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Research Article

The Role of Omentin-1 and Fibroblast Growth Factor-23 in Iraqi Patients with Prostate Cancer during Chemotherapy

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Abstract

Background: Omentin-1 is mainly expressed in stromal vascular cells of adipose tissue and can also be expressed in airway goblet cells, mesothelial cells, and vascular cells. Fibroblast growth factor 23 (FGF-23), generated by bone cells, regulates phosphate and vitamin D metabolism by regulating phosphate reabsorption in the kidneys and inhibiting vitamin D activation. Vitamin D is a fat-soluble vitamin that regulates calcium absorption, bone health, and immunological function. Prostate cancer is a significant health concern for men worldwide. Several studies demonstrated a link between these variables and cancer as they exert important anti-inflammatory, antioxidative, and anti-cancer functions.
Objectives: To assess the impact of Omentin and FGF-23 biochemical functions, as well as the anti-cancer properties of vitamin D.
Patients and methods: This is a case-control study on serum samples collected from Iraqi prostate cancer patients after receiving chemotherapy at Al Amal Center in Imam Hussein Medical City in Karbala between November 2022 to May 2023. The serum samples were collected from two groups: The control group consisted of 30 healthy males. The patient group consisted of 30 prostate cancer patients after receiving chemotherapy, both groups aged 45 – 80 years. The two groups were matched for body mass index. ELISA technology was used to estimate serum levels of the aforementioned biochemical parameters with vitamin D.
Results: The patient group had a significantly higher FGF-23 level than the control group (309.5±41.65) versus (163.1±22.4). On the other hand, Irisin, Omentin and Vitamin D mean serum concentrations were significantly lower in-patient group (15.2±4.24), (13.8±4.28) and (4.4±0.69), respectively, compared to control group (60.8±3.5), (38.8±6.59), and (18.9±2.36), respectively. (ROC) curve analysis identified the best AUC values of FGF-23, Omentin, and Vitamin D (0.995, 0.959, 0.937), respectively, which suggests a high level of accuracy.
Conclusions: These parameters may serve as critical indicators for prostate cancer patients and their disease progress. Vitamin D insufficiency is a risk factor for these individuals.
Keywords: Chemotherapy; Irisin; FGF-23; Omentin-1; Prostatic cancer.

Introduction

Prostate cancer (PCa) is the world's second most frequent malignant cancer and the fifth highest cause of cancer-related death among males (1,2). Prostate tumours develop and spread slowly, postmortem examinations revealed that, many cases over the age of 50 years (and a number of younger men), who died of other conditions, also had prostate cancer that had never manifested when they were alive. In many cases, neither they nor their healthcare providers were aware of their condition (3). Within an average age at onset of 68 years, PCa is the most frequent male illness. Men over the age of 75 account for two-thirds of all prostate cancer fatalities. (4). Despite advances in medical technology, identifying and curing prostate cancer remains a significant challenge. Men are frequently unaware of the possible hazards of prostate cancer until it is too late, although early diagnosis is essential for effective treatment. As a result, many men die from the illness needlessly (5). More than 3.3 million men in the United States have been diagnosed with prostate cancer and are still living. However, Prostate cancer kills around one out of every 44 men (6). Omentin or interlectin-1, is a novel adipocytokine of 313 amino acids. Circulating omentin-1 can be applied as an indicator for bone metabolism (7), inflammatory illnesses, malignancies, sleep apnea syndrome, preeclampsia, cancer, and polycystic ovary syndrome (8). It can also be regarded as an acute-phase response with a therapeutic potential (9). Fibroblast growth factor-23 (FGF-23) is a 251 amino acid, 32 kDa glycoprotein. It is a member of endocrine FGFs, FGF-19, and FGF-21(10, 11). FGF-23 induces the growth of prostate tumors. FGF-23 autocrine synthesis promotes the proliferation of certain types of cancers, such as breast cancer, prostate cancer, and renal cell carcinoma (12-14). Irisin is a 112 amino acid-glycoprotein (15, 16). It is produced by different tissues, including skeletal muscle and fat cells. Irisin transforms white adipose tissue, which stores energy, into beige or brown adipose tissue, that is more metabolically active (17). Recent investigations revealed a relationship between irisin and cancer (18). Serum irisin levels are considerably lower in breast cancer patients (19), obesity-related cancer, and

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hormone-related malignancies such as prostate cancer compared to healthy people (20). Thus, the current study is designed to assess the impact of Omentin and FGF-23 biochemical functions, as well as the anti-cancer properties of vitamin D.

Patients and methods

A case-control study was carried out. Patients' and controls' serum samples were collected and tested at Al Amal Oncology Center in Karbala between November 2022 to May 2023. Irisin, Omentin-1, FGF-23 and Vitamin D levels were examined by using the following Kits: (SEN576Hu, SEA933Hu, SEA746Hu, CEA920Ge), respectively, Cloud-Clone Corp, USA. The ELISA technology was used to estimate serum levels of the aforementioned biochemical parameters with vitamin D. Inclusion criteria for cases and controls: The study included 60 samples, of which 30 samples were taken from (prostate cancer patients receiving either Cabazitaxel© or Taxotere© chemotherapy), and 30 healthy controls. The cases and controls were selected between the ages of 45-80 years. Exclusion criteria for cases and controls: Prostate cancer patients and controls with other diseases such as cardiovascular disease, thyroid disorder, and diabetes **were excluded.** Ethical approval was obtained from the research committee in the Kerbala Directorate of Health/ Ministry of Health (Ref: 206 in 27/November 2022). Statistical methods: The results were expressed as mean± SD. The student t-test was used to compare mean values between cases and controls. P-values of <0.05 were considered to be significant. Pearson correlations between variables were determined using a simple linear regression model. The SPSS package - version 28 was used for data storage and analysis (21).

Results

Table 1 shows a lower mean concentration of Irisin in the patients' group (15.2±4.25), compared to the healthy group (60.8±3.5), (p <0.001). The mean level of serum FGF-23 in PCa patients treated with chemotherapy was (309.5±41.65) compared to that of healthy controls (163.1±22.4), (p <0.001), The mean level of serum omentin concentration was significantly lower in patients (13.8±4.28) compared to the healthy control group (38.8±6.59), (p <0.001) with. The mean Vitamin D concentrations of the patients were (4.4±0.69) compared to that of the control group (18.9±2.36), *(p <0.001).*

Table 1: Mean concentrations of Irisin, Omentin, FGF-23, and Vitamin D in prostatic cancer patients and controls

Parameters	Mean ± SD		*P value*
	Controls (No. 30)	Patients (No.30)	
Irisin pg/ml	60.8±3.5	15.2±4.25	0.0001
Omentin ng/ml	38.8±6.59	13.8±4.28	0.0001
FGF-23 pg/ml	163.1±22.4	309.5±41.65	0.0001
Vitamin D ng/ml	18.9±2.36	4.4±0.69	0.0001

The correlations between FGF-32 and Omentin with other parameters among prostate cancer patients on chemotherapy were shown in Tables (2 and 3). Table 2 shows a non-significant correlation between FGF-23 with all parameters, negatively with Omentin and Irisin. However, positively with Vitamin D.

Table 2: Correlation between FGF-23 with Irisin, Omentin & Vitamin D

	Factors	Irisin	Omentin	Vitamin D
FGF-23	R (Pearson)	-0.028	-0.351	0.042
	P	0.883	0.058	0.824

Table 3 shows a non-significant correlation between Omentin with other parameters, positive with Irisin, but negative with Vitamin D.

Table 3: Correlation between Omentin with Irisin & Vitamin D

	Factor	Irisin	Vitamin D
Omentin	R (person)	0.13	-0.173
	P	0.493	0.360

The Receiver Operating Characteristic (ROC) curve assessment for Irisin, FGF-23, Omentin and Vitamin D across every group is demonstrated in Table 4. It depicts the relationship between sensitivity and specificity in the ROC curve. The findings recorded an exceptional Area Under the Curve (AUC) value of the aforementioned parameters with the following details: 0.734, 0.955,0.959 and 0.937, respectively. This indicates a good level of discrimination between the groups for Irisin, and high level for other parameters.

Table 4: The Receiver Operating Characteristic (ROC), sensitivity, and specificity of Irisin, FGF-23, Omentin and Vitamin D

Variables	Area under the curve	Sensitivity	Specificity	95% C.I.		Cut off value
				L.B.	U.B.	
Irisin	0.734	0.852	0.718	0.644	0.824	21.793
FGF-23	0.955	0.953	0.998	0.993	0.988	252.66
Omentin	0.959	0.935	0.962	0.929	0.990	25.788
Vit D	0.937	0.927	0.952	0.902	0.963	15.5

Discussion

The lower Irisin level among PCa patients in the current study is in line with the results of Tekin et al, which showed that Irisin regulates cell division and proliferation in prostate cancer cells (22). The significantly lower Irisin levels are in prostatic cancer patients in the current study may indicate that it can be used as a biomarker with PSA, as also suggested by other studies (23). Irisin levels can be at their lowest in prostate cancer patients who have just

received chemotherapy, due to the marked weight loss and muscle mass loss (24). FGF-23, which is largely generated by osteocytes in the bone, is essential for controlling the metabolism of phosphate and vitamin D (25). To assist in keeping the body's mineral balance, it works on the kidneys and parathyroid gland. Phosphate excretion in the urine is enhanced as a result of FGF-23's reduction of phosphate reabsorption in the kidneys (18). Additionally, it prevents the kidneys from producing calcitriol, an active form of vitamin D that contributes to even lower phosphate levels (26). It is well known that FGF-23 levels rise when active vitamin D (calcitriol) signaling is insufficient. This happens to lessen intestinal phosphate absorption and increase urine phosphate excretion (27). Analysis of human tumour samples, in vitro experiments, and animal models have provided strong evidence that fibroblast growth receptors (FGFRs) are crucial for PCa development. The higher FGF-23 concentration in of prostate cancer patients in the current study is consistent with the results of the study of Teishima *et. al*. (28). Androgen-inhibiting therapy for PCa patients contributes to osteoporosis and thus increases FGF-23 values, as indicated by Hussein *et. al*. (29). All the aforementioned facts support the findings of the current study of higher serum FGF-23 levels in prostate cancer patients compared to controls.

Intelectin-1) ITLN1)might help with the growth and spread of tumors. Since the blood levels of ITLN1 are very changeable and vary depending on the kind of cancer, the expression of ITLN1 in the local tumor might be more indicative of malignant behavior (15). Generally, testosterone and estradiol levels tend to fall with hormonal prostate cancer treatment associated with chemotherapy, such as androgen deprivation therapy (ADT). Since testosterone and other androgens are frequently required for the formation of prostate cancer cells, ADT seeks to reduce the quantities of these substances in the body. Estradiol is produced in part from testosterone through the process of aromatization; thus, a drop in testosterone synthesis or its effects also results in a fall in estradiol levels (30). Omentin and the ratio of estradiol to testosterone were also shown to be positively correlated. Perhaps because of the drop in this proportion following hormonal or chemotherapeutic treatments, the concentration of Omentin-1 may also be reduced (31). A major side effect of hormone therapy for prostate cancer is a drop in both testosterone and estradiol levels, which is frequently accompanied by other diverse effects such as changes in energy levels, bone health, muscle mass, and sexual function. Based on variables namely the kind of hormonal therapy, the length of the treatment, and individual variations in hormone metabolism, the precise magnitude of the drop and its impact might vary across people (32). Vitamin D insufficiency and racial inequities are linked to a slew of ailments, including cancer, putting a strain on the healthcare system (33-35). There are two types of Vitamin D: D2 [ergocalciferol] and D3 [cholecalciferol]. Human skin produces Vitamin D3 in reaction to UVB light, but Vitamin D2 is acquired from plant sources such as edible (UV-exposed) mushrooms in our diets, albeit at varied amounts and with lower efficacy (36). The two types of Vitamin D are physiologically inactive and must be changed to 2(OH)D in liver by Vitamin D-25-hydroxylase (37). The predominant form of Vitamin D in circulation is 25 (OH)D, and its measurement in the clinical environment offers information about one's Vitamin D status (38). FGF-23 inhibits the production of 1,25(OH)2D in the kidney by suppressing the transcription of the Vitamin D-activating enzyme 1-hydroxylase (CYP27B1). A rise in FGF-23 results in a reduction in the levels of serum Vitamin D in prostate cancer patients (39). Although Vitamin D is commonly recognized due to its function in the equilibrium of minerals management, its deficiency is additionally connected to the emergence and progression of some cancer forms. New Vitamin D-mediated molecular processes that govern cancer cell self-regeneration, and demise have been discovered in recent epigenomic, transcriptomic, and proteomic investigations. Tumor microenvironmental research has further introduced dynamic links between the immune system and the anti-neoplastic capabilities of Vitamin D. The preponderance of research demonstrates that low circulation Vitamin D ratios are linked to an elevated risk of tumors; whereas supplemental intake alone or in conjunction with additional chemo-immunotherapeutic medications may enhance clinical results even more (40).

Limitations

The study was and only two tumor centers (Al-Amal) at Imam Hussein Medical City in Karbala, and Oncology Hospital Teaching in Baghdad.

Conclusion

FGF-23, Omentin and Vitamin D may be useful biomarkers and indicators for prostate cancer patients and their disease progress. Vitamin D insufficiency is a risk factor for these individuals. They might serve as biomarkers to accurately predict the progression of the stage of prostate cancer.

Authors' declaration

Conflicts of Interest: The authors declare no conflict of interest. We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current research, have been given permission for re-publication attached to the manuscript.

Authors sign on ethical consideration's approval- Ethical Clearance: The local ethical committee approved the project in (Karbala Directorate of Health/ Ministry of Health (Ref: 206 in 27/November 2022).

Conflict of Interest/ None

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Authors' contributions

Study conception & design: (Ammar H. Alqazzaz & Anwar F. Al-Taie). Literature search: (Ammar H. Alqazzaz & Anwar F. Al-Taie).
Data acquisition: (Ammar H. Alqazzaz & Anwar F. Al-Taie). Data analysis & interpretation: (Ammar H. Alqazzaz & Anwar F. Al-Taie). Manuscript preparation: Ammar H. Alqazzaz). Manuscript editing & review: (Ammar H. Alqazzaz & Anwar F. Al-Taie).

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دور الأومينتين-1 وعامل نمو الخلايا الليفية-23 (FGF-23) في المرضى العراقيين المصابين بسرطان البروستاتا أثناء العلاج الكيميائي

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الخلاصة:
الخلفية: يتم التعبير عن Omentin-1 بشكل رئيسي في الخلايا الوعائية اللحمية للأنسجة الدهنية ويمكن التعبير عنه أيضًا في الخلايا الكأسية للمجرى الهوائي والخلايا الظهارية المتوسطة والخلايا الوعائية. تم العثور على Omentin-1 كمضاد للإلتهابات ومضاد للأكسدة ومضاد للسرطان. ينظم عامل نمو الخلايا الليفية 23، الناتج عن الخلايا العظمية، إستقلاب الفوسفات وفيتامين (د) عن طريق تنظيم إعادة إمتصاص الفوسفات في الكلى وتثبيط تنشيط فيتامين (د). فيتامين (د) قابل للذوبان في الدهون وينظم إمتصاص الكالسيوم وصحة العظام والوظيفة المناعية. يتم الحصول عليه في الغالب عن طريق التعرض لأشعة الشمس والتغذية. يعد سرطان البروستاتا مصدر قلق صحي كبير للرجال في جميع أنحاء العالم. وهو ثاني أكثر أنواع السرطان شيوعًا لدى الرجال. وأظهرت العديد من الدراسات وجود صلة بين هذه المتغيرات والسرطان لأنها تمارس وظائف مهمة مضادة للإلتهابات ومضادات الأكسدة ومضادة للسرطان.
الأهداف: تم تصميم دراسة حالات وعينة ضابطة لدراسة تقييم تأثير الأومينتين والوظائف البيوكيميائية لـ FGF-23، بالإضافة إلى الخصائص المضادة للسرطان لفيتامين (د).
المنهجية: تم جمع عينات البحث من الذكور العراقيين المصابين بسرطان البروستاتا. المرضى الذين تم تشخيصهم بعد تلقي العلاج الكيميائي في مركز الأمل في مدينة الإمام الحسين الطبية في كربلاء للفترة بين تشرين الثاني ٢٠٢٢ الى آيار ٢٠٢٣. وكانت العينات عبارة عن مصل من الذكور الذين تتراوح أعمارهم بين 45 و 80 سنة، و 30 عينة لمجموعة الأصحاء الذين كانوا مرافقين لمرضى سرطان البروستاتا و 30 عينة للمرضى بعد العلاج الكيميائي، وتمت مطابقة الحالات والعينة الضابطة أيضًا حسب مؤشر كتلة الجسم. تم استخدام تقنية ELISA لحساب مستويات مصل العوامل الكيميائية الحيوية المذكورة أعلاه مع فيتامين (د).
النتائج: وجد ارتفاع كبير للغاية في مستويات FGF-23 (41.65±309.5) (p <0.001)عند المرضى، مقارنة بالمجموعة الضابطة (163.1 ± 22.4). كما وجد انخفاض معنوي في تركيز الإيريسين والأومنتين وفيتامين (د) في الأمصال لدى المرضى (4.24±15.2)(p <0.001) ، (4.28±13.8) (p <0.001)و (4.4± 0.69) (p <0.001)على التوالي، مقارنة بمجموعة الاصحاء الضابطة (3.5 ± 60.8)، (6.59 ± 38.8)، و (2.36 ± 18.9)، على التوالي. حدد تحليل منحنى (ROC) أفضل قيم AUC لـ FGF-23، وOmentin، وفيتامين (د) (0.995)، (0.959)، 0.937)، على التوالي، مما يشير إلى مستوى عالٍ من الدقة.
الإستنتاجات: قد يكون الأومينتين-1 وعامل نمو الخلايا الليفية-23 وفيتامين (د) علامات حيوية ومؤشرات مفيدة لمرضى سرطان البروستاتا ولتطور مرضهم. يعد نقص فيتامين د أحد عوامل الخطر بالنسبة لهؤلاء الأفراد.
مفتاح الكلمات: سرطان البروستات، العلاج الكيمائي، الاومنتين-1، عامل نمو الخلايا الليفية-23، الايريسين

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Research Article

Preparation and Characterization of Isradipine as Surfactant-Free Dry Emulsion

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Abstract:

Background: The prevalence of hypertension in Iraqi children is increasing, and most drugs are not suitable for children, leading to unsafe off-label use. Liquid dosage forms are favorable for pediatric patients due to their dose flexibility. Isradipine, the preferred oral therapy for severe hypertension in pediatrics, belongs to the biopharmaceutical classification system class II. Its oral bioavailability is approximately 15 to 24 %, so it needs to be made as a surfactant-free emulsion for improved oral bioavailability and pediatric safety, but it lacks physicochemical stability. Surfactant-free dry emulsion is a promising solution to all these challenges.

Objectives: The study aims to create a stable, eco-friendly, and surfactant-free oral oil in water emulsion of Isradipine for pediatric patients, protecting it from hydrolysis, oxidation, and photosensitivity and increasing its solubility and absorption.

Methods: The study used Corn oil for solubilizing Isradipine, and then the surfactant free emulsion was stabilized by different percentages of β-cyclodextrin. Eight formulas were prepared using a homogenizer and mixed for 5 minutes at 10,000 rpm and 25ºC. The surfactant free emulsion (SFE) formulas were evaluated for organoleptic attributes, thermodynamic stability, viscosity, pH, drug content, droplet size distribution, and in-vitro dissolution. Then, the selected formula was lyophilized with 15gm Mannitol using a freeze-drying system. The experimental results were expressed as a mean triplicate sample ± standard deviation (SD) and were analyzed according to a one-way analysis of variance (ANOVA).

