-19; most cases were reported in Baghdad (2233 cases), followed by Basra (747 cases) and Najaf (318 cases). ،Although the samples are relatively large compared to our study, they reached the same. (13)

current study revealed that 87.8% of participants have no smoking history. But This does not mean smoking does not affect people with COVID-19 or increase the severity of the disease. These percentages could be because most of the participants in this study were women and young. Tobacco includes additives that disrupt the regular epithelial lining of the respiratory system and impair mucociliary clearance. This may be because tobacco smoke suppresses the feature of innate immune cells, consisting of respiratory epithelium, alveolar surfactant, macrophages, neutrophils, and lymphocytes. This can make smokers more vulnerable to developing complications of COVID-19

like pneumonia. (Htun *et al.*, 2021)

Regarding the presentation of COVID-19, current data reported Fever in 78.5%, cough in 51.7%, headache in 78.5%& fatigue in 66.7%which were the most prevalent symptoms of patients. When compared with a previous study in China and Myanmar, the same results were concluded: fever (88.0%), followed by dry cough (70.2%), and fatigue (42.8%) (Guan *et al.*, 2020). Another study recorded percentages of fever at 54.1%, loss of smell at 50.3%, and cough at 30.9%. (Htun *et al.*, 2021) The initial manifestation of fever in COVID-19 in the first week during the viral phase of the disease is likely a manifestation of the body's immune response to viral replication to boost immunity. (Gul, Htun and Inayat, 2021)

Medical comorbidities distribution among study group present results showed that past medical history of hypertension presented in 11.8% of patients while diabetes mellitus represented 9%, asthma 5.5% & and CVD 4.9%. Other comorbidities were present in lower percentages. Compared with a similar study in China, out Of the 856 patients, 242 (28.3%) had comorbidities, including hypertension 142 [16.6%], diabetes mellitus 64 [7.5%], cardiac disease 13 [1.5%], chronic hepatitis B 27 [3.1%].

, malignancies 8 [0.9%], chronic kidney disease 7 [0.8%], and COPD 5 [0.6%]. (Ye *et al.*, 2020)

The reason why people with high blood pressure may be at higher risk of contracting the coronavirus is unknown. One possibility is a connection between high blood pressure and the immune system. Long-term health problems and aging weaken the immune system, making it less able to fight off the virus. Almost two-thirds of people over sixty have high blood pressure. Another possibility is that the higher risk is not due to high blood pressure but to some drugs used to treat ACE inhibitors and angiotensin receptor blockers(17).

COVID-19 infection disrupts homeostasis and glucose metabolism in patients with and without D.M. due to developing a cytokine storm (C.S.), ACE2 suppression,
and direct damage to pancreatic β-cells. (18) COVID-19 is associated with a high inflammatory burden, known as the inflammatory or cytokine storm, which causes vasculitis, myocarditis, and cardiac arrhythmia, which can exacerbate heart damage. (Aggarwal *et al.*, 2020)

Present findings showed that the history of hospitalization was positive in 37 patients with past medical comorbidities, which is a highly significant statistical result compared to patients with no comorbidities. These findings were similar to the study conducted in January 2020, where 41 patients who tested positive for COVID-19 were admitted to a hospital in Wuhan, China, with slight variation in the number of patients due to a large sample (Sanyaolu *et al.*, 2020). In another study in Saudi Arabia, out of over l 35,284 COVID-19 patients, 81.8% were adults, and 21.7% were hospitalized. Compared to non-hospitalized patients, hospitalized patients had more women (52.1% vs. 47.3%, and an elevated incidence of diabetes mellitus, arterial hypertension, coronary artery disease, cancer, COPD, and asthma (Abolfotouh *et al.*, 2022).

Current data showed that those patients admitted to hospital 18 required ICU admission. Among them, 7 have hypertension, 4 have asthma & 3 have D.M.

Compared with previous studies in Saudi Arabia showed that patients have significantly higher rates of diabetes, hypertension, coronary artery disease, cancer, COPD, and asthma (Abolfotouh *et al.*, 2022).

Present data recorded that all ICU patients 18 required C-PAP 3.6%, while the other hospitalized patients 69 were on mask13.6%. Steroid treatment was given to 9% of patients with COVID-19. C-PAP therapy is commonly used in patients who have stopped breathing due to severe COVID-19 pneumonitis, including patients who are not likely to benefit from invasive mechanical ventilation (22). Corticosteroid use in patients with severe acute respiratory syndrome coronavirus 2 delayed viral clearance and did not result in a convincing improvement in survival. Therefore, corticosteroids should be cautiously used to treat COVID-19 (Wang *et al.*, 2021). A past study in China revealed that out of 214 patients, 34 (9%) were treated with corticosteroids (Li *et al.*, 2020).

In conclusion, the need for hospitalization was higher among COVID-19 patients with comorbidities, especially hypertension followed by diabetes,
asthma & CVD.

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