Results: Among all the prepared surfactant-free emulsion formulas, F4 containing 8g of beta-cyclodextrin (β-CD) and 15g of Corn oil was chosen as the optimum SFE formula due to its small particle size range of 1451±0.01 nm, respectable pH, good organoleptic attributes, excellent thermodynamic stability, acceptable viscosity 1869.5±1.54 mg/ml, acceptable drug content percentage, and drug release in 90minuets. F4 was freeze-dried and then further evaluated for Flow and compressibility Properties.

Conclusion: The study found that surfactant-free emulsion provided an important pediatric dosage form for the oral water-insoluble drug. It improves the dissolution rate and solubility of Isradipine.

Keywords: Beta-cyclodextrin; Corn oil; Pickering emulsion**;** Surfactant-free dry emulsion; Surfactant-free emulsion.

Introduction:

Hypertension is the third most common chronic pediatric disease, with a prevalence in Iraqi children increasing from 1.7% (1) in 2006 to 19.6% in recent years (2). Factors contributing to this increase include family history, low birth weight, high body mass index, insulin resistance, and sympathetic nervous system activation (3, 2). Hypertension is also common in Iraqi children with type 1 diabetes (4) and dyslipidemia among Iraqi teenagers (5).

Pediatric patients have unique pharmacokinetics, pharmacodynamics, administration routes, toxicity, and taste preferences compared to adults. This necessitates the development of convenient formulations for children of all age groups due to their varying responses to active substances and excipients (6, 7).

The majority of drugs in the market are not suitable for children, leading to unsafe off-label or extemporaneous compounding practices. Thereby, crushing hard tablets containing the active pharmaceutical ingredient can alter the rate of drug dissolution and absorption, increasing risks of inaccurate dosing, hospitalization, elevated healthcare costs, contamination, and death would be increased. Therefore, it is necessary to have convenient formulations for children in all age groups (8, 9). The optimal pediatric formulations should have low dosing frequency, appropriate dosage forms for

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different age groups, convenient administration, minimal impact on ifestyle, use of
non-toxic, well-tolerated excipients, taste masking, easy production, elegant, stable, and cost-efficient manufacturing (7).

Liquid dosage forms are advantageous for pediatric patients and infants due to their dose flexibility and ease of swallowing (9). Emulsions are dispersions of two immiscible liquids that are thermodynamically unstable and need to be stabilized by surfactants (10) However, synthetic emulsifiers in these systems can cause health problems and toxic symptoms with prolonged use. Clinical tests show that anionic emulsifiers may bind to proteins, enzymes, and phospholipid membranes, leading to adverse effects such as enzyme dysfunction, protein structure modification, and phospholipid changes in the cell membrane (11).

Pickering emulsion is a surfactant-free emulsion (SFE) stabilized by solid particles (12). These non-toxic, biocompatible, and biodegradable stabilizers are edible, natural substances, readily available and inexpensive. This unique structure for SFE endows them with good stability, excellent biocompatibility, and environmental friendliness (13).

Liquid emulsion offers several advantages over other dosage forms, including improved oral bioavailability, but it exhibits a lack of physicochemical stability. Dry emulsion formulations are a promising solution to these challenges (14).

Dry emulsions are prepared using lyophilization, this process prolongs shelf-life by shielding the drug from oxygen, light, and water. Dry emulsions are also easier to handle and storage than liquid emulsions (15).

Isradipine is a calcium channel blocker drug. It is the drug of choice for oral therapy of hypertensive crisis. The usual dose of Isradipine for pediatrics is 0.05–0.1mg/kg/dose/8hr up to 5 mg/dose (16). Isradipine is a class II drug according to the biopharmaceutical classification system (17).

The study aims to create a stable, eco-friendly, and oral oil in water surfactant-free dry emulsion (SFDE) of Isradipine for pediatric patients, protecting it from hydrolysis, oxidation, and photosensitivity and increasing its solubility and absorption.

Materials and Methods:

Isradipine and Native β-CD were purchased from Hyper Chem Company, China. Olive oil supplied by Pomace Olive oil Oilex ,S.A. Spain. Avocado oil and almond oil were obtained from Now (USA). Corn, grape, sesame, sunflower, soybean, canola, and cottonseed were bought from Shaanxi Guanjie Technology CO, LTD ,China. HCl was purchased from Avantor Performance Materials. Sodium Dodecyl Sulfate (SDS) was purchased from Panreac Barcelona, Spain. Methanol was obtained from Sigma-Aldrich, Germany. Janeen supplied deionized water for chemical and laboratory materials in Baghdad, Iraq.

Methods

Solubility Study of Isradipine

Isradipine's saturated solubility was tested in various oils: Sesame oil, Olive, Sunflower, Almond oil, Soybean oil, Canola oil, Grape seed, Cotton seed, Avocado oil, and Corn oil. An excess amount of Isradipine powder was added to 5 grams of each oil in small plain tubes to measure the solubility. These tubes were tightly closed and placed in an isothermal shaker water bath at 25 ± 0.5 °C for 48 hours (18). After 48 hours, the samples were centrifuged at 3000 rpm for 20 minutes. The supernatant layer of each sample was then filtered using a 0.45 µm filter membrane. Once filtered, the samples were diluted with methanol, and the solubility was evaluated at λ max at 326nm using a UV-visible spectrophotometer (19).

Formulations of surfactant-free emulsions of Isradipine

Isradipine SFE was prepared by using β-CD in different weights as stabilizers instead of surfactants with a selected oil based on a solubility study as the oil phase, as seen in Table 1. The drug dose incorporated in each of these formulations was 2.5 mg of Isradipine/5 mg of SFE. The method of preparation is the mechanical method, where the specified Weight of the drug is dissolved in the oil (as the oil phase), while in another beaker, the specialized amount of β-CD with deionized water (as the aqueous phase), then while continuously mixing the aqueous phase by using a homogenizer (Witeg HG-15D), dropped the oil phase containing drug slowly on it then the homogenizer still mixed for 5 minutes at 10,000 rpm at 25ºC to obtain surfactant free o/w emulsion. The percent of each component is based on a ternary phase diagram and references (20, 21). As shown in the Table 1.

The selected formula was lyophilized with 15gm of Mannitol by using a drying system (Labconco, USA) to obtain SFDE (20).

Table 1. Components of surfactant-free emulsion for Isradipine

F. NO.	β-CD (g)	Corn oil(g)	Water (g)	drug(mg)
F1	2	15	83	50
F2	4	15	81	50
F3	6	15	79	50
F4	8	15	77	50
F5	2	20	78	50
F6	4	20	76	50
F7	6	20	74	50
F8	8	20	72	50

Evaluation of the prepared surfactant-free emulsion

Evaluation of organoleptic attributes

The study evaluated the organoleptic attributes of formulations, including color and odor, through visual and olfactory evaluation. The texture of emulsions was assessed by pressing a small amount between the thumb and index finger. At the same

time, consistency was evaluated based on homogeneity. The ease of removal of emulsions was also assessed after washing the body part with tap water (22).

Thermodynamic stability studies: Due to their different densities between oil and aqueous phases, emulsions rapidly separate into oil and water layers, making them thermodynamically unstable systems. The stability of emulsions means their ability to maintain their properties. Their strength depends on phenomena like flocculation, sedimentation, creaming, phase inversion, Ostwald ripening, and coalescence, which contribute to their destabilization (10)**.**

a) Observation of phase separation: Ten ml of the prepared emulsions were stored in tubes fixed vertically at room temperature (25 ± 2 °C) and evaluated for instability phenomena after 1, 2, 4, 6, and 24 h of preparation (23).

b) Heating-cooling cycle: Six cycles of temperature changes (4 ºC to 45 ºC) were conducted in a refrigerator, then storage at each temperature for 48 hours, and the stability of the formulations was examined at temperatures (24).

c) Freeze-thaw cycle: By this test, the emulsion was stored at -5 ºC (in a fridge) for 24 hrs. Then, at 27 ºC (at room temperature) for 24 hrs. Then, in an oven at 40 ºC for 24 hrs. The results were recorded for further studies (25).

Viscosity determination: The viscosity of a prepared SFE sample was determined without dilution using a digital viscometer and spindle number 4, which was inserted into a glass beaker at different speeds: 6, 12, 30, and 60 rpm (26).

Particle size distribution determination: The SFE's mean particle size distribution was measured using a laser particle size analyzer instrument (Malvern Instruments Ltd Great Britain) by taking the angle of detection at 90º and 25 ºC after being diluted fivefold with double-deionized water before measurements (20).

pH measurement: The pH measurement was done using a pH meter. A glass electrode was dipped in SFE emulsion, and the reading was noted (27). The measurement was repeated three times for each sample, and the result was presented as mean ±SD.

Drug content estimation: Accurately, 5gm of each SFE formula which contains 2.5mg of Isradipine was dissolved in 100 ml Methanol, then filtered using a 0.45 µm filter syringe and suitably diluted. The contents of Isradipine were determined using a UV/Vis spectrophotometer at the selected λ max 328 nm (17).

The in-vitro dissolution profile of Isradipine SFE: The in-vitro dissolution test of Isradipine SFE was conducted using a paddle assembly type II dissolution test apparatus. Each formula equivalent to 2.5 mg of Isradipine was placed in a dialysis bag. The paddle rotated at 50 rpm at 50 rpm at 37 ± 0.5°C in 250ml of dissolution medium, 0.1N HCl with SDS 1%w/v, to ensure sink condition. An aliquot of 5 ml samples was drawn at predetermined time intervals, and compensated by an equal volume of fresh dissolution medium then, samples were assayed spectrophotometrically using a UV-spectrophotometer at 328 nm. The same procedure was made for the pure drug

Selection optimum Isradipine surfactant-free emulsion: The best formula of Isradipine SFE was selected according to the results that are obtained from the evaluation tests that included intrinsic stability, drug content, pH, particle size, viscosity, accelerated stability, and in vitro release study. Then this formula will be dried by lyophilization to form surfactant free dry emulsion (SFDE).

Evaluation of selected optimum Isradipine lyophilized surfactant free dry emulsion

Flow Properties

These properties were determined in terms of angle of repose, Bulk density, Tapped density, Carr's index, and Hausner's ratio for the SFDE formula (29).

Determination angle of repose

It is a method for assessing the flow properties of powder. It was determined using the fixed funnel method, by permitting a powder to flow throughout a funnel and pass freely onto a surface. The height and diameter of the resultant cone were measured and the angle of repose was calculated from this equation:

$$\text{Tan}(\theta) = h/r$$

Where: h is the height of the powder cone and r is the radius of the powder cone (29).

Bulk density: It is a ratio of the powder mass to bulk volume. The bulk density depends on particle size distribution, shape, and cohesiveness of particles. The weighted amount of the powder was carefully poured into the graduated measuring cylinder through the large funnel and volume was measured, which is the initial bulk volume. Then it was expressed in g/ml. Bulk density was calculated by the following equation (30).

$$\text{Bulk Density} = \text{Weight of powder} / \text{Bulk volume}$$

Tapped density: The graduated cylinder containing a known mass of mixture was tapped for a permanent time. The volume was measured, and the tapped density was calculated by the following equation (30).

$$\text{Tapped Density} = \text{Weight of powder} / \text{Tapped volume}$$

Carr's index (compressibility index)

Carr's index indicates the flow properties of the powder. It was expressed in percentage and was calculated by the following equation (31):

$$\text{Carr's index} = [(\text{Tapped density} - \text{Bulk})/ (\text{Tapped density})] \times 100$$

Hausner's ratio: Hausner's ratio is an indirect index of powder flow. It was calculated by the following equation (31):

$$\text{Hausner's ratio} = (\text{Tapped density})/(\text{Bulk density})$$

Scanning electron microscopy (SEM): By Scanning electron microscope, the morphological

features, including (shape and surface characteristics) of SFE, were evaluated (19).

Statistical analysis

The experimental results were expressed as the mean of triplicate samples ± standard deviation (SD) and were analyzed using one-way analysis of variance (ANOVA) in SPSS software. Results were considered significant if $p < 0.05$ and non-significant if $p > 0.05$.

Results

Solubility study

Isradipine solubility was indicated to be highest in Corn oil (4.9 mg/ml) in comparison to other oils in Table 2, so it was selected as an oil phase for preparing SFE for Isradipine.

Table 2. Saturation solubility of Isradipine in different oils.

Oil	Solubility (mg/ml)*
Sesame oil	1.4±0.01
Olive market	1.41±0.02
Sunflower	1.4±0.13
Almond oil	2.2±0.12
Soybean oil	2±0.06
Canola oil	2.4±0.06
Grape seed	4.7±0.03
Cotton seed	4.6±0.10
Avocado	4.4 ±0.04
Corn oil	4.9±0.017

***Data are presented as mean ± SD of n= 3.**

Evaluation of the prepared surfactant-free emulsion

Evaluation of organoleptic attributes: All formulations freshly prepared have a yellowish-white color. Their appearance is homogenous, with a smooth texture, and no lumps were detected after 24 and 48 hrs, and odorless. They offer smoothness to the touch. After applying all twelve samples to the hand, they were readily removed by washing the body part with running water.

Thermodynamic stability studies

a) Observation of phase separation: The study found that F1 exhibits phase separation after 24 hours, indicating they cannot be further investigated. However, other formulas remained thermodynamically stable during this time, maintaining emulsion stability without phase separation, flocculation, sedimentation, creaming, or phase inversion.

b) Heating-cooling cycle: All formulas pass this test except F2 and F6 so only the formulations that remained stable at these temperatures were exposed to further studies.

c) Freeze-thaw test

F2 and F6 showed oiling-off after 2 cycles of freeze-thaw provided that evidence of coalescence was already evident so that all formulations passed this test except F2 and F6. So there are no further studies for F2 and F6

Viscosity determination: The viscosity range of the investigated formulas is 1665.9-311 mP, as shown in Table 3.

Particle size distribution determination: The particle size range of the investigated formulas is 1451-4112 nm, as shown in Table 3.

Table 3. Viscosity (mP) and Mean droplet size of Isradipine SFE

F. NO.	Viscosity (mP) *	Mean droplet size (nm) *
F3	1665.9±1.62	1825±0.1
F4	1869.5±1.54	1451±0.01
F7	2621±1.38	4121±0.3
F8	3115 ± 1.64	3109±0.02

***Data are presented as mean ± SD of n= 3.**

Determination of pH: The pH related to all the formulations has been determined via pH meter in triplicate at 25 ± 1 °C and indicated to be in the range of 6.2-6.7.

Drug content: The drug content related to all prepared Isradipine SFE was more than 95% and there has been no considerable difference between different formulations ($p > 0.05$),

In vitro drug release: The study reveals a flexible duration time for Isradipine release from each formula, with F4 completely releasing Isradipine after 90 minutes, while F3, F7, and F8 took more than 120 minutes without completing the release. Pure Isradipine showed 15.4% drug release at the end of 120 minutes, as shown in Figure 1.

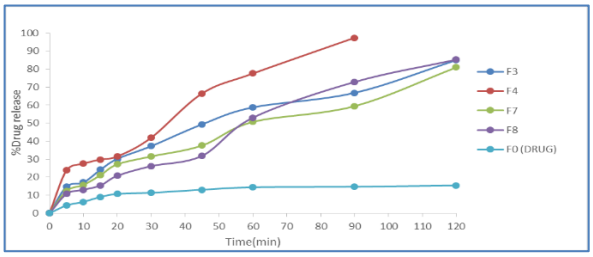

Figure 1. A comparative dissolution profile of Isradipine SFE (F3, F4, F7, F8, and pure Isradipine) in 250ml of 0.1 N HCl (containing 1% SDS) dissolution medium at 37°C.

Selection optimum Isradipine surfactant-free emulsion: Based on previous results, F4 was chosen as the best Isradipine SFE formula since it had a globule size range of 1451±0.01 nm, respectable pH, good organoleptic attributes, excellent thermodynamic stability, acceptable viscosity of 1869.5±1.54 mP, great % drug content and In vitro release 100% in 90 minutes. The selected formula was subjected to further studies.

Evaluation of selected optimum Isradipine lyophilized surfactant-free emulsion

Flow Properties

The flow properties of the selected surfactant-free dry emulsion formula were evaluated. The results are shown in Table 4.

Table (4): The Flow Properties of a surfactant-free dry emulsion of Isradipine.

Parameter	Result*
Angle of repose	34.01 ± 0.015
Bulk density(g/cm3)	0.39 ± 0.024
Tapped density(g/cm3)	0.54 ± 0.014
Carr's index %	27.074 ± 0.163
Hausner's ratio	1.37 ±0.005

***Data are presented as mean ± SD of n= 3.**

Scanning electron microscopy (SEM): The SEM shows the spherical shape of spherical droplets of Corn oil that are surrounded by β-CD as shown in Figure 2.

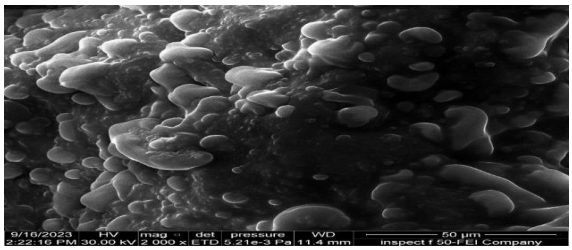

Figure 2. SEM of the selected formula (F4).

Discussion:

According to the solubility result, the choice of corn oil as the oil phase for surfactant-free emulsion of Isradipine based on the highest Isradipine solubility in corn oil to obtain stable formulas with high drug miscibility and superior drug loading (32)**.** Moreover, all prepared formulations were accepted in color, odor, and texture so can be passed to the next evaluation (22).

Thermodynamic stability studies: The results of this study demonstrated that all the prepared SFE formulations were stable except for (F1). The reason for this was the irreversible adsorption of β-CD on oil droplets causes β-CD in the continuous phase to cover the oil droplets' surface, preventing aggregation and improving emulsion stability (33). F1 has an insufficient concentration of solid particles enough to form a robust, dense layer surrounding the oil particles, leading to phase separation (34). Regarding the stability, the formulations that are stable against storage in extreme conditions and ensure the system remains dispersed with no separation (35). All formulas pass this test except F2 and F6. According to Freeze-thaw which is commonly used parameter to evaluate the stability of emulsions, a higher oil phase volume or higher particle concentration has better freeze-thaw stability than that of a lower particle concentration and lower oil phase volume (36). F2 and F6 showed oiling-off after 2 cycles of freeze-thaw, providing that evidence of coalescence was already evident (37), so all formulations passed this test except F2 and F6. The viscosity study found that formulas F4 and F8 had more viscous emulsions than formulas F3 and F7, respectively, due to F4 and F8 having higher amounts of β-CD. This is because excess particles form a network structure around each droplet, improving emulsion stability and increasing emulsion viscosity (33). Systems with a higher volume fraction of dispersed phase have the viscosity of SFE increased by increasing the number of particles (34). Formulas F7 and F8 had more viscous emulsions than the group of formulas F3 and F4, respectively, due to higher oil content (32). This is because as oil content increased, the number of emulsified oil droplets increased, which caused a decrease in the aggregation of oil droplets and resulted in smaller droplet sizes, leading to increased interfacial area, allowing more interactions between one particle and another and increased emulsion viscosity (38).

Particle size distribution determination: The particle size range of the investigated formulas is 1451-4112nm, with variations attributed to the amount of β-CD and oil. The formulas F3 and F7 had larger particle sizes than formulas F4 and F8, respectively, with the same amount of oil due to having a lower amount of β-CD. The increase in particle size by decreasing surfactant concentration can be explained by the partial coverage of oil droplets by solid particles, leading to coalescence and large droplets (39, 40).

Formulas F7 and F8 had larger particle sizes than F3 and F4, respectively, with the same amount of β-CD due to higher oil content and increased oil volume, leading to droplet coalescence and droplet size increase (41, 42).

The pH range of all formulations (6.2-6.7). The acceptable range of pH for oral solutions is (2-9); therefore, all formulations have accepted pH values (43).

Drug content: The drug content of all prepared Isradipine SFE formulas was within an acceptable range (95.0%- 105.0%), which meets British pharmacopeia requirements and indicates that the drug has not precipitated in any of the prepared formulations (44).

In vitro drug release: The percentage of drugs released from SFE of Isradipine formulation increased with the increase in the percentage of β-CD. This was observed in F4 and F8, which have smaller particle sizes than F3 and F7, respectively. Decreased particle size results in an increased surface area for drug transfer, which enhances drug release absorption and overall promotes drug bioavailability (45). As the percentage of oils increased in the SFE of Isradipine formulation, there was a decrease in the percentage of drugs released. Specifically, F3 and F4 had higher drug release compared to F7 and F8. The findings suggest that Isradipine released more from formulas with lower oil content because these formulations had a lower viscosity (32). Pure

Isradipine showed the lowest release at the end of 120 minutes due to its practically insoluble (17).

Flow Properties and SEM analysis: The flow properties of the selected formula indicate that the surfactant free dry emulsion of Isradipine has passable flowability and poor compressibility (46). These flow properties are typical for powder with lipophilic core material and have previously been observed in dried oil-based powders.

SEM analysis revealed the absence of drug crystals, indicating complete solubilization of the drug within the emulsion (20).

Conclusions

The study found that SFDE provided an important pediatric dosage form for the oral water-insoluble drug. SFDE, which was prepared from Corn oil, β-CD was an encouraging method for improving the dissolution rate and solubility of Isradipine and could serve as a prototype for developing other hydrophobic drug formulations using surfactant-free emulsion drug delivery systems.

Authors' declaration

We confirm that all the Figures and Tables in the manuscript are ours. Besides, the figures and images, which are not ours, have been given permission for re-publication attached to the manuscript.

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Authors' Contributions

Study conception & design: (Zahraa M. Naji & Fatima J. Jawad). Literature search: (Zahraa M. Naji). Data acquisition: (Zahraa M. Naji). Data analysis & interpretation: (Zahraa M. Naji). Manuscript preparation: (Zahraa M. Naji). Manuscript editing & review: (All Authors).

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تحضير وتوصيف الأسرادبين كمستحلب جاف خال من المادة المستحلبة

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الخلاصة:
خلفية البحث: يتزايد انتشار ارتفاع ضغط الدم بين الأطفال العراقيين، ومعظم الأدوية المتوفرة ليست مناسبة للأطفال، مما يؤدي إلى استخدام غير مصرح به وغير آمن .تعد أشكال الجرعات السائلة مفيدة للمرضى الأطفال والرضع بسبب مرونتها في الجرعة وسهولة بلعها .إسراديبين، العلاج الفموي المفضل لارتفاع ضغط الدم الشديد في للأطفال ينتمي الدواء للأدويه المصنفه من الدرجة الثانية حسب نظام تصنيف الصيدلانيات البيولوجي. التوافر البيولوجي له منخفض بحوالي15-24٪. ويحتاج إلى أن يكون مستحلبًا خاليًا من المادة المستحلبه (SFE) لتحسين التوافر البيولوجي الفموي ,وسلامة الأطفال ولكنه يفتقر إلى الاستقرار الكيمياء الفيزيائية .يعتبر SFE الجاف حلاً واعدًا لجميع هذه التحديات.
الاهداف : تهدف الدراسة إلى إنشاء مستحلب دهني مائي فموي للأسرادبين فموي مستقر وصديق للبيئة وخالي من المادة المستحلبة للأطفال، وحمايته من التحلل المائي والأكسدة والحساسية للضوء، وزيادة قابليته للذوبان وامتصاصه.
المواد وطرق العمل: استخدمت الدراسة زيت الذرة لتذويب الإسراديبين. حضر SFE بنسب مختلفة من البيتا-سيكلودكسترين. تم تحضير ثمان صيغ باستخدام جهاز التجانس وخلطها لمدة 5 دقائق عند 10000 دورة في الدقيقة و 25° م. تم تقييم صيغ SFE للصفات الحسية، ودراسة الاستقرار الديناميكي الحراري، وتحديد اللزوجة، وقياس الأس الهيدروجيني، ومحتوى الدواء، وتوزيع حجم القطرات، ودراسة تحرر الدواء. ثم تم تجميد الصيغة المختارة باستخدام المانيتول وذلك باستخدام نظام التجفيف بالتجميد.
النتائج: من بين جميع صيغ SFE المحضرة، تم اختيار F4 التي تحتوي على 8 غم من β-CD و 15 غم من زيت الذرة، كأفضل صيغة SFE بسبب نطاق حجم الجسيمات الصغيرة 1451 ± 0.01 نم، والدرجة الحمضية المحترمة، والصفات الحسية الجيدة، والاستقرار الديناميكي الحراري الممتاز، واللزوجة المقبولة 1869.5 ± 1.54 ملغم/مل، ونسبة محتوى الدواء المقبولة، وأعلى تحرردواء. تم تجفيف F4 بالتجميد ثم تم تقييمها لاحقًا لتدفقها وقابليتها للضغط.
الاستنتاجات: وجدت الدراسة أن SFEالجاف قدم شكل جرعة دوائيه فموية مهمة للأطفال للدواء الغير القابل للذوبان في الماء.
مفتاح الكلمات: مستحلب بيكرنك,المستحلب الخال من المادة المستحلبة,المستحلب الجاف الخال من المادة المستحلبة,بيتاسايكلودكسترين, زيت الذرة.

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Research Article

Comparison of Quality of Life and Treatment Satisfaction among Sample of Iraqi Patients Using Anticoagulant Therapy (Warfarin or non-vitamin K Antagonist Oral Anticoagulants

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Abstract:

Background: Oral anticoagulation medication, warfarin and non-vitamin K antagonist oral anticoagulants (NOAC) may require long term use which may affect patients' satisfaction with their treatment and their quality of life (QOL).

Objective: To compare the quality of life and treatment satisfaction among groups of patients using different anticoagulant therapies (warfarin and NOAC).

Methods: A cross-sectional study was performed at Ibn Al-Bitar Hospital for cardiac surgery in Baghdad in the period from December 2022 to May 2023. The study population included a convenient sample of patients receiving either warfarin or non-vitamin k antagonist oral anticoagulant treatment. The Arabic version of the short form 12 (SF-12) questionnaire and the Anti-Coagulant Treatment Satisfaction Scale (ACTS) questionnaire were used to assess the quality of life and satisfaction with treatment respectively.

Results: The study included 181 patients in total. The mean physical and mental quality of life scores for study participants were 42.3±9.92 and 52.6±10.36 respectively. There was no significant difference in the QOL between patients taking warfarin and those on non-vitamin k antagonist oral anticoagulants treatment. The mean total satisfaction score was 65.4±6.73. Patients receiving non-vitamin k antagonist oral anticoagulants had significantly higher satisfaction compared to those receiving warfarin. The physical score correlated significantly with gender, educational level, employment status, number of chronic medications, and number of chronic diseases. The total satisfaction score correlated significantly with gender, number of chronic medications, number of side effects, and duration of anticoagulation. There was a significant correlation between the QOL and treatment satisfaction.

Conclusion: Treatment with non-vitamin K antagonist oral anticoagulants showed comparable QOL and higher treatment satisfaction than that of warfarin. Better treatment satisfaction can improve patients' QOL which may ultimately enhance their adherence to treatment.

Keywords: Iraqi patients; NOAC; Quality of life; Satisfaction; Warfarin.

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Introduction

With the aging population, the number of people on anticoagulants has been increasing. (1) Oral anticoagulants (OACs) have distinctive ways of action and are indicated for several different conditions including prevention and treatment of thromboembolic diseases in stroke secondary to atrial fibrillation (AF), pulmonary embolism, deep vein thrombosis, myocardial infarction, and valvular heart disease. (2) Vitamin-K antagonist (VKA) warfarin, has been available for over 50 years and is still prescribed. Several factors can make warfarin therapy challenging, like its narrow therapeutic index, the need for frequent laboratory assessment of the international normalized ratio (INR), food-drug and drug-drug interactions, and a slow onset and offset of action. (3) Despite that, warfarin is still a commonly used OAC, because of its affordability and availability. Non-vitamin k antagonist oral anticoagulants (NOACs) have only recently been utilized in clinical settings. (4) NOACs include dabigatran, a direct thrombin inhibitor, and the direct factor Xa inhibitors apixaban, edoxaban, and rivaroxaban. These drugs have reliable anticoagulation, shorter onset and offset of action, predictable pharmacokinetic parameters, fewer drug-food and drug-drug interactions, consistent dosing regimens and they don't require routine laboratory monitoring. (5) However, being newer OACs, there is no enough evidence on their usage in patients during hemodialysis, cancers, additionally to their lack of effectiveness in specific clinical situations, such as antiphospholipid syndrome or mechanical heart valves. (6-7) While OACs effectively treat the disease, they also possess several characteristics that may significantly impact patients' compliance, satisfaction, and QOL. These may include restrictions in activity and diet, requirements of regular laboratory testing, and bleeding adverse effects. As a result, this may adversely affect treatment. Quality of Life is defined by the World Health Organization

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(WHO) as "an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns". (8) Health-related QOL is a crucial measure that helps medical practitioners understand patients' perspectives on diseases. (9) Socio-demographic and socio-economic changes, treatment outcomes and different patient care planning may affect patient's QOL while on OACs. (10) Treatment with an OAC affects patients' QOL as it is linked to a higher risk of bleeding, and needs a change in lifestyle with no objective relief in symptoms. (11). Patient satisfaction remains an important factor that influences patients' adherence to their treatment plans. Satisfaction with the treatment is defined as "an individual's rating of important attributes of the process and outcomes of his/ her treatment experience, which involves the interaction of expectation, preference, and satisfaction". (12) Treatment satisfaction includes aspects of both the therapeutic process and outcome. Factors that are linked to treatment satisfaction are treatment preference, medication beliefs, and adherence. (13) A high level of satisfaction with treatment is crucial as it has been linked to better QOL. (14). Globally, many studies have compared the QOL and satisfaction with the treatment between users of warfarin versus NOACs. A study to assess satisfaction with treatment and QOL in patients taking OACs for atrial fibrillation showed that, when compared to warfarin groups, NOAC users report better satisfaction and similar QOL. (15) Another study conducted to compare satisfaction with treatment and QOL in patients on warfarin versus NOACs found that there was no statistically significant difference in the QOL between the warfarin and NOAC groups. Nevertheless, patients receiving NOACs showed significantly higher satisfaction level compared to those receiving warfarin. (16) In Iraq. there were no previous studies assessing QOL or treatment satisfaction with OAC therapy, although there were some studies assessing QOL in other chronic diseases (17-20). One study assessed the knowledge of patients using OACs for AF. (21) this study aimed to compare the quality of life and treatment satisfaction among patients using anticoagulant therapy (warfarin and NOAC).

Patients and Methods

Study design: The study was designed as a single-center, cross-sectional study. It was performed at Ibn Al-Bitar Hospital for cardiac surgery in Baghdad in the period from December 2022 to May 2023.

Study population and sampling technique

The study population included adult patients who were 18 years of age or older, receiving oral anticoagulation therapy with warfarin or NOACs (rivaroxaban or apixaban) for at least two months, irrespective of the underlying medical condition. They were able to speak and understand Arabic and verbally agreed to participate in this study. They were a convenient sample of patients.

Individuals who were unable to speak had a history of mental illness, refused to participate in the study, provided incomplete responses, and those with end-stage liver disease or end-stage renal disease or malignancy were excluded from this study.

Data Collection: After receiving verbal consent, data was collected from the participants through face-to-face interviews. A data collection sheet was employed to collect the information needed for the study which included: Socio-demographic characteristics (age, gender, marital status, occupational status, educational level, smoking habit, body mass index (BMI)). The collected clinical characteristics of the patients included: Indication for anticoagulant, medical history, medication history, type of anticoagulant used, duration of anticoagulant, concomitant use of antiplatelet drugs, and adverse effects (bleeding). The QOL was assessed using the Arabic version of the Short Form 12 Health Survey (SF12) questionnaire. (22) The SF-12, which consists of 12 items, is a valid substitute for the SF-36 in large-scale surveys of general and specific populations. (23) SF-12 includes eight dimensions: General health, physical functioning, social functioning, bodily pain, mental health, vitality, role limitations resulting from emotional issues, and role limitations resulting from physical health issues. The SF-12 questionnaire's scoring system was based on the SF-12 scoring system developed by Ware and colleagues (24) A weighted number was given to each physical and mental item of the SF-12 questionnaire. The mean physical component score (PCS) and mental component score (MCS) were derived by using specific online calculations (SF-12 – OrthoToolKit ®). These means were used as measures of physical QOL and mental QOL. Treatment satisfaction was assessed using the Arabic version of the Anti-Coagulant Treatment Satisfaction Scale (ACTS) questionnaire. (25) ACTS is a condition-specific tool that is designed to measure patient satisfaction with anticoagulant therapy. ACTS contains 15 items; twelve items to assess treatment burdens and three items to evaluate treatment benefits. A five-point rating system is used to assess the patients' experiences with anticoagulant treatment over the last four weeks (1 being not at all, 2 being a little, 3 being moderately, 4 being quite a bit, and 5 being extremely). The ACTS burden scores are reverse-scored, with higher scores indicating less burden and ranging from 12 to 60. On the other hand, the benefit scores are directly scored, ranging from 3 to 15. Greater satisfaction with treatment is indicated by higher ACTS Burdens and Benefits scores. (26)

Statistical analyses:

The Statistical Package for Social Sciences (SPSS) version 25 was used to analyze the data. Descriptive statistics were performed on all study items. Continuous variables were expressed as means ±

standard deviation (SD), while categorical variables were expressed as frequencies and percentages. The independent T-test was utilized to compare the differences in the means of continuous variables between the two treatment groups (NOAC vs warfarin). One-way ANOVA was used to measure the difference in means of the continuous variables (total scores) across demographics with more than two categories. Pearson correlation was used to measure the relationships between the continuous variables. A P-value of less than 0.05 was considered to be statistically significant.

Results

Socio-demographic and clinical data of the participants: A total of 181 patients participated in the current study. The patients had a mean age of 57.1 ± 10.72 years and a mean BMI of 28.7 ± 5.09 kg/m2. Males constituted 60.2% of the patients, 92.8% were married, and 58.6% had primary or secondary education, table 1.

Table 1: Distribution of the participants by socio-demographic characteristics

Variables	Categories	Frequency (%)
Gender	Male	109 (60.2)
	Female	72 (39.8)
Education level	No formal education	41 (22.7)
	Primary school	45 (24.9)
	Secondary school	61 (33.7)
	College	34 (18.8)
Marital status	Married	168 (92.8)
	Unmarried	13 (7.2)
Employment status	Employed	52 (28.7)
	Retired	35 (19.3)
	Unemployed	94 (51.9)
Cigarette smokers	Non-smoker	106 (58.6)
	Smoker	20 (11.0)
	Ex-smoker	55 (30.4)
Alcohol drinker	Yes	1 (0.6)
	No	180 (99.4)

	Minimum	Maximum	Mean±SD
Age (years)	24.0	78.0	57.1±10.72
BMI (kg/m2)	16.3	46.90	28.7±5.09

The mean duration of anticoagulant use was (6.0±8.11) years. More than half (56.9%) of the patients were taking warfarin with the most frequent indication for use being AF (56.4%). Non-valvular AF was the most frequent type of AF (40.3%). Hypertension was the most frequent chronic disease among the participants (45.3%). Moreover, 73.8% of the participants were on chronic use of beta-blockers, and 81.2% had no concomitant use of antiplatelet drugs (aspirin or clopidogrel). Epistaxis was the most frequent adverse effect of anticoagulants (23.2%), table 2.

Table 2: Distribution of the participants by their clinical characteristics

Variables	Categories	Frequency (%)
Anticoagulants type	Warfarin	103 (56.9)
	Apixaban	46 (25.4)
	Rivaroxaban	32 (17.7)
Indications for anticoagulant use	Atrial fibrillation	102 (56.4)
	Prosthetic valve	99 (54.7)
	Other indications*	9 (5.0)
AF type	Non-valvular AF	73 (40.3)
	Valvular AF	29 (16.0)
Prosthetic valve type	Aortic	64 (35.4)
	Mitral	55 (30.4)
	Aortic & mitral	20 (11.0)
Number of chronic diseases	0	25 (13.8)
	1	72 (39.8)
	2	61 (33.7)
	≥3	23 (12.7)
Number chronic medications	0	10 (5.5)
	1	14 (7.7)
	2	22 (12.2)
	3	29 (16.0)
	4	42 (23.2)
	5	29 (16.0)
	≥6	35 (19.3)
Antiplatelet drugs	None	147 (81.2)
	Clopidogrel	19 (10.5)
	Aspirin	11 (6.1)
	Asiprin & clopidogril	4 (2.2)
Bleeding adverse reactions of anti-coagulants (minor bleeding) †	Epistaxis	42 (23.2)
	Bleeding gums	39 (21.5)
	Hematuria	11 (6.1)
	Menorrhagia	6 (3.3)
	Other adverse effects £	12 (6.6)

	Minimum	Maximum	Mean±SD
Duration of anticoagulants (years)	0.17	50.00	6.0±8.11

***** Other indications: deep venous thrombosis, atrial flutter, and pulmonary embolism.
† Minor bleeding: Not requiring treatment according to WHO bleeding scale.
£ Other adverse effects: Bruising, melena, bleeding per rectum, hematemesis, hemoptysis, ecchymosis, otorrhagia.

Assessment of quality of life: The mean physical component score (PCS) of all participants was 42.3±9.92 (range: 21.7-62.9). There was no statistically significant difference in PCS subscale of QOL between participants taking warfarin and NOACs (P=0.052). The mean mental component score (MCS) of all participants was 52.6±10.36 (range: 14.5-66.1). There was no statistically significant difference in MCS between patients receiving warfarin and NOACs treatment (P = 0.322), table 3.

Table 3: The quality of life across the study groups

Variable	Anticoagulant	N	Mean of score±SD	P-value*
PCS	Warfarin	103	43.6±9.72	0.052
	NOAC	78	40.7±10.00	
MCS	Warfarin	103	51.9±10.88	0.322
	NOAC	78	53.5±9.62	
Variable	N	Minimum score	Maximum score	Mean±SD
PCS	181	21.7	62.9	42.3±9.92
MCS	181	14.5	66.1	52.6±10.36

Relationships of sociodemographic and clinical variables to QOL: Patients' gender, educational level and employment status had statistically significant effects on the PCS. The mean PCS was significantly higher in males compared to females (44.5 ± 9.54 vs 39.0 ± 9.62, p = 0.000). Patients with college education had significantly higher PCS compared to those with other educational levels (45.7 ± 9.13, p = 0.006). Patients who were employed had significantly higher PCS compared to those with other employment status (45.4 ± 9.98, p = 0.011). There was no significant difference between QOL according to the patient's age, marital status, cigarette smoking and BMI. PCS had significant negative correlations with the number of chronic diseases (r = - 0.314, p = 0.000) and chronic medications (r = - 0.153, p = 0.041). When the number of chronic diseases and medications decreases, the PCS increases. **Assessment of treatment satisfaction:** The mean total anticoagulant treatment satisfaction scale (ACTS) score for the whole study participants was 65.4±6.73 (range: 42-75). There was a statistically significant difference in the total ACTS score between patients receiving warfarin treatment and those receiving NOACs treatment (P=0.000). There was a statistically significant difference in the burden ACTS score between the two study groups (P=0.000) whereas there was no significant difference in the benefit ACTS score (P=0.083), table 4.

Table 4: Anticoagulant treatment satisfaction across the study groups

Variable	Anti-coagulant	N	Mean of score±SD	P-value*
Total ACTS score	Warfarin	103	62.6±7.45	0.000
	NOAC	78	69.1±2.92	
Total burden ACTS score	Warfarin	103	50.0±6.34	0.000
	NOAC	78	55.8±1.96	
Total benefit ACTS score	Warfarin	103	12.7±2.37	0.083
	NOAC	78	13.2±1.90	
Variable	N	Min. score	Max. score	Mean±SD
Total ACTS score	181	42.00	75.00	65.4±6.73
Total burden ACTS score	181	30.00	60.00	52.5±5.73
Total benefit ACTS score	181	3.00	15.00	12.9±2.19

Relationship of sociodemographic and clinical characteristics of treatment satisfaction

There was a statistically significant difference in the total ACTS score according to the patients' gender with males having significantly higher ACTS scores than females (66.5 ± 6.15 vs 63.8 ± 7.25, p = 0.007). There was no significant difference between treatment satisfaction and the patient's age, education level, employment status, marital status, cigarette smoking, and BMI. The total ACT and burden ACT scores had significant negative correlations with the duration of anticoagulation (r= - 0.214 p=0.004, r= - 0.255 p=0.001) and the number of adverse effects (r= - 0.227 p = 0.002, r= - 0.240 p=0.001) respectively. In contrast, they had a significant positive correlation with the number of chronic medications used by the patients (r= 0.153 p=0.041, r= 0.149 p=0.047) respectively.

Correlation of QOL with treatment satisfaction: There was a statistically significant correlation between the two study parameter scores. The total ACTS scores, burden ACTS scores and benefit ACTS scores had significant positive correlations with QOL (PCS & MCS). Regarding PCS, the total ACTS score, burden ACTS score, and benefit ACTS score showed a positive correlation of 0.329 (P=0.000), 0.310 (P=0.000), and 0.199 (P=0.007) respectively. Concerning MCS, the total ACTS score, the burden ACTS score, and the benefit ACTS score displayed a substantial positive correlation of 0.385 (P=0.000), 0.338 (P=0.000), and 0.298 (P=0.000) respectively.

Discussion

The primary focus of clinical trials and observational studies has been on evaluating the safety and effectiveness of anticoagulant medications but not on QOL or treatment satisfaction. Similar to the results of the current study on QOL of patients taking warfarin or NOACs, other studies reported the same findings of non-significant differences between mean PCS or MCS. (16,27,28) This may be due to the fact that anticoagulants do not provide objective relief for symptoms. Furthermore, QOL assessed after two months of treatment may lead patients to adapt to the treatments. (16) The finding of the current study of a non-significant difference between patients' age and PCS and MCS is in line with other studies that assessed QOL in other chronic diseases where there was no significant difference of age with QOL. (29-30) However, Fang et al. showed there was a

significant difference between higher MCS scores and lower PCS scores with older age. (27) The finding of the current study of a significant relationship between gender and PCS (males having a higher QOL than females) are in line with other studies which showed that both PCS and MCS are higher in males than females. (27,31,32) This may be due to physiological and biological factors that contribute to the differences between genders, as females tend to experience more discomfort and pain. It is believed that males may give less attention to healthcare than females. Previous Iraqi studies found no significant association between gender and QOL. (33) The finding of the current study that showed the level of education had a significant positive relationship with the PCS domains but not with MCS domains of QOL, was in line with other studies showing a direct correlation between higher education levels and high QOL score. (10,31,34) Highly educated people were found to live longer lives with better health than those with low educated, suggesting that higher levels of education improve self-care and self-motivation, which improve health status. (35) The finding of the current study that showed a significant higher score in PCS were observed in employed patients is in agreement with the results of another study that showed higher PCS and MCS scores in employed patients. (31) This may be due higher monthly incomes which in turn improve the living conditions resulting in a better QOL. In addition, employment provides social support and engagement in social activities. (36) Comorbidities were found to significantly impair the physical domain of the QOL in the current study, which is consistent with an earlier study that reported comorbidities significantly impact the QOL. (37) Patients with comorbidities tend to have a lower positive perception of their QOL. They can contribute to decreased self-care, daily activities and mobility. Patients with comorbidities reported higher levels of discomfort and depression than patients without comorbidity. (38) The current study found that patients on NOACs had higher satisfaction levels than patients on warfarin, with both higher mean ACTS burden scores and higher ACTS benefit scores, in consistence with earlier studies. (16,39) This is because dosing and administration of NOACs are simple, with no need for INR monitoring or drug/food restrictions, and a lower risk of fatal bleeding. (5) Patients on warfarin may have a substantial burden as a result of these limitations.

The current study found a significantly higher mean satisfaction score in men than women. Woman may have lower satisfaction due to the increased risk of side effects from menstruation and pregnancy, and a greater responsibility and workload at home. Women are perceived to have a lower health perception and QOL than men in several chronic clinical conditions. (14) These results were similar with those of another study which showed that men have higher satisfaction scores in most domains than women. (39) In contrast, a study conducted in Canada, showed that women had higher satisfaction scores than men. (40) The current study results found a significantly negative correlation between the duration of therapy and the burden of ACTS and total ACTS scores, in line with other studies. (14) Frequently used anticoagulants for long duration with bleeding side effects, limitation in physical activity, and restriction of some drugs or foods can all have a negative effect on patient's QOL and lower their compliance and satisfaction levels. The number of side effects experienced by the patients had a significant negative correlation with satisfaction, in line with other studies that showed the side effects of warfarin can negatively affect patient satisfaction levels. (41,42) It is thought that the bleeding history of patients may restrict them by increasing their obligations, such as taking medications regularly and not stopping INR follow-ups, which can lead to decreased satisfaction. (42) The significant positive correlation between satisfaction and number of chronic medications found in the current study is in disagreement with a previous study which showed a significant negative correlation of poly-medication with the ACTS burden scale and a non-significant association with the ACTS benefit scale. (14) This may be due to differences between study populations, duration of treatment, and sample size. In the current study, QOL was significantly correlated with satisfaction. In contrast, previous studies showed no significant correlation between them. Ingre et al found no significant correlation between QOL and satisfaction. (11) Michaël et al reported no significant association between the three parameters (QOL, satisfaction, and adherence). (15)

Conclusion

Treatment with NOACs showed comparable QOL and greater satisfaction with treatment than warfarin. Better satisfaction with treatment can improve patients' quality of life which may ultimately enhance their treatment adherence.

Authors' declaration

We confirm that all Tables in the manuscript are mine.

The project was approved by the local ethical committee in (College of Pharmacy, University of Baghdad) according to the code number (RECAUBLP4120225). In addition, approval of the Ibn Al-Bitar Hospital was obtained. While verbal agreement was obtained from patients to participate in the study.

Conflict of interest: None

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Authors' Contribution

Study conception & design: (Tuqa Maitham AL-Ameen & Basma Zuheir Al-Metwali). Literature search: (Tuqa Maitham AL-Ameen). Data

acquisition: (Tuqa Maitham AL-Ameen). Data analysis & interpretation: (Tuqa Maitham AL-Ameen & Basma Zuheir Al-Metwali). Manuscript preparation: (Tuqa Maitham AL-Ameen & Basma Zuheir Al-Metwali). Manuscript editing & review: (Tuqa Maitham AL-Ameen & Basma Zuheir Al-Metwali).

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مقارنة نوعية الحياة والرضا عن العلاج بين عينة من المرضى العراقيين الذين يستخدمون العلاج المضاد للتخثر (الوارفرين أو مضادات التخثر الفموية غير المضادة لفيتامين ك)

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الخلاصة

الخلفية: قد يتطلب العلاج المضاد للتخثر بما في ذلك الوارفرين ومضادات التخثر الفموية غير المضادة لفيتامين ك (NOAC) استخدامًا طويل الأمد مما قد يؤثر على رضا المرضى عن علاجهم ونوعية حياتهم.

الهدف: مقارنة نوعية الحياة والرضا عن العلاج بين المرضى الذين يستخدمون العلاج المضاد للتخثر (الوارفارين ومضادات التخثر الفموية غير المضادة لفيتامين ك).

المنهجية: أجريت هذه الدراسة المقطعية في مستشفى ابن البيطار لجراحة القلب في بغداد في الفترة ما بين كانون الأول 2022 إلى أيار 2023. شمل مجتمع الدراسة عينة مناسبة من المرضى الذين يتلقون علاج الوارفرين أو مضادات التخثر الفموية غير المضادة لفيتامين ك (NOACs) وقد تم استخدام النسخة العربية من الاستبيان القصير (SF-12) واستبيان مقياس الرضا عن العلاج المضاد للتخثر (ACTS) لتقييم جودة الحياة والرضا عن العلاج على التوالي.

النتائج: شملت الدراسة 181 مريضا، كان متوسط درجة جودة الحياة الجسدية والعقلية لديهم (42.3 ± 9.92) و (52.6 ± 10.36) على التوالي. ولم يكن هناك فرق كبير في جودة الحياة بين المرضى الذين يتلقون الوارفرين وأولئك الذين يتلقون مضادات التخثر الفموية غير المضادة لفيتامين ك (NOAC). اما متوسط درجة الرضا الإجمالي فقد كان (65.4 ± 6.73). كان المرضى الذين يتلقون مضادات التخثر الفموية غير المضادة لفيتامين ك أكثر رضاً عن العلاج بشكل ملحوظ مقارنةً بمرضى الوارفرين (P = 0.000). ترتبط نتائج جودة الحياة البدنية بشكل كبير بالجنس ومستوى التعليم والحالة الوظيفية وعدد الأمراض المزمنة وعدد الأدوية المزمنة. اما درجة الرضا الإجمالية فترتبط بشكل كبير بالجنس وعدد الأدوية المزمنة وفترة اخذ العلاج المضاد لتخثر الدم وعدد الآثار الجانبية. وقد كان هناك ارتباط كبير بين جودة الحياة والرضا عن العلاج (P = 0.000).

الاستنتاج: أظهر العلاج باستخدام مضادات التخثر الفموية غير المضادة لفيتامين ك جودة مماثلة ورضا أعلى مقارنة بالمرضى الذين يتلقون علاج الوارفارين. إن الرضا الأفضل عن العلاج يمكن أن يحسن نوعية حياة المرضى مما قد يعزز في النهاية التزامهم بالعلاج.

الكلمات المفتاحية: وارفرين، مضادات التخثر الفموية غير المضادة لفيتامين ك، جودة الحياة، الرضا، المرضى العراقيين.

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Research Article

Evaluation of the Level of Electrolytes in Children with Steroid Sensitive or Steroid Resistant Nephrotic Syndrome

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Abstract:

Background: Childhood idiopathic nephrotic syndrome is one of the most common conditions pediatric nephrologists encounter globally. Nephrotic syndrome is characterized by proteinuria, hyperlipidemia, and edema. The degree and duration of proteinuria have an impact on serum electrolyte levels. However, local data is limited.

Objectives: To assess and compare the degree of electrolyte imbalance and its relationship to kidney functions indicators during relapse and remission in children with idiopathic nephrotic syndrome.

Methods: In this case-control study, blood samples were collected from 80 Iraqi children with an age range of (2-14) years. They were divided into three groups: Group I (20 individuals with steroid-sensitive nephrotic syndrome (SSNS)), Group II (20 individuals with steroid-resistant nephrotic syndrome (SRSN)), and Group III (40 healthy individuals as the control group). Serum electrolyte levels (Na, Ca, Cl, and K) were measured by an ion-selective electrode (9180 electrolyte analyzer). Blood creatinine and urea were measured by a Cobas c311 autoanalyzer during the relapse and remission phase. The patients were clients of the pediatric nephrology consultation center at the Children's Teaching Hospital / Baghdad Medical City and Al-Batoul Teaching Hospital for Women and Children from 15 February to 20 August 2022. The controls were healthy children who medical history was reviewed to eliminate a history of kidney disease and underwent a comprehensive physical examination. Controls were recruited from a network of family, friends, relatives, and the National Autism Center/ Child Protection Teaching Hospital affiliated with Medical City/Baghdad.

Results: Serum Calcium levels showed a clear decrease in all SSNS and SRSN patients compared to the control group. The levels of Sodium and Chloride were significantly lower than the control group during the relapse phase. The results of the relapse phase of SRNS patients indicated higher serum potassium concentration compared with the control group and the SSNS patient group, with a statistically significant difference).

Conclusion: All children with idiopathic nephrotic syndrome had hypocalcemia in the relapse and remission phase. SRNS cases had hyperkalemia, Sodium and chloride fluctuated between low levels during the relapse phase and normal levels during the remission phase.

Keywords: Electrolyte; Estimated glomerular filtration rate (eGFR); Idiopathic nephrotic syndrome.

Introduction:

Nephrotic syndrome is becoming more widespread and is currently the second most common condition in pediatric nephrology [1]. The clinical manifestation of glomerular illness known as "nephrotic syndrome" is typified by severe proteinuria exceeding 3.5 grams per 24-hour period [2]. Increased permeability of the glomerular capillary walls is the cause of proteinuria which results in the loss of albumin (hypoalbuminemia) (≤2.5 g/dL), edema, and hyperlipidemia (cholesterol >200 mg/dL) which comprise the trio of nephrotic disorders [3,4]. Steroid treatment is the first line of therapy, but even with this kind of care, relapses are common (1–20 relapses throughout childhood), and they can cause serious morbidities [5,6]. Relapse is associated with many complications such as hypertension, cataract, osteoporosis, and growth retardation [7,8]. Approximately 80% of children with nephrotic syndrome fully recover from their proteinuria after taking prednisolone for four to six weeks [9]. Steroid-

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resistant nephrotic syndrome (SRNS) is a condition that affects about 20% of children with nephrotic syndrome who do not improve after a prescribed course of prednisolone. It is more rapidly progressing than steroid-sensitive nephrotic syndrome (SSNS) and is linked to a higher chance of coexisting problems [10,11]. Furthermore, with an increasing number of cases reported globally, SRNS is one of the main causes of end-stage renal disease and chronic renal failure in children [12,13]. The categorization of chronic kidney disease in children and adults is mostly based on (eGFR) in conjunction with predetermined cut-off values. Sixty mL/min/1.73 m^2 is the primary cut-off eGFR number used to characterize chronic kidney disease (CKD). This value is also applied to children older than 2 years, adolescents, and young adults in whom abnormal GFR begins at less than 75 (mL/min/1.73 m^2) [14]. Children with nephrotic syndrome frequently experience problems with the metabolism of their electrolytes [15]. The degree and duration of proteinuria affect the electrolyte levels in the serum. Changes in serum electrolyte levels can result in a range of symptoms, from minor ones like exhaustion, lethargy, and cramping in the muscles to serious ones like irregular heartbeat, disorientation, convulsions, and even death [16].

Global studies indicate a correlation between electrolyte imbalance and nephrotic syndrome. Local studies are few, though. The electrolyte imbalance in children with nephrotic syndrome in both remission and relapse will be ascertained in this investigation. In addition to highlighting recent findings, this study will persuade medical professionals to treat patients with idiopathic nephrotic syndrome more comprehensively.

The current study aims to assess and compare the degree of electrolyte imbalance and its relationship to kidney function indicators during relapse and remission in children with idiopathic nephrotic syndrome.

Patients and Methods:

The patients are Iraqi children suffering from idiopathic nephrotic syndrome, aged 2 to 14 years, who were seen in the pediatric nephrology consultation center at the Children's Teaching Hospital / Baghdad Medical City and Al-Batoul Teaching Hospital for Women and Children from 15 February to 20 August 2022. The total number of participants in this study is 80, of whom there were 40 patients and 40 controls. The patients were classified into two groups: Group I (20 renal patients with SSNS) and Group II (20 renal patients with SRNS). Group III (40 healthy control children who were age and sex-matched to the patients). A control group of healthy children was established following a rigorous medical history review to eliminate participants with a history of kidney disease. All controls underwent a comprehensive physical examination. Controls were recruited from a network of family, friends, relatives, and the National Autism Center/Child Protection Teaching Hospital affiliated with Medical City/Baghdad.

Nephrotic syndrome was diagnosed according to the following criteria:

- The steroid-sensitive children were those who showed no proteinuria on early morning urine dipsticks (less than 1+) during the first four weeks of daily prednisolone medication (2 mg/kg/day or 60 mg/m^2 and a maximum daily dose of 60 mg/day).
- The steroid-resistant children were those who, after eight weeks of daily prednisolone or four-six weeks of daily prednisolone regimen (2 mg/kg/day or 60 mg/m^2 and a maximum daily dose of 60 mg/day) followed by another four-six weeks of alternate day prednisolone regimen (1.5 mg/kg/day or 40 mg/m^2 and a maximum daily dose of 50 mg/day), did not achieve remission (more than 1+ proteinuria on early morning urine dipstick) [17].

Relapse: Heavy proteinuria is defined as a corresponding to 3+ or 4+ (protein excretion = 300 mg/dL or 2.0-5.0 mg/hour) by urine dipstick test for 3 consecutive days after remission, edema, hypoalbuminemia (less than 2.5 g per dL), and hyperlipidemia [18].

Remission: The absence of proteinuria for ≥ 3 consecutive days or < 1+ (negative or trace protein; corresponding to negative or trace < 10 mg/dL protein) on urine dipstick [19].

The exclusion criteria included acute kidney injury, and nephrotic syndrome due to systemic diseases such as viral infections, lupus nephritis, or diabetes. The following information was collected from all children: Complete medical history including nephrotic syndrome symptoms, illness duration, and steroid medication response. Investigations included serum electrolytes (Na, Ca, Cl, and K) levels, urine protein, serum creatinine, blood urea, and estimated glomerular filtration rate (eGFR).

Blood sample collection and biochemical analysis

Five milliliters of venous blood were collected in gel tubes. After clotting, they were centrifuged at 3000 rpm for 10 min. The level of serum electrolytes was determined by the ion-selective electrode principle of (9180 Electrolyte Analyzer). The serum creatinine, blood urea, and serum albumin were determined at the same day by auto analyzer Cobas c311 supplied by SIEMENS Dimension. Then, the estimated glomerular filtration rate (eGFR) was calculated according to the Schwartz formula [20] with height measured in cm and creatinine (mg/dL), a bedside calculation of 0.413* (height/serum creatinine).

Statistical analysis

Frequencies and percentages were used to describe categorical data. Minimum and maximum values, along with the mean and standard deviation (SD) were calculated for continuous data. The Kolmogorov-Smirnov test was used to assess the normality of the data. One-way Analysis of Variance (ANOVA) was done to compare the differences between the means of the three groups. The differences between two selected groups in multiple pairwise comparisons using post-hoc tests (Games-Howell in equal variances assumed and Bonferroni for equal variances not assumed) were presented as p-values. The Chi-square test was used for categorical variables. P values of < 0.05 were considered statistically significant. Pearson's correlation test was used to determine the relationship between the two parameters. We used SPSS software (version 23.0) to perform statistical analyses.

Results:

No statistically significant associations were found between gender, age, and body mass index (BMI) and the three study groups, Table 1.

Table 1: Description of selected demographic variables in the study groups

Variables	Total (n=80)	SSNS (n=20)	SRNS (n=20)	Controls (n=40)	p- value
Gender					0.855[a]
Males	48 (60%)	12 (25%)	13 (27.1%)	23 (47.9%)	
Females	32 (40%)	8 (25%)	7 (21.9%)	17 (53.1%)	
Age, years					
mean±SD	8.2 ± 3.68	7.6 ± 3.95	9.6 ± 3.03	7.9 ± 3.75	0.168[b]
Range	(2-14)	(2-14)	(4-14)	(2-14)	
BMI					
mean±SD	19.5+1.37	19.1±1.01	20.1±1.63	19.3±1.31	0.050[b]
Range	(16.8-22.7)	(17.6-21.3)	(17.9-22.7)	(16.8-22.0)	

a: *Chi-square test was used.*
b: *The ANOVA test was used to determine whether the mean difference was significant at the 0.05 level of analysis.*

Laboratory measurements for the patients in the relapse phase are shown in Table 2. Significant differences were found between the mean values of serum electrolytes (Na, Cl, K, and Ca), Creatinine, eGFR and blood urea in the three groups. Lower sodium, calcium and chloride concentrations were observed in the SSNS and SRNS groups in comparison to the controls ($p < 0.0001$ for all pairwise comparisons). The levels of proteinuria in the relapse phase were heavy proteinuria as the equivalent of 3+ or 4+ by urine dipstick (protein excretion = 300 mg/dL or 2.0-5.0 mg/hour).

Table 2: Biochemical laboratory results for patients in the relapse phase

Parameters	Groups	Mean±SD	Min.– Max	P-Value
Na (mmol/L)	SSNS	126.9±5.65	117- 136	<0.001
	SRNS	127.3±7.20	114- 141	
	Control	138.9±3.23	134-144	
Ca (mg/dL)	SSNS	5.9±0.85	4.5-7.6	<0.001
	SRNS	6.0±1.15	4.1-7.8	
	Control	9.3±0.69	8.3-10.8	
K (mmol/L)	SSNS	5.9±0.33	5.4-6.5	<0.001
	SRNS	5.6± 0.40	5.1- 6.3	
	Control	4.5±0.57	3.3- 5.4	
Cl (mmol/L)	SSNS	93.6+2.44	89.5-97.6	<0.001
	SRNS	94.5+3.05	88.8-99.2	
	Control	100.6+3.51	95.3-108.2	
Urea (mg/dL)	SSNS	27.2±4.72	19.3-35.6	0.036
	SRNS	29.3±6.43	18.1-38.5	
	Control	25.5±4.89	18.4-33.9	
Creatinine (mg/dL)	SSNS	0.5±0.08	0.4-0.7	<0.001
	SRNS	0.667±0.14	0.4-0.9	
	Control	0.5±0.09	0.4-0.7	
eGFR (mL/min/1.73 m²)	SSNS	94.1±24.68	56.4-131.6	0.009
	SRNS	84.1±16.82	48.9-113.1	
	Control	103.2±23.21	55.5-152.1	

The ANOVA test was used to determine whether the mean difference was significant at the 0.05 level of analysis.
***= Highly significant difference at 0.05 level.*

As for patients in remission (Table 3), the SSNS patients had all their electrolytes return to normal levels, except for calcium, which continued to be lower than the control group. Sodium, chloride, and potassium were not significantly different when compared to the controls (p=0.182, p=0.884, and p=0.083 respectively). On the other hand, patients in the SRNS group during the remission phase, where the concentrations of both sodium and calcium remained low, with a statistically significant difference compared to the control group (p<0.0001, and p<0.0001, respectively), while the concentrations of chloride and potassium did not show any statistically significant difference compared to the control group (p = 0.931, and p = 0.101 respectively).

Table 3: Biochemical laboratory results for patients in the remission phase

Parameters	Groups	Mean±SD	Min.– Max	P-Value
Na (mmol/L)	SSNS	137.1±3.05	131-142	<0.001
	SRNS	134.2±4.17	127-141	
	Control	138.9±3.23	134-144	
Ca (mg/dL)	SSNS	8.2+0.76	7.2-9.4	<0.001
	SRNS	6.7+0.58	5.8-7.8	
	Control	9.3+0.69	8.3-10.8	
K (mmol/L)	SSNS	5.1±1.01	2.7-6.7	0.017
	SRNS	5.1±1.12	3.33-7.2	
	Control	4.5±0.57	3.3- 5.4	
Cl (mmol/L)	SSNS	101.6±3.44	94.1-105.7	0.576
	SRNS	101.0±3.64	95.2-107.7	
	Control	100.6±3.51	95.3-108.2	
Urea (mg/dL)	SSNS	25.3±4.19	19.1-33.2	<0.001
	SRNS	26.8±4.51	18.3-34.4	
	Control	25.5±4.89	18.4-33.9	
Creatinine (mg/dL)	SSNS	0.5±0.10	0.4-0.8	0.028
	SRNS	0.6±0.12	0.4-0.9	
	Control	0.5±0.09	0.4-0.7	
eGFR mL/min/1.73 m²	SSNS	94.8±16.57	68.1-120.2	0.009
	SRNS	85.9±15.95	55.4-110.8	
	Control	103.2±23.21	55.5-152.1	

The ANOVA test was used to determine whether the mean difference was significant at the 0.05 level of analysis.
***= Highly significant difference at 0.05 level.*

The eGFR values for all patients (SSNS) and (SRNS), whether in remission or relapse, were normal, greater than (75 ml/min/1.73 m²). A negative correlation was found between eGFR and all electrolytes except for Chloride (r = 0.035, p=0.883) in the SSNS patients in the relapse phase, while the results of SRNS patients in the relapse phase indicate a positive correlation for electrolytes, except for potassium, which had a negative relationship with eGFR (r = -0.465, p=0.039). The correlation was weakly negative between eGFR and both urea and creatinine for SSNS patients, while it was strongly negative for SRNS patients. On the other hand, the results for SSNS patients in the remission phase indicate a positive correlation with all measured variables, except for creatinine, which was negative (r=-0.484, p=0.030). A positive correlation between serum concentrations of (Na, Cl, and K), while they were negative with calcium (r=-0.141, p=0.554). The results of SRNS patients indicate a strong negative correlation between eGFR and both creatinine and urea in SRNS patients in remission, table 4.

Table 4: Correlation between eGFR and biochemical variables in SSNS and SRNS groups

Parameters	Relapse phase				Remission phase			
	SSNS		SRNS		SSNS		SRNS	
	r	P	r	P	r	P	r	P
Na (mmol/L)	-0.005	0.983	0.195	0.411	0.259	0.271	0.106	0.657
K (mmol/L)	-0.147	0.537	-0.465*	0.039	0.119	0.617	0.032	0.894
Cl (mmol/L)	0.035	0.883	0.194	0.413	0.252	0.283	0.173	0.467
Ca (mg/dL)	-0.071	0.767	0.178	0.454	0.024	0.919	-0.141	0.554
Urea (mg/dL)	-0.215	0.362	-0.736**	0.000	0.194	0.413	-0.564**	0.010
Creatinine (mg/dL)	-0.310	0.184	-0.873**	0.000	-0.484*	0.030	-0.841**	0.000

***. Correlation is significant at the 0.01 level (2-tailed).*
**. Correlation is significant at the 0.05 level (2-tailed).*

Discussion:

In the current study, the BMI was not different between the study groups and was always on the low side. Shah et al. noted that in adults and children with proteinuric glomerulopathies, obesity was linked to a

lower rate of proteinuria remission from nephrotic syndrome [21]. The lower sodium, calcium, and chloride concentrations observed in the SSNS and SRNS groups in comparison to the control subjects are consistent with the results of previous studies [22,23] which showed a significantly lower serum sodium and calcium concentration. However, Basu et al disagree with our results, indicating that sodium and potassium concentrations were at normal levels in patients in the relapse stage [24]. Previous studies have also indicated that low albumin levels might still cause total calcium to appear lower during the relapse stage. However, the ionized calcium (active form) also remains normal during relapse [16]. The current study showed that the highest serum potassium concentration was observed in the SRNS group, and there were significantly higher potassium levels in SSNS patients than in the control group. This is consistent with the results of Ydegaard who found that patients in relapse suffer from high serum potassium. Through reabsorption/ excretion via the kidneys and gastrointestinal tract, serum potassium concentration is regulated, so an increase in potassium concentrations is an indicator of damage to the kidneys caused by the nephrotic syndrome [25]. Regarding kidney function indicators, the higher creatinine and urea concentrations in SRNS patients than the SSNS and controls, and their normal levels in the SSNS are consistent with the results of Thakor [26]. The results showed that relapsed eGFR scores were normal, but when combined with protein concentrations, they indicated a moderate level of risk to kidney function. According to Andersen, between the acute and remission periods, there was no discernible change in eGFR in patients with acute nephrosis [27]. The normal creatinine concentrations and eGFR values in the remission phase for the SSNS group, the absence of significant differences between urea concentrations between the three groups, the higher creatinine concentration and the low eGFR values in the SRNS group compared to the controls are consistent with the findings of Esezobor that when compared to children with SRNS, children with SSNS had greater eGFR and lower serum creatinine [10]. Current guidelines on chronic kidney disorders from Kidney Disease Improving Global Outcomes (KDIGO) call for the assessment of albuminuria to determine the amount of proteinuria and that combined with the calculation of eGFR values to base the final decision on the assessment of renal function of patients [28]. Elevated urinary protein excretion during the relapse phase, in conjunction with eGFR values of 94.1 ml/min/1.73m² for SSNS patients and 84.1 ml/min/1.73m² for SRNS patients, aligns with KDIGO guidelines, suggesting a moderate risk to renal function. In the remission phase when considering proteinuria along with eGFR values according to the recommendations of (KDIGO), there is little risk to kidney function because there is no proteinuria (protein secretion +1, or 30 mg/dL or 1.0–2.0 mg/hour). The eGFR values are higher during this phase than those for patients in the relapse phase.

Conclusion

All children with idiopathic nephrotic syndrome had hypocalcemia in the relapse and remission phase. SRNS cases had hyperkalemia, Sodium and chloride fluctuated between low levels during the relapse phase and normal levels during the remission phase. Kidney function, represented by eGFR, was within normal limits in the remission phase but at a moderate risk level in the relapse phase due to its association with proteinuria.

Authors' declaration:

At this moment, we confirm that all the Figures and Tables in the manuscript are ours. Authors sign on ethical consideration's Approval-Ethical Clearance: Approval was obtained from the Scientific Research Committee of the Diyala Health Department to conduct this research according to the code number (4408 on 27/1/2022). Also, the local ethical committee in the Medical City-Baghdad/Children Welfare Teaching Hospital approved the project according to code number (7345 on 15/2/2022).

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Authors' Contributions

Study conception & design: (Ahmed H. Alwan& Nawal M.J. Al-Shammaa). Literature search: (Ahmed H. Alwan). Data acquisition: (Zahraa M. Naji). Data analysis & interpretation: (Ahmed H. Alwan). Manuscript preparation: (Ahmed H. Alwan). Manuscript editing & review: (Nawal M.J. Al-Shammaa).

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تقييم مستوى الألكتروليتات عند الأطفال المصابين بمتلازمة الكلى الحساسة أو المقاومة للستيرويد

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الخلاصة:
الخلفية: متلازمة الكلى مجهولة السبب عند الأطفال هي واحدة من أكثر الحالات شيوعًا التي يواجهها أطباء أمراض الكلى عند الأطفال على مستوى العالم. تتميز متلازمة الكلى ببيلة بروتينية وفرط شحميات الدم والوذمة. تؤثر درجة ومدة بيلة البروتين على مستويات الإلكتروليتات في المصل. ومع ذلك، فإن البيانات المحلية محدودة.
الأهداف: تقييم ومقارنة درجة إختلال توازن الشوارد وعلاقتها بمؤشرات وظائف الكلى أثناء مرحلتي الإنتكاس والهدوء عند الأطفال المصابين بمتلازمة الكلى مجهولة السبب.
المرضى والمنهجية: تضمنت هذه الدراسة للحالة والشواهد جمع عينات الدم من 80 طفلاً عراقيًا تتراوح أعمارهم بين (2-14) عامًا. تم تقسيمهم إلى ثلاث مجموعات: المجموعة الأولى (40 فردًا سليمًا كمجموعة تحكم)، المجموعة الثانية (20 فردًا مصابًا بمتلازمة الكلى الحساسة للستيرويد (SSNS))، والمجموعة الثالثة (20 فردًا مصابًا بمتلازمة الكلى المقاومة للستيرويد (SRSN)). تم قياس مستويات الإلكتروليتات في المصل (Na و Ca و Cl و K) بواسطة قطب كهربائي إنتقائي للأيونات (محلل إلكتروليتات 9180). تم قياس الكرياتينين واليوريا في الدم بواسطة جهاز التحليل التلقائي Cobas c311 أثناء مرحلة الإنتكاس و مرحلة الهدوء. كان المرضى من مراجعي مركز استشارة أمراض الكلى للأطفال في مستشفى الأطفال التعليمي / مدينة الطب ببغداد ومستشفى البتول التعليمي للنساء والأطفال من 15 فبراير إلى 20 أغسطس 2022. تم إنشاء المجموعة الضابطة من الأطفال الأصحاء بعد مراجعة دقيقة للتاريخ الطبي لاستبعاد المشاركين الذين لديهم تاريخ من أمراض الكلى. خضع جميع الضوابط لفحص بدني شامل. تم تجنيد الضوابط من شبكة من العائلة والأصدقاء والأقارب ومركز التوحد الوطني / مستشفى حماية الطفل التعليمي التابع لمدينة الطب / بغداد.
النتائج: أظهرت مستويات الكالسيوم في المصل إنخفاضًا واضحًا في جميع مرضى SSNS و SRSN مقارنة بمجموعة التحكم (. كانت مستويات الصوديوم والكلوريد أقل بشكل ملحوظ من مجموعة التحكم أثناء مرحلة الإنتكاس. أشارت نتائج مرحلة الإنتكاس لمرضى SRNS إلى تركيز أعلى للبوتاسيوم في المصل مقارنة بمجموعة التحكم ومجموعة مرضى SSNS، مع وجود فرق ذي دلالة إحصائية.
الإستنتاج: يعاني جميع الأطفال المصابين بمتلازمة الكلى مجهولة السبب من نقص كالسيوم الدم، سواء في مرحلة الإنتكاس أو مرحلة الهدوء، في حين يعاني المرضى المصابون بمتلازمة الكلى المقاومة للستيرويد من ارتفاع بوتاسيوم الدم؛ تتقلب أيونات الصوديوم والكلوريد بين الإنخفاض أثناء مرحلة الإنتكاس والعودة إلى وضعها الطبيعي أثناء مرحلة الهدوء.
الكلمات المفتاحية: الإلكتروليتات، معدل الترشيح الكبيبي المقدر (eGFR)، متلازمة الكلى مجهولة السبب.

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Research Article

Evaluation of the Role of Arginase1 Enzyme in Type 2 Diabetics with and without Retinopathy

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Abstract

Background: Oxidative stress plays a major role in the pathogenesis of diabetes mellitus by damaging cellular organelles and enzymes in blood such as arginase1, insulin and glutathione s-transferase; increasing lipid peroxidation such as malondialdehyde and increasing insulin resistance which can lead to diabetic complications such as diabetic retinopathy.

Objectives: To explore the relationship of oxidative stress to the development of diabetic retinopathy by measuring the levels of Arginase1, the activity of glutathione s-transferase enzyme, and the levels of malondialdehyde as a secondary product of lipid peroxidation (biomarker for oxidative stress).

Methods This study was conducted from November 2022 to January 2023 at the Ibn Al-Haitham Teaching Eye Hospital in Baghdad, the University of Baghdad / Department of Chemistry and the National Diabetes Centre for Treatment and Research at Al-Mustansyrriah University. This study was conducted on 120 subjects distributed as follows: 40 non-diabetic obese controls, 40 type 2 diabetics with no retinopathy, and 40 type 2 diabetic retinopathy patients, between 30 and 65 years of age. All groups were subjected to tests: measuring fasting blood glucose (FBG), HbA1c, lipid profile (cholesterol, triglycerides, HDL- cholesterol, LDL- cholesterol, and VLDL cholesterol), serum total arginase1, malondialdehyde glutathione s -transferase, body mass index (BMI), and waist-to-hip ratio.

Results: Mean arginase1 levels were significantly higher in diabetic patients than in diabetic retinopathy and control groups. The mean oxidative stress marker malondialdehyde concentration was significantly higher in diabetic retinopathy patients than in type 2 diabetics and the control group. The mean glutathione s-transferase activity in diabetic retinopathy patients was significantly higher than in the control group and type 2 diabetics.

Conclusion: There is a relationship between oxidative stress and the development of diabetic retinopathy, where the levels of arginase1 and malondialdehyde increased and the activity of glutathione s –transferase enzyme increased as a result of oxidative stress and inflammation associated with complications of type 2 diabetes.

Keywords: Arginase1; Diabetic Retinopathy; Type 2 Diabetes mellitus; Oxidative Stress; Lipid Profile

Introduction

Diabetes mellitus (DM) is one of the most common chronic metabolic diseases marked by high blood sugar (hyperglycemia) which may lead to life-threatening, debilitating, and expensive consequences and to a lower life expectancy (1). Type 2 diabetes mellitus (T2DM), makes up over 90% of all instances of the disease and is characterized by insulin resistance (IR) and β-cell dysfunction at the beginning of the disease. DM and its consequences have been linked to oxidative stress, and inflammatory responses (2), which lead to damages, malfunctions, and failure in different systems. DM patients develop long-term health problems in the kidneys, blood vessels, nerves, heart, and eyes (diabetic retinopathy) (3). Cellular damage, oxidative stress, and reactive oxygen species (ROS) caused by hyperglycemia contribute to diabetes complications; one of which is retinopathy4 Diabetic retinopathy (DR) is a neurovascular condition that is generally asymptomatic, but ophthalmoscopic examination shows microaneurysms and leakage of microscopic arteries, which may cause the retina to expand and to cause vision loss (5). An important factor in the development of DM is oxidative stress, because of glucose oxidation, nonenzymatic protein glycation, and the oxidative breakdown of glycated proteins that follow, and an overabundance of free radicals. The significant increases in the formation of free radicals may be assessed indirectly by the presence of products of lipid peroxidation, mainly malondialdehyde (MDA) (6), which is frequently used as a biomarker for evaluating oxidative stress, making elevated circulating MDA levels a risk factor in patients with diabetic retinopathy (7). Additionally, due to oxidative stress, Glutathione S-Transferase

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(GST) activity increases in DM, and this enzyme is significantly associated with the detoxification. It is primarily involved in the neutralization of reactive oxygen species (ROS) by enzymatic conjugation with the scavenger peptide glutathione (GSH)(8). Arginase and GST are involved in hormone synthesis, intracellular transport, and oxidative stress resistance (9). DM disrupts the majority of the body's enzyme functions as well as other essential elements involved in diabetic complications. Arginase is a key enzyme whose activity rises with DM, causing retinal tissue damage because it affects the synthesis of nitric oxide, which is essential for endothelial function (10). An enzyme called arginase hydrolyzes arginine to produce urea and ornithine. It is present in every cell and tissue and is crucial for the early onset of vascular problems and diabetic retinopathy in T2DM, which is now among the main causes of blindness and death (11). Diabetes causes a decrease in retinal blood flow, which is thought to be implicated in the development of diabetic retinopathy. The purpose of the study is to measure the levels of arginase1, MDA, and GST activity to determine the relationship between arginase1 and oxidative stress in T2DM patients with retinopathy as well as the function of arginase1 in the development of inflammation that causes diabetic complications.

Patients and Methods

This study was conducted between November 2022 to January 2023 at the Ibn Al-Haitham Teaching Eye Hospital in Baghdad, the Department of Chemistry, College of Science for Women, University of Baghdad, and the National Diabetes Centre for Treatment and Research at Al-Mustansyrriah University. This study was conducted on 120 subjects distributed as follows: 40 non-diabetic obese controls, 40 type 2 diabetics with no retinopathy, and 40 type 2 diabetic retinopathy patients, between 30 and 65 years of age. The diagnosis of DR is based on the symptoms of blurred vision, floaters and flashes, and loss of vision. Additionally, a physical examination of the fundus pictures and a computer diagnostic method were used in the diagnosis. Patients with chronic liver, kidney, heart failure, type 1 diabetes, and gestational diabetes were excluded. They were excluded as the current study was designed to include T2DM patients only, with those having DR as a complication of the disease, to show the effect of high blood glucose on the retina and the enzymes specified in the study. Patients with T2DM, with and without retinopathy were included. Each participant (patient and control) had 10 mL of venous blood taken with a disposable needle. The blood was divided into two tubes: The first was a gel tube to collect serum (when the blood clotted, it was spun at 3000 rpm for 10 minutes at room temperature), and the second was an ethylene diamine tetra acetic acid (EDTA) tube and tested for HbA1c. The total quantity of Arginase and Malondialdehyde in human blood was measured using an enzyme-linked immunoassay (ELISA) kit from Elabscience-USA. The FUJIFILM NX600 was used to determine fasting blood glucose (FBG) after a minimum of eight hours of fasting and lipid profile (cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, and VLDL-cholesterol). HbA1c was determined using the Finecare HbA1c rapid quantitative test. The spectrophotometer was used to measure the activity of GST manually by measuring the conjugation of 1-Chloro2,4 dinitro benzene (CDNB) with Glutathione (GSH). The 1 ml assay mixture contained 0.5 mM CDNB, 1 mM Reduced (GSH), and 100 mM potassium phosphate buffer, pH 6.5. The rate of increase in absorbance at 340 nm was measured for 5 mm at 37°C against a blank containing the reaction mixture without enzyme (13). Body Mass Index (BMI) was calculated from the equation [weight in kilograms/ height in square meters] and waist-to-hip ratio (waist centimeter hip centimeter) was also measured. The data was analyzed using the Statistical Packages for Social Sciences (SPSS Version 26) (14). The correlation coefficient (r), the ANOVA test for differences between three independent variables and estimates via analysis of the linear regression between the values were used. The data was shown as mean± SE. Statistically, significance was at (P-value < 0.05).

Results

Table 1 shows that diabetics with retinopathy had higher mean levels of HbA1C, TG, and VLDL-C than the other two groups. As for other biochemical parameters, there were no consistent differences between the three groups. Significant differences between the three groups were found in cholesterol and HDL-C, while highly significant differences were found in FBS, HbA1C, and LDL-C. Non-significant differences in TG and VLDL were found.

Table (1): Mean ± SE of biochemical parameters in the study groups

Parameters	Study Groups - Mean ± SE - (Median)			P-value
	Control	Diabetes mellitus	Retinopathy Diabetics	
FBS (mg/dL)	98.4±1.44 [a] (100.5)	216.9±12.89 [b] (205)	199.4±13.65 [b] (180)	**0.0001
HbA1c%	5.2±0.07 [a] (5.25)	8.1±0.22 [ab] (8.15)	9.8±1.62 [b] (8.4)	**0.004
Cholesterol (mg/dL)	196.4±3.86 [b] (200)	174.8±8.26 [a] (165)	191.6±6.20 [ab] (189.5)	*0.045
TG (mg/dL)	190.1±9.85 [a] (196)	178.5±13.10 [a] (169.5)	201.0±18.04 [a] (176.5)	0.530
HDL-C (mg/dL)	36.7±0.95 [a] (36.05)	42.0±1.60 [b] (39.5)	37.3±1.55 [a] (35)	* 0.016
LDL-C (mg/dL)	121.4±3.99 [a] (124)	94.9±5.99 [b] (97.5)	113.5±5.43 [a] (113.5)	**0.002
VLDL-C (mg/dL)	37.6±2.06 [a] (39)	35.2±3.72 [a] (30.5)	39.9±3.61 [a] (34.1)	0.587

* Statistically significant, ** statistically highly significant

The arginase1 levels ng/mL show statistically highly significant differences between the groups. Arginase level in the DM and retinopathy groups compared to the control (*p*-value ≤0.05). Table 2 shows highly significant differences between the three groups in the (mean± SE) of arginase 1, MDA and GST. Those with DR had a higher mean Arginase 1 than the controls but lower than the other diabetics, while they had the highest means of MDA and GST than the two other groups.

Table (2): Arginase1, MDA levels and GST activity in patients and control groups

Biochemical Parameters	Study Groups – Mean ± SE - (Median)			P-value
	Control	Diabetes mellitus	Retinopathy Diabetics	
Arginase1 (ng/ml)	41.8±1.66 [a] (38.56)	113.8±4.12 [b] (112.6)	85.0±4.90 [c] (82.19)	**0.0001
Malondialdehyde (ng/ml)	366.7±13.24 [a] (387.40)	581.7±16.53 [b] (605.96)	627.2±22.71 [b] (585.93)	**0.0001
GST activity (IU/L)	7.7±0.94 [a] (6.25)	6.5±0.75 [a] (4.95)	17.0±2.69 [b] (8.8)	**0.0001

** Statistically highly significant

Correlation of Arginase1 and Other Clinical Variables: The correlation coefficient of arginase1 levels with various anthropometric and biochemical variables for the three study groups are shown in Table 3. There was a negative correlation between arginase1 and weight, height and a positive correlation between arginase1 and cholesterol in the control group and a negative correlation between arginase1 and MDA in the retinopathy group.

Table (3): Correlation between Total arginase1 and Study Parameters

Parameters	Correlation of Arginase1 (ng/ml) in the study groups					
	Control		Diabetes mellitus patients		Retinopathy Diabetes patients	
	R	P	R	P	R	P
Age (years)	0.166	0.306	-0.163	0.316	0.136	0.402
Weight (kg)	-0.360*	0.022	0.092	0.571	-0.195	0.228
Height (cm)	-0.327*	0.039	-0.044	0.788	0.064	0.697
BMI (Kg/m2)	-0.192	0.235	0.095	0.560	-0.301	0.059
W/H ratio	-0.104	0.523	-0.111	0.495	-0.244	0.129
FBS (mg/dL)	-0.261	0.103	-0.118	0.470	0.103	0.527
HbA1C %	0.077	0.636	0.301	0.059	-0.009	0.954
Cholesterol (mg/dL)	0.342*	0.031	-0.139	0.391	0.286	0.074
TG (mg/dL)	0.105	0.519	-0.151	0.352	0.046	0.779
HDL-C (mg/dL)	-0.019	0.907	0.075	0.647	0.056	0.729
LDL-C (mg/dL)	0.288	0.071	-0.064	0.694	0.296	0.063
VLDL-C (mg/dL)	0.121	0.456	-0.066	0.686	0.047	0.775
Malondialdehyde (ng/ml)	0.103	0.525	-0.266	0.098	-0.336*	0.034
GST activity (IU/L)	0.270	0.093	0.162	0.318	-0.037	0.822

* Correlation is significant, **Correlation is highly significant P= p-value, R= Regression

ROC analysis for Arginase1: The arginase1 ROC analysis yielded an excellent result with an area under the curve of (0.948), indicating a flawless ROC test. Table (4 and Figure 1) show the area under the curve for arginase1 in T2DM retinopathy patients.

Table (4): Area under the curve of arginase1 between DR and control group

Area Under the Curve - Test Result Variable(s): Arginase1							
Area	Std. Error[a]	Asymptotic Sig.[b]	Cut-Off Point	Sensitivity	1 – Specificity	Asymptotic 95% Confidence Interval	
						Lower Bound	Upper Bound
.948	.028	.000	60.40	.850	.050	.893	1.000
a. Under the nonparametric assumption							
b. Null hypothesis: true area = 0.5							

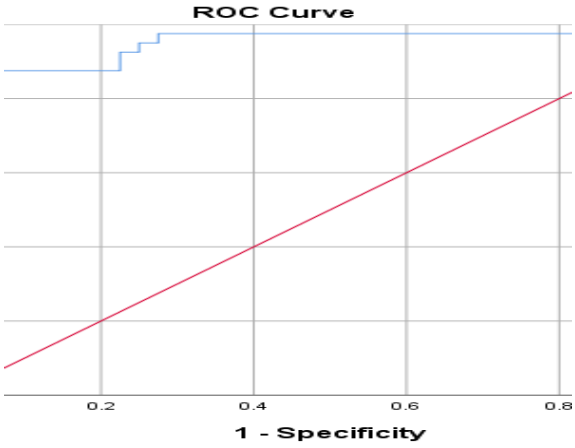

Figure 1 ROC analysis of arginase1 - DR and control

Correlation of Malondialdehyde enzyme levels and other clinical variables: The correlation coefficient of malondialdehyde levels with various anthropometric and biochemical variables for the three study groups are shown in Table (5). A positive correlation was found between height and MDA and a negative correlation was found between MDA and BMI in DM group. A positive correlation was found between MDA and cholesterol in the control group. A negative correlation was found between MDA and arginase1 in the DR group.

Table 5: Correlation between MDA and Study Parameters

Parameters	Malondialdehyde (ng/ml)					
	Control Group		Diabetes mellitus patients Group		Retinopathy Diabetes patients Group	
	R	P	R	P	R	P
Age (years)	0.099	0.544	-0.043	0.791	0.001	0.993
Weight (kg)	-0.097	0.553	-0.129	0.428	0.092	0.572
Height (cm)	-0.049	0.766	0.341*	0.031	0.043	0.793
BMI (Kg/m2)	-0.056	0.732	-0.329*	0.038	0.110	0.498
W/H ratio	0.123	0.450	0.287	0.073	-0.013	0.934
FBS (mg/dL)	0.230	0.153	-0.009	0.956	0.046	0.779
HbA1C %	-0.027	0.868	-0.014	0.932	-0.092	0.572
Cholesterol (mg/dL)	0.325*	0.040	-0.187	0.247	0.008	0.961
TG (mg/dL)	0.033	0.839	-0.091	0.578	-0.008	0.963
HDL-C (mg/dL)	0.281	0.080	-0.262	0.103	0.078	0.631
LDL-C (mg/dL)	0.259	0.107	-0.027	0.871	-0.014	0.932
VLDL-C (mg/dL)	0.036	0.826	0.174	0.282	-0.007	0.967
Arginase1 (ng/ml)	0.103	0.525	-0.266	0.098	-0.336*	0.034
GST activity (IU/L)	-0.060	0.714	0.241	0.134	-0.074	0.650

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.
P = p value, R= Regression

Correlation of GST Activity and other Clinical Variables: The correlation coefficients between Glutathione S-Transferase and various anthropometric and biochemical variables for the three study groups are shown in table (6). A positive correlation was found between GST and height and a negative correlation was found between BMI and GST in the DM group and a positive correlation was found between GST and cholesterol in the control group and a negative correlation was found between Arginine 1 and GST in the DR group.

Table (6): Correlation between GST and Study Parameters

Parameters	Glutathione S-Transferase					
	Control Group		Diabetes Mellitus Patients Group		Retinopathy Diabetes patients Group	
	R	P	R	P	R	P
Age (years)	0.099	0.544	-0.043	0.791	0.001	0.993
Weight (kg)	-0.097	0.553	-0.129	0.428	0.092	0.572
Height (cm)	-0.049	0.766	0.341*	0.031	0.043	0.793
BMI (Kg/m2)	-0.056	0.732	-0.329*	0.038	0.110	0.498
W/H ratio	0.123	0.450	0.287	0.073	-0.013	0.934
FBS (mg/dL)	0.230	0.153	-0.009	0.956	0.046	0.779
HbA1C %	-0.027	0.868	-0.014	0.932	-0.092	0.572
Cholesterol (mg/dL)	0.325*	0.040	-0.187	0.247	0.008	0.961
TG (mg/dL)	0.033	0.839	-0.091	0.578	-0.008	0.963
HDL-C (mg/dL)	0.281	0.080	-0.262	0.103	0.078	0.631
LDL-C (mg/dL)	0.259	0.107	-0.027	0.871	-0.014	0.932
VLDL-C (mg/dL)	0.036	0.826	0.174	0.282	-0.007	0.967
Arginase1 (ng/ml)	0.103	0.525	-0.266	0.098	-0.336*	0.034
GST activity (IU/L)	-0.060	0.714	0.241	0.134	-0.074	0.650

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level. P = p value, R= Regression

Discussion

DM results from the insufficient amount or activity of insulin produced by the pancreas which leads to elevated blood glucose levels. Insulin resistance and insufficiency is associated with an increased risk of microvascular damage (15). For the glycemia-associated risks of microvascular and macrovascular consequences of diabetes mellitus, the glycated hemoglobin HbA1c is regarded as the gold standard (16). The levels of HbA1c and the duration of (DM) are significant risk factors for diabetic retinopathy (17). Overweight or obesity increases the chance of developing T2DM and insulin resistance. We included obese individuals in the control group to determine arginase1 levels in all groups and its relationship to oxidative stress and inflammation by measuring the effectiveness of GST as an antioxidant enzyme and malondialdehyde as products of lipid peroxidation in this group of patients. The data show that, except for lower the HDL levels. Patients have hyperlipidemia in terms of lipid features. The lipid profile of obese controls was abnormal, and the patients' groups had high levels of VLDL because hyper-insulinemia and that agree with other study they said hyperglycemia enhance the liver's synthesis of VLDL-C. Plasma VLDL-C particle turnover may increase plasma VLDL-C concentrations have while plasma HDL-C concentrations dropped (18). Diabetes eye complications have become one of the leading causes of blindness due to an increased risk of microvascular illness, among other complications including retinopathy, neuropathy and nephropathy all of which can lead to disability, dependency, accelerating morbidity, and mortality. Diabetes causes disruptions in most of the body's enzyme activities and other vital factors that are involved in diabetes complications, one of which is arginase1 its activity increases with diabetes mellitus, leading to retinal problems because it has an effect on the production of nitric oxide, which is crucial for endothelial function (19). Moreover, arginase1 levels rise when obesity-related BMI rises influencing how T2DM condition develops (20). T2DM patients with problems like retinopathy had considerably higher MDA levels than those without problems. MDA, being the most major risk factor, might be used in combination with antioxidants to assess oxidative stress in T2DM patients (21). Individuals with T2DM with retinopathy had higher levels of serum GST activity, indicating a significant presence of oxidative stress in those patients and that agree with the other study that say implying that GST is an important biochemical instrument that could provide significant insight into the oxidative stress prevalent in a variety of diseases (22). A possible explanation is the DM group had higher arginase1 activity than the control group. Arginase1 may contribute to the development of DM and its repercussions because of its regulatory role in β-cell functioning and vascular dysfunction by influencing L-arginine metabolism, inflammatory responses, and oxidative stress (23). A key mechanism in the development of microangiopathy is the enhanced lipid peroxidation and peroxyl radical production brought on by hyperglycemia and dyslipidemia in DM (24). In this study malondialdehyde levels were high in diabetic retinopathy patients due to oxidative stress, which was assessed by lipid peroxidation marker, antioxidant enzyme status, and type 2 diabetes mellitus patients with and without retinopathy and compared with a control non-diabetic group. In this study also Arginase1 increases in obesity and aging that agree with another study that say the obesity increases the quantity of arginase1 in the vascular wall, which is directly correlated with the degree of vascular wall remodeling (25). Like another study that found a relationship between arginase and cholesterol. In cases where increased levels of the enzyme arginase serve as a useful indicator of a

person's propensity for cardiac abnormalities, blood total cholesterol has a positive and significant relationship with cardiovascular disease (CVD) (26). This is a positive accurate result confirming that arginase has a good relationship with T2DM patients with retinopathy that agreement with other studies that found the increased Arginase activity in diabetes a key pathogenic factor, is associated the ROC curve is used in those studies to distinguish between individuals with type 2 diabetes and those with the development of obesity-related type2 diabetes and associated vascular disease (27). In this study the high MDA levels in diabetic patients who suffer from obesity (BMI>30) and the higher plasma lipid peroxidation marker, MDA, indicate that the obese and centrally obese T2D had more oxidative stress. This study agrees with another study that says the oxidative stress increased MDA in T2D patients who are obese or centrally obese supports "reductive stress" induction (28). Cholesterol and BMI were strongly correlated, that agree with other study that explained by residual cholesterol inducing atherosclerosis in the overweight and obese groups by accumulating in the artery wall (29). The high BMI group had significantly higher MDA levels, and there was a positive correlation between MDA levels and BMI, this result agree with other study which found a high MDA levels is linked to higher atherogenic lipid profiles and enhanced oxidative stress30. In this study, the levels of MDA and arginase1 were high in a diabetic retinopathy group and that related with oxidative stress in diabetic complications. This study found the MDA levels were higher in DR patients than in DM patients who did not have DR this agrees with another study that says as a byproduct of lipid peroxidation, malondialdehyde (MDA) is one of the most extensively used biomarkers for assessing oxidative stress 31. Arginase induces premature senescence it induces endothelial dysfunction and increases oxidative stress (32). The expression of glutathione S-transferases in type 2 diabetes patients showed extremely significant alterations when compared to diabetic and control groups this agree with the other study that found the increases in free radicals are a result of abnormalities in cellular metabolism in diabetes when these free radicals interact with other vital biological elements, they can lead to diabetic retinopathy (DR), a side consequence of diabetes33. In diabetics, a higher triglyceride index (TG) is related to the prevalence of retinopathy and might be utilized to evaluate metabolic state in clinical settings in another study (34). In this study, GST activity increased in diabetic retinopathy indicating marked oxidative stress in these patients that agree with other study said the hyperglycemia-induced hyperproduction of reactive oxygen species causes microvascular problems in diabetes mellitus. Glutathione S-transferases have key detoxifying and antioxidant properties (35). In this research, T2DM patients saw an increase in small HDL particles. That agree with other numerous investigations demonstrate that HDL's capacity to inhibit inflammatory signals is dramatically diminished in this patient population (36). In this study, the DR group had elevated VLDL levels that similar with other study that explain the changes in lipid profile have a major impact on micro-vascular risk in T2DM. These indices can serve as new indicators for spotting micro-vascular problems linked to diabetes. Thus, to control on DR, examination of these indicators can be included to the evaluation of lipid profile (37).

Conclusion

There is a relationship between oxidative stress and the development of diabetic retinopathy, where the levels of arginase1 and malondialdehyde increased and the activity of glutathione s –transferase enzyme increased as a result of oxidative stress and inflammation associated with complications of type 2 diabetes.

Authors' declaration:

Conflicts of Interest: The authors declare no conflict of interest.

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's **Ethical Clearance:** Ibn Al-Haitham Teaching Eye Hospital, the Department of Chemistry, College of Science for Women, University of Baghdad and the National Diabetes Centre for Treatment and Research at Al-Mustansyrriah University in 22/11/2022 according to the code number (158347).

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Authors' Contributions:

Study conception & design: (Maha F Yaseen & Fayhaa M. Khaleel). Literature search: (Maha F Yaseen & Fayhaa M. Khaleel). Data acquisition: (Maha F Yaseen). Data analysis & interpretation: (Maha F Yaseen & Fayhaa M. Khaleel). Manuscript preparation: (Maha F Yaseen). Manuscript editing & review: (Maha F Yaseen & Fayhaa M. Khaleel).

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دور الارجنيز وبعض العلامات الحيويه في مرضى السكري من النوع الثاني الذين يعانون من اعتلال الشبكية والذين لا يعانون منه

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الخلاصة:
الخلفية: يلعب الإجهاد التأكسدي دورا رئيسيا في التسبب في مرض السكري عن طريق إتلاف العضيات الخلوية والإنزيمات في الدم مثل الأرجنيز 1 والإنسولين والكلوتاثايون اس ترانسفيريز ونتيجة لذلك ترتفع مستويات بيروكسيد الدهون مثل المالونداياليديهايد وتزداد مقاومة الإنسولين التي يمكن أن تؤدي إلى مضاعفات مرض السكر ومنها إعتلال الشبكية السكري.
الأهداف: إكتشاف علاقة الإجهاد التأكسدي بتطور إعتلال الشبكية السكري عن طريق قياس مستويات إنزيم الأرجنيز 1 ونشاط إنزيم الكلوتاثايون اس ترانسفيريز ومستويات المالونداياليديهايد كناتج ثانوي لبيروكسيد الدهون (علامة حيوية للإجهاد التأكسدي).
المرضى والمنهجية: أجريت الدراسة في الفترة من تشرين الثاني 2022 إلى كانون الثاني 2023 في مستشفى إبن الهيثم للعيون وقسم الكيمياء في جامعة بغداد كلية العلوم للبنات والمركز الوطني لعلاج وابحاث السكري في الجامعة المستنصرية وقد أجريت الدراسة على 120 شخصا موزعين على ثلاثة مجموعات: 40 في مجموعة السيطرة يعانون السمنة وليسوا مصابين بالسكري و40 في مجموعة مرضى السكري النوع الثاني غير المصابين بإعتلال الشبكية السكري و40 مريض سكري من النوع الثاني مصاب بإعتلال الشبكية السكري تتراوح أعمارهم من 30 الى 65 عاما. خضعت جميع المجموعات إلى إختبارات قياس الكلوكوز في دم الصائم، السكر التراكمي، ملف الدهون ويشمل (الدهون الثلاثية TG،الكوليسترول الحميد HDL، LDL والكوليسترول VLDL) وقياس مستويات الأرجنيز والمالونداياليديهايد وفعالية الكلوتاثايون اس ترانسفيريز، قياس مؤشر كتلة الجسم BMI ونسبة الخصر إلى الورك.
النتائج : كان متوسط مستويات الأرجنيز 1 أعلى بكثير في مرضى السكري منه في مرضى إعتلال الشبكية السكري ومجموعة السيطرة ($P<0.05$) وكان متوسط تركيز علامة الإجهاد التأكسدي المالونداياليديهايد أعلى بكثير في مرضى إعتلال الشبكية السكري منه في مرضى السكري النوع الثاني غير المصابين بإعتلال الشبكية ومجموعة السيطرة ($P<0.05$) وكان متوسط نشاط إنزيم الكلوتاثايون اس ترانسفيريز أعلى في مرضى إعتلال الشبكية السكري منه في مجموعة السيطرة ومجموعة مرضى السكري غير المصابين بإعتلال الشبكية السكري ($P<0.05$).
الاستنتاج: توجد علاقة بين الإجهاد التأكسدي وتطور إعتلال الشبكية السكري، حيث ازدادت مستويات إنزيم ألأرجنيز 1 والمالونداياليديهايد وازداد نشاط إنزيم الكلوتاثايون اس ترانسفيريز نتيجة الإجهاد التأكسدي والإلتهابات المرتبطة بمضاعفات مرض السكري من النوع الثاني.
الكلمات المفتاحية: مرض السكري من النوع الثاني، إعتلال الشبكية السكري، الأرجنيز 1، الإجهاد التأكسدي، صورة الدهون.

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Open Access Research Article

Clinical and Cephalometric Assessments in Grade II and Grade IV Adenoid Hypertrophy: A Cross-Sectional Study

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Abstract

Background: Adenoid hypertrophy is one of the most common causes of nasal obstruction, and physicians use different methods to investigate it to reach a definite diagnosis.

Objectives: This study aims to determine whether there are clinical differences between grade II and IV adenoid hypertrophy and whether there is a positive correlation between adenoid-nasopharyngeal ratio and endoscopic examination findings.

Methods: This study was carried out on 120 patients; they were confirmed with five clinical tests (graded mirror, water-retention, lip-seal, deep-breath, and functional tests) and lateral cephalometric radiographs to measure the adenoid-nasopharyngeal ratio. Kruskal-Wallis test was used for the mirror test, while the Chi-squared test was used for the rest to detect the differences among groups. Spearman's correlation coefficient test was used to determine the correlation between the adenoid-nasopharyngeal ratio and endoscopic findings.

Results: The age range of the patients was 6-12 years [mean age = 9.13 ± 1.97 years], 60.8% male, 39.2% female. Kruskal-Wallis and Chi-squared tests showed a statistically significant difference with $P < 0.01$ between groups. A strong positive significant correlation at $P < 0.01$, Spearman's test 0.94 was found between adenoid-nasopharyngeal ratio and endoscopic findings.

Conclusion: Lateral cephalogram and nasal endoscopy can detect most pathologies associated with airway blockage. There are clinical differences between grade II and grade IV adenoid hypertrophy, and there is a good correlation between the adenoid-nasopharyngeal ratio measured by lateral cephalogram and endoscopic findings.

Keywords: Cephalometrics; Cross-sectional; Endoscopy; Grade II adenoid; Hypertrophy; Lip-seal; Water-retention.

Introduction

At the median of the posterior wall of the nasopharynx, there is a collection of lymphatic tissues named the adenoids (pharyngeal tonsils). They represent the superior part of Waldeyer's ring. Because of their location and structure, the adenoids capture any virus or bacteria that enters the human body through the nasal routes (1).Adenoid size increases between three and six years old and is related to the reduction of the growth of the nasopharynx. Then, after six, the adenoid stops growing under normal conditions, and the nasopharyngeal cavity widens; later on, some expansion of the adenoid could happen (due to the fibrous tissue involution) (2).Adenoid hypertrophy could block the choanae, causing symptoms such as mouth breathing, hyponasal speaking, and snoring. It could also produce otitis media with effusion (3) and conductive hearing loss (4). Craniofacial changes caused by chronic adenoid hypertrophy cause a facial feature named the adenoid face, characterized by a long face, retrognathic mandible, underdevelopment of the maxilla, constructed upper arch, and incompetent lips (5-7). Orthodontic correction of those changes would greatly depend on age of the patients (8) Various methods have been used to detect adenoid hypertrophy; some of them were invasive; others provided digital imaging; the first one includes manual finger pressure, posterior rhinoscopy by a special mirror, rigid or flexible nasopharyngoscopy; the second includes ultrasonography, lateral cephalogram, computed tomography (CT) or Magnetic resonance imaging (MRI) (9). The most effective and accurate method is the flexible fiberoptic endoscopy since it gives a direct visualization of the nasal and nasopharyngeal area, giving more precise measurements of the size of the adenoids (10). Since there were insufficient studies that compare grade II and grade IV adenoid hypertrophy using clinical tests, the current cross-sectional study was designed to do so in addition to the use of adenoid-nasopharyngeal ratio (ANR) for cephalometric assessments of adenoids.

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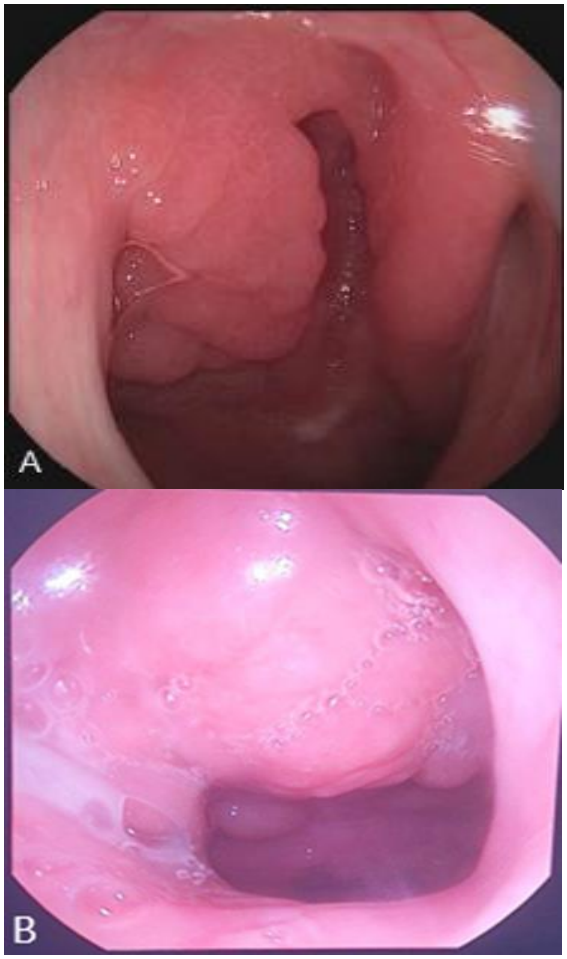
Subjects and Methods:

A cross-sectional study was carried out on 120 patients with 73 males and 47 females aged 6–12 years who were selected from the ear, nose, and throat (ENT) department at Imam Al-Hussein Medical City in Karbalaa and examined by a specialist otolaryngologist to detect the adenoids. The ENT specialist examined each patient to include or exclude patients using a flexible Storz Karl Storz Endoscope machine (model 495xx, 69495xx) under topical anesthesia with 2% xylocaine (Figure 1). The patients were allocated into three groups based on the endoscopic classification system of adenoids proposed by Cassano et al. (11) Grade I corresponds to the adenoid tissue covering less than 25% of the choanal openings; grade II corresponds to the adenoid tissue covering less than 50% of the choanal openings, with the Eustachian tube visible; grade III corresponds to a covering of about 75%, with the Eustachian tube slightly involved; and grade IV refers to a covering of over 75%, with the Eustachian tube completely covered (Complete choanal obstruction). The 120 patients were divided into control, grade II, and grade IV groups with 40 subjects in each group. The control group is free from any systemic disease and from adenoids diagnosed by the otolaryngologist using the endoscope. Subjects with systemic diseases, handicaps, previous maxillofacial trauma, past adenoidectomy, and grade I and III adenoids were excluded.



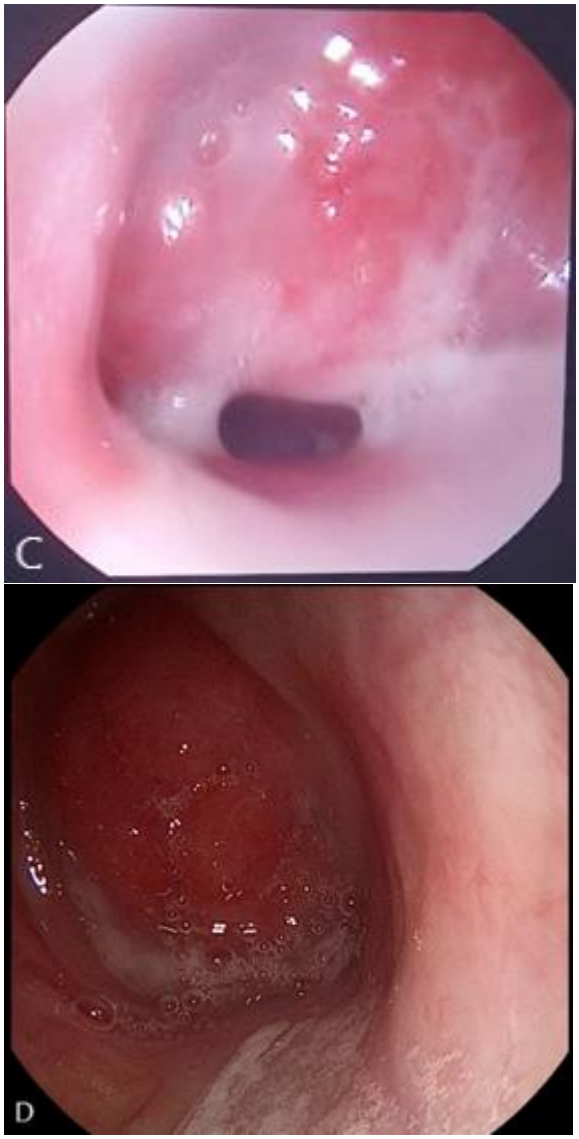


Figure 1: Endoscopic view shows adenoid hypertrophy A. Grade I, B. Grade II, C. Grade III, D. Grade IV.

Informed consent had been taken from each patient participated in the study. Five clinical tests were used in this study. In the *graded mirror test*, the water vapor marked on the mirror from the exhaled air with a marker to measure the area of interest (Low nasal flow: up to 30 mm; Average nasal flow: 30–60 mm; High nasal flow: above 60 mm) (Figure 2). While in the *water-retention test*, the patient was asked to hold the water (about 15ml) still in his mouth for 3 minutes without swallowing. In the *lip-seal test*, the patient was asked to seal his lips completely for 3 minutes. In the *deep-breath test*, the patients were instructed to breathe deeply through their noses while keeping their lips closed. Those who breathe through their noses have better than average voluntary function of the alar muscles, which change the size and shape of the external nares during inhalation. Mouth breathers, on the other hand, don't change the shape and size of

their nostrils when they breathe via the nose, and they even occasionally compress their external nares while inhaling. Lastly, in the *functional test*, the patients were instructed to bend their knees quickly ten times. They were considered pure nose breathers if they were able to maintain quiet nasal breathing for the 30 seconds following the exercise (12, 13).

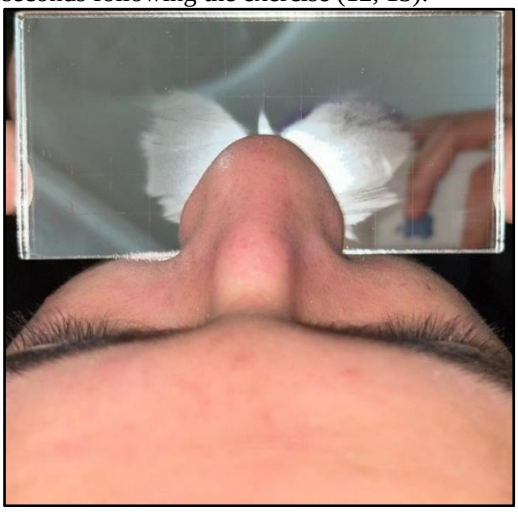


Figure 2: The steam halo generated on the graded mirror.

Lateral cephalometric radiograph has been taken for the included patients in the department of radiology at Imam Al-Hussein Medical City in Karbalaa, with the use of the Hyperion X9 Pro professional 3-in-1 full-touch imaging system by the same x-ray specialist at centric occlusion in natural head position (14) with the head fixed by ear rods laterally and a plastic stopper (nasion support) on the bridge of the nose anteriorly, fixing the Frankfort plane horizontally (15). The x-ray beam's central ray entered through the subject's right external auditory meatus and left from the left side meatus. ANR was measured using the guidelines from Fujioka et al. (16) to measure the Adenoid hypertrophy. The width of the adenoid depth (AD) will be measured by drawing a line at a right angle from the straight part of the anterior margin of the basi-occiput to the part of the adenoid that is the most rounded. The nasopharyngeal depth (ND) will be measured by drawing another line from the sphenoid-occipital synchondrosis to the posterosuperior edge of the hard palate. ANR will then be calculated by dividing AD by ND (16, 17) (Figure 3).Tracing processes have been made by the researcher using Autodesk AutoCAD 2020 (18).

Statistical analysis

Kruskal-Wallis test was used for the mirror test, while the Chi-squared test was used for the rest to detect the differences between the control group, grade II, and grade IV adenoid. Spearman's correlation coefficient test was used to determine the correlation between the ANR and endoscopic findings. Analysis was performed using a statistical package for social science (SPSS version- 26). A *P*-value < 0.05 was considered a statistically significant difference.

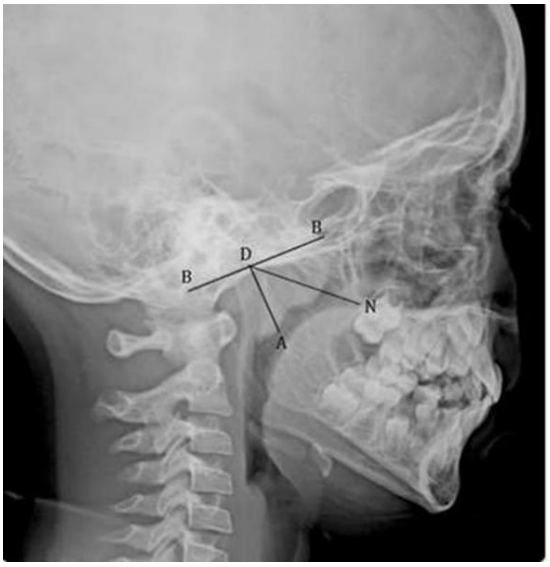


Figure 3: Cephalometric radiograph, where BB is the line drawn along the straight part of the anterior margin of the basiocciput; AD is the adenoid depth; ND is the nasopharyngeal depth (17).

Results

This study was conducted on 120 participants (mean age = 9.13 ± 1.97 years, range 6-12 years, 60.8% male, 39.2% female). Different results were found across the study; for the graded mirror test, 38 subjects from the control group had a high nasal flow, two subjects had average nasal flow, and nobody was found to have low nasal flow. While in grade II, ten subjects had a high nasal flow, 29 subjects had an average nasal flow, and only one subject had a low nasal flow. In grade IV, three subjects had a high nasal flow, 28 subjects had an average nasal flow, and only nine subjects had a low nasal flow (Table 1).

Table 1: Descriptive statistics for the graded mirror test

Group	Graded mirror test	Frequency	%
Control	0	0	0
	1	2	5.0
	2	38	95.0
Grade II	0	1	2.5
	1	29	72.5
	2	10	25.0
Grade IV	0	9	22.5
	1	28	70.0
	2	3	7.5

* 0 for Low nasal flow, 1 for Average nasal flow, and 2 for High nasal flow.

Kruskal-Wallis Test showed a statistically significant difference between groups (Table 2).

Table 2: Kruskal-Wallis test for comparison among groups for the mirror test

Kruskal-Wallis test		
Pairwise Comparisons of Groups		
Between groups	Statistics	*P*-value
Grade IV- Grade II	16.53	0.018
Grade IV- Control	55.89	0.000
Grade II- Control	39.36	0.000

*Highly significant when *P*-value < 0.01.

For the water-retention and lip-seal tests, all subjects from the control group passed the two tests successfully, while 37 subjects from grade II passed the test, and half of the subjects from grade IV passed. For the deep-breath test, all the subjects in the control group could take a deep breath successfully; 37 subjects from grade II passed the test, while only 16 subjects from grade IV passed. Meanwhile, for the functional test, the control group could do it; 36 subjects from grade II passed the test, while only 12 subjects from grade IV passed (Table 3). Chi-Squared test showed a highly significant difference at *P*-value < 0.01 across groups (Table 4). A very strong correlation was found between ANR and endoscopic findings at *P*-value < 0.01 and Spearman's correlation coefficient equal to 0.94.

Table 3: Descriptive statistics of the distribution of clinical examination variables among groups

Variable	Group	Result	Frequency	%
Water-retention test	Control	1	40	100.0
	Grade II	0	3	7.5
		1	37	92.5
	Grade IV	0	20	50.0
		1	20	50.0
Lip-seal test	Control	1	40	100.0
	Grade II	0	3	7.5
		1	37	92.5
	Grade IV	0	20	50.0
		1	20	50.0
Deep-breath test	Control	1	40	100.0
	Grade II	0	3	7.5
		1	37	92.5
	Grade IV	0	24	60.0
		1	16	40.0
Functional test	Control	1	40	100.0
	Grade II	0	4	10.0
		1	36	90.0
	Grade IV	0	28	70.0
		1	12	30.0

*0 describes subjects who could not pass the test. *1 describes subjects who could.

Table 4: Chi-squared test of the distribution of clinical examination among groups

Variable	Group	Result	Observed count	Expected count	Chi-squared test	*P*-value
Water-retention test	Control	0	0	7.7	37.54	0.000 (HS)
		1	40	32.3		
	Grade II	0	3	7.7		
		1	37	32.3		
	Grade IV	0	20	7.7		
		1	20	32.3		
Lip-seal test	Control	0	0	7.7	37.54	0.000 (HS)
		1	40	32.3		
	Grade II	0	3	7.5		
		1	37	32.3		
	Grade IV	0	20	7.7		
		1	20	32.3		
Deep-breath test	Control	0	0	9	49.03	0.000 (HS)
		1	40	31		
	Grade II	0	3	9		
		1	37	31		
	Grade IV	0	24	9		
		1	16	31		
Functional test	Control	0	0	10.7	58.64	0.000 (HS)
		1	40	29.3		
	Grade II	0	4	10.7		
		1	36	29.3		
	Grade IV	0	28	10.7		
		1	12	29.3		

HS: highly significant at $P < 0.01$

Discussion:

Hypertrophy of the lymphoid tissues plays an essential role in the constriction of the nasal airways (17, 19), and to aid the breathing process, the child substitutes nasal breathing with oral breathing and becomes a mouth breather (20). Clinical recognition of mouth breathers could be done by different approaches (13, 21). The most common breathing tests used in the dental office by the orthodontists are the lip-seal, mirror, and lastly, water-retention tests (13). Significant differences at *P*-value < 0.01 were found among groups for the five clinical tests. Although the graded mirror test was used to access nasal patency and is very useful in unilateral nasal obstruction, done by a special mirror with equal divisions of squares, it was not standardized (13). Water retention and lip-seal tests are easy and do not need any equipment to diagnose oral breathing. Still, there needed to be more agreement on the manner of application, so to standardize the breathing tests, three minutes was the optimum time chosen in this study (22). The choice of this longer period was essential because mouth breathers might breathe through their noses even when there was an obstruction, but only for a short period, depending on the level of nasal obstruction (13). Morais-Almeida et al. (23)

suggested an absence of lip-seal caused by hypotonicity of the orbicularis oris muscle. Kalaskar et al. (24) added that facial divergence, long face, short lips, and oral breathing cause incompetent lips. Maintaining the lip closure for up to three minutes was considered a training process used by the patient to restore nasal breathing throughout the test day after day at home after removing the causative factor of nasal obstruction (13). So, the lip-seal test in the study could be efficient for the patients after that. The two tests, the deep-breath test and the functional test, could be misleading, especially in children, because most children do not do it well, and it could be not easy. Applying only one test to check the airway's patency could be unreliable; it is better to choose at least two tests as an initial step for the diagnosis of a mouth breather, just like the mirror test with the water-retention or lip-seal test could be a good suggestion (13). Attaining nasal breathing is especially important for children since they are growing, and oral breathing could have deleterious effects on craniofacial features (25). In the case of adenoid hypertrophy, there are various methods to detect adenoid size (26); the palpation method and the posterior rhinoscopy are old methods. They are hard to do and traumatic to the patient, and greatly depend on the patient's cooperation, the results do not correlate well with the endoscopic findings, and they give wrong results of the adenoid size (26). Most otologists suggested the endoscopic examination to check airway patency; using the nasopharyngoscope could be very efficient to measure adenoid size (10), especially before adenoidectomy (9, 27); this procedure was mandatory in this study. Although the endoscopic examination is quick and informative and produces a 3D image, it is limited to the clinical specialist and cannot be done by the primary care physician. Also, the incomplete relaxation of the soft palate causes inaccuracy in the diagnostic results (9). In addition, endoscopic examination gives a negative result for nasal patency (28). Multiple radiologic imaging techniques could be used to estimate adenoid tissue size. The lateral cephalometric radiograph is simple, cost-effective, and with low radiation, mostly used to measure the adenoid size (29-31). However, MRI could detect adenoid size in different age groups (32). Moreover, CBCT could be used to examine the craniofacial area to reach a correct diagnosis and treatment (33-35). Fujioka et al. (16) suggested the use of the A/N ratio to measure the adenoid size; it depends on the measurement of the maximum convexity of the adenoids arising from the straight part of the basiocciput in an anteroinferior direction divided by the anteroposterior diameter of the nasopharyngeal area. In this study, the A/N ratio was strongly correlated with endoscopic findings, agreed with Moideen et al. (17). However, lateral cephalograms might overestimate the adenoid size (36); according to the systematic review by Major et al. (37), who suggested measurement of the size of the airways instead of the adenoid. Some limitations could be present with the lateral x-ray, and those greatly affect the results, such as head position, patient respiration, and phonation. The soft palate elevates and reduces the nasopharyngeal cavity during crying, mouth breathing, and swallowing. So, it is better to perform the exposure at an extended head position and the end of inspiration without crying or swallowing, and this is not easy for children (38). In addition, it is a 2D image and might have superimposition, artifacts, and errors (34).

Conclusions

Clinical examination is the primary step in detecting long-term airway blockage. The primary diagnoses could detect most pathologies, however, sometimes they need confirmations, which include the lateral cephalogram and nasal endoscopy. There are clinical differences between grade II and grade IV adenoid hypertrophy, and there is a good correlation between ANR measured by lateral cephalogram and endoscopic findings.

Authors' declaration

Conflicts of Interest: None.
We hereby confirm that all the Figures and Tables in the manuscript are mine/ ours. Besides, the Figures and images, which are not mine /ours, have been given permission for re-publication and attached to the manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in the College of Dentistry/University of Baghdad according to the code number (818 on 18/5/2023).

Conflicts of interest/ None.
Funding/ None

Authors' contributions

Afnan R. Hammood: Study conception & design, literature search, data acquisition, data analysis & interpretation, manuscript preparation.
Hayder F. Saloom: Conceptualization, methodology, validation, formal analysis, resources, data curation, visualization, supervision, project administration, manuscript editing & review.

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التقييمات السريرية وقياسات الرأس في الدرجة الثانية والدرجة الرابعة من تضخم اللحمية الانفية: دراسة مقطعية

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الخلاصة:
خلفية البحث: يعد تضخم اللحمية الانفية أحد الأسباب الأكثر شيوعًا لانسداد الأنف، ويستخدم الأطباء طرقًا مختلفة لفحصه للوصول إلى تشخيص محدد.
هدف الدراسة: تهدف هذه الدراسة إلى تحديد ما إذا كانت هناك اختلافات سريرية بين تضخم اللحمية الانفية من الدرجة الثانية والرابعة وما إذا كان هناك علاقة إيجابية بين نسبة اللحمية إلى البلعوم الأنفي ونتائج الفحص بالمنظار.
طريقة البحث: تم إجراء دراسة مقطعية على 120 مريضاً؛ تم تأكيدهم من خلال خمس اختبارات سريرية (اختبار المرآة المتدرجة، واختبار احتباس الماء، واختبار اغلاق الشفة، واختبار التنفس العميق، والاختبار الوظيفي) والصور الشعاعية الرأسية الجانبية لقياس نسبة اللحمية الأنفية الى البلعوم. تم استخدام اختبار كروسكال واليس لاختبار المرآة، بينما تم استخدام اختبار مربع كاي للباقي للكشف عن الاختلافات بين المجموعات. تم استخدام اختبار معامل ارتباط سبيرمان لتحديد العلاقة بين نسبة اللحميه الانفية الى البلعوم والنتائج بالمنظار.
النتائج: كان عمر المرضى 6-12 سنة [متوسط العمر = 9.13 ± 1.97 سنة]، 60.8% ذكور، 39.2% إناث وأظهرت اختبارات كروسكال واليس ومربع كاي وجود فرق ذو دلالة إحصائية مع ($P < 0.01$) بين المجموعات. تم العثور على علاقة إيجابية قوية وذات دلالة إحصائية عند قيمة ($P < 0.01$)، واختبار سبيرمان 0.94 بين نسبة اللحميه الانفية الى البلعوم والنتائج بالمنظار
الاستنتاج: يمكن لتصوير الرأس الجانبي والتنظير الأنفي اكتشاف معظم الأمراض المرتبطة بانسداد مجرى الهواء. هناك اختلافات سريرية بين الدرجة الثانية والدرجة الرابعة من تضخم اللحميه الانفية، وهناك علاقة جيدة بين نسبة اللحمية إلى البلعوم الأنفي المقاسة بواسطة مخطط الرأس الجانبي والنتائج بالمنظار.
الكلمات المفتاحية: قياسات الرأس، المقطع العرضي، التنظير، اللحمية من الدرجة الثانية، مرآة متدرجة، تضخم، ختم الشفة، احتباس الماء.

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Research Article

Exploring the Link between Iron Status and Catalase Activity in Type 2 Diabetes Mellitus

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Abstract:

Background: Catalase is an antioxidant enzyme found in all living organisms that is responsible for the degradation of hydrogen peroxide, a type of harmful compounds known as reactive oxygen species (ROS), into harmless oxygen and water. It is necessary for the cell protection from ROS-induced oxidative damage in type 2 diabetes mellitus (T2DM) individuals. T2DM is rapidly rising in prevalence worldwide, emerging as a significant public health challenge. It can disrupt iron regulation in the bloodstream, leading to the production of ROS, which can damage the cells.

Objective: To explore the correlation between iron status and catalase activity in T2DM patients, and the possibility of using it as a predictor for the onset and severity of diabetes.

Methods: One hundred and fifty participants were included in the study, comprising 50 healthy volunteers who served as the control group (C) and 100 cases diagnosed with T2DM who were divided into three groups based on the duration of their disease: Group A1 (n=38; < 5 years), A2 (n=37; 5-10 years) and A3 (n=25; >10 years). The participants were recruited from the Al-Kindi Teaching Hospital, Baghdad, during the period from October 2022 to the end of January 2023. The study assessed various blood markers in all participants, including: Fasting blood glucose (FBG), glycosylated hemoglobin A1c (HbA1c), Iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), ferritin, transferrin, saturation of transferrin (S.Trans) and catalase activity (CAT).

Results: Compared to the control group, patients with T2DM showed significantly higher levels of FBG, HbA1c, and ferritin, notably lower levels of iron, TIBC, UIBC, and S.Trans. Interestingly, only the A2 group had significantly lower transferrin levels compared to control. There was a significant decrease in catalase activity across all patient groups. Additionally, a positive correlation was observed between iron levels and catalase activity in all patient groups.

Conclusion: Increases in ferritin level might be a risk factor for developing T2DM. The observed association between lower iron levels and reduced catalase activity in T2DM is intriguing, and can serve as a future predictor for the onset and severity of diabetes, warranting further investigation.

Keywords: Catalase activity; Ferritin; Iron; Transferrin; Type 2 Diabetes.

Introduction:

Iron (Fe) is an essential element that is necessary for health and appropriate physiological function for almost all living organisms. Iron's significant functions are oxygen transport and hematopoiesis. It can remarkably act as an electron donor and acceptor (1). Iron may also damage cells by catalyzing the production of free radicals and oxidative stress (2). Plasma transferrin has long been recognized to play an essential role in these activities, primarily by transporting iron in a soluble, non-toxic form across human tissues and organs (3). Transferrin is the body's iron-binding protein; its levels rise as iron demand increases. However, serum iron is difficult to detect and analyze in isolation due to the diversity and consistency of variances in all body iron (4). On the other hand, many studies realize that serum iron affects glucose metabolism even when there is no appreciable iron overload or deficit (5). Serum ferritin levels are frequently used as an indicator of body iron storage, and in epidemiological research, greater ferritin levels and dietary iron consumption predict the possibility of type two diabetes mellitus (T2DM) (6,7). As an iron carrier, ferritin, a cytosolic protein, is released into the serum at trace levels (8). Moreover, ferritin is correlated with blood glucose levels and insulin resistance (9). Type 2 diabetes mellitus, a serious and chronic metabolic disorder marked by insufficient insulin production by the body or does not effectively respond to the insulin it produces, is becoming increasingly common and poses a serious threat to public health worldwide (10,11). Chronic hyperglycemia of T2DM is associated with the long-term harm, malfunction,

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and failure of several organs, including the eyes, kidneys, heart, nerves, and blood vessels (12,13). In Iraq, diabetes is the most common public health issue, where it is among the highest prevalence rates in the Middle East (14,15). The adult diabetes prevalence in Iraq is 10.4% which means that more than three million people have diabetes (16). Numerous human experimental research studies have provided evidence of a strong correlation between T2DM and changes in the metabolism of several trace elements. Low levels of trace elements, including iron, magnesium, chromium, and others, have been associated with insulin resistance, decreased insulin release, and glucose intolerance in T2DM. These trace elements are essential for optimal insulin synthesis and function (4, 17). Experts believe systemic iron excess to be a contributing factor to impaired glucose metabolism. T2DM is produced by oxidative stress on pancreatic β-cells, resulting in insulin insufficiency, cell death, and reduced insulin output. Alternatively, it can be caused by iron excess and hepatic dysfunction, leading to insulin resistance (18-20).

T2DM is a polygenic condition caused by insulin receptor defects and is characterized by oxidative stress, which involves the generation of reactive oxygen species (ROS). ROS can react with DNA, proteins, and lipids, causing these molecules to be destroyed (21). Iron and oxidative stress are inextricably related. Increased oxidative stress is frequently linked with T2DM patients, which can lower serum iron levels (22). The Fenton reaction produces highly hazardous free radicals such as hydroxide and the superoxide anion, which triggers lipid peroxidation. To operate as a pro-oxidant agent, iron must be accessible. Iron is liberated from ferritin by reducing agents, which convert Fe^{3+} to Fe^{2+} (23). Interestingly, iron is a potent oxidant that can accelerate the generation of a lot of reactive oxygen radicals, which in turn influence the secretion of insulin and disrupt the process of glucose metabolism by controlling the signal transduction of islet β-cells. Iron is also crucial for the mitochondria, which can influence insulin secretion levels and promote adenosine triphosphate (ATP) synthesis, both of which can result in disorders related to glucose metabolism (7,18). Moreover, iron influences insulin action reciprocally. It interferes with insulin action by inhibiting liver production of glucose. The insulin metabolism and hepatic extraction is reduced which leads to peripheral hyperinsulinemia, when iron stores increase (24).

The human body has many defense mechanisms that work together to prevent harm from ROS. These mechanisms are referred to as antioxidants. Included in these defense mechanisms are enzymatic antioxidants like glutathione peroxidase (GPx) and catalase (CAT), which both detoxify hydrogen peroxide (H_2O_2), and superoxide dismutase (SOD), which breaks down superoxide anion (O^{2-}) (25). The Se, Cu–Zn, and Fe metals are cofactors for the GPx, cytoplasmic SOD, and CAT enzymes, respectively (23,26). Catalase (EC 1.11.1.6; CAT) is a crucial component of the body's antioxidant defense, essential to protect cells from the detrimental effects of ROS. This enzyme is present in practically all living species. It protects against oxidative stress, which has been linked to a variety of clinical disorders such as diabetes, atherosclerosis, cataracts, cancer, ischemia/ reperfusion damage, nutritional deficiencies, and aging (27,28). Iron is a constituent of several metalloenzymes, including catalase (EC 1.11.1.6) and peroxidase (EC 1.11.1.7) (29). So, both enzymes could be used as biochemical indicators of iron availability. Thus, understanding the relationship between iron status and catalase activity in T2DM patients may provide valuable insights into the underlying mechanisms of the disease and potentially open possibilities for novel therapeutic interventions.

Cases and Methods:

According to the standard American Association criteria, a total of 100 patients with T2DM, between 20-70 years of age were divided into three groups based on the duration of diabetes:

Group1: A1 consists of (38) patients who had diabetes (<5 years)

Group2: A2 consists of (37) patients who had diabetes (5-10 years)

Group 3: A3 consists of (25) patients who had diabetes (>10 years)

Fifty age-matched healthy volunteers were enrolled as the control (C) group. The patients and controls were recruited from Al-Kindi Teaching Hospital, Baghdad, during the period from October 2022 to the end of January 2023 and were diagnosed by the physician specialist to be free of diabetic complications for diabetes patients and free of diabetes for controls.

Exclusion criteria: T1DM, alcohol drinking, smoking, and other complications of the disease, such as anemia, hepatitis, retinopathy, neuropathy, nephropathy, and cardiovascular diseases (CVD) have been excluded, as they may be confounders of the variables addressed in this study.

Blood samples (7 ml) were obtained from the patients and controls after fasting overnight for (8-12) hours. The blood samples were separated into aliquots (2 and 3 ml). The first aliquot was placed in a tube containing EDTA to calculate HbA1c. The second aliquot was placed in a plain gel tube and allowed to stand at room temperature for 10 minutes before being centrifuged at 3000 rpm for 10 minutes to collect serum, which was transferred to Eppendorf tubes and stored at -20 °C until tested.

Fasting blood glucose (FBS) was quantified using an enzymatic colorimetric technique using a commercially available kit (Spinrecat, Spain). The HbA1c was measured by immunoturbidimetric assay with an automatic analyzer (Spinrecat, Spin 240) for the directed kit (PZ Cormay, Poland). Iron and total iron-binding capacity (TIBC) concentrations in

serum were determined using kits (Human, Germany). The UIBC was determined using the following equation:

UIBC (µg /dl) = TIBC (µg /dl) – Iron conc. (µg /dl)

The transferrin can be determined indirectly by using the following equation (30):

Transferrin (µg /dl) = 0.7 x TIBC (µg/dl)

The saturation of transferrin with Iron was determined using the following equation:

Saturation of transferrin (%) = (Iron conc. / TIBC) x 100

Using an available Cobas kit, ferritin serum levels were analyzed using an immunoturbidimetric by an automatic platform (Cobas C311- Germany). The CAT activity in human serum was measured using a spectrophotometric method dependent on ammonium molybdate (31).

Statistical analysis: The data were analyzed using SPSS (version 22) and presented in mean ± standard deviation (±SD). A one-way analysis of variance (ANOVA) was used to compare the groups. The difference between groups was statistically highly significant if the $p<0.01$, significant if the $p<0.05$, and non-significant if the $p>0.05$. Pearson correlation coefficient was used to determine the correlations between variables.

Results:

Table 1 shows the demographic characteristics of all studied groups. The controls were 50% males and 50% females; while the patients were distributed as follows: Males and females were 58% and 42% in A1, 43% and 57% in A2, 48% and 52% in A3.

Table 1: The demographic characteristics of the study groups

Characteristics		Groups			
		C	A1	A2	A3
Number		n=50	n=38	n=37	n=25
Age (year)	Mean±SD	43.1±11.05	42.8±9.89	54.3±10.47	57.2±11.24
Sex	Male (%)	25 (50)	22 (58)	16 (43)	12 (48)
	Female (%)	25 (50)	16 (42)	21 (57)	13 (52)
DM Duration (year)	Mean±SD	-	2.0±1.12	7.9±2.24	17.4±5.66

Table 2 illustrates the mean FBG and HbA1c in patients and control groups. The results showed significantly higher FBG levels in A1, A2, and A3 groups compared to the controls. In addition, they were significantly higher in A2 and A3 groups than in A1. The HbA1c levels in groups A1, A2, and A3 were significantly higher than those of the control group, and A3 showed a significantly higher level compared to A1.

Table 2: The mean±SD FBG and HbA1c levels of all study groups

Parameters (Mean±SD)	Groups			
	C (n=50)	A1 (n=38)	A2 (n=37)	A3 (n=25)
FBG (mg/dl)	92.2 ± 8.58	186.6 ± 78.34 ^{a**}	228.7 ± 72.09 ^{ab**}	237.4 ± 89.02 ^{ab**}
HbA1c %	4.4 ± 0.79	7.7 ± 2.05 ^{a**}	7.9 ± 1.93 ^{a**}	8.7 ± 1.32 ^{a**b*}

*$p<0.05$, **$p<0.01$
a: significant difference between C with A1, A2 and A3
b: significant difference between A1with A2 and A3

The Iron status parameters, including iron, TIBC, UIBC, transferrin, saturation transferrin (S.Trans), and ferritin in patients and control groups, are presented in Table 3. A highly significantly lower serum iron in all study groups (A1, A2 and A3) was detected compared to the controls. Compared to groups C and A1, a substantially lower TIBC level was found in groups A2 and A3. Non-significant differences were found between UBIC levels for all patient groups compared to the controls, while groups A2 and A3 showed a considerably lower level compared to the A1.

In A2, a highly significantly lower transferrin level was found compared to the controls. Compared to group C, a highly significantly lower S.Trans level was found in A1 and A2 groups. Moreover, ferritin levels were significantly higher in A1 and A2 groups compared to the controls, table 3.

Table 3: The Iron status parameters of all study groups

Parameters	Groups			
(Mean±SD)	C (n=50)	A1 (n=38)	A2 (n=37)	A3 (n=25)
Iron (µg/dl)	86.6±27.52	66.8 ± 25.45 [a**]	64.6 ± 23.31 [a**]	73.4 ± 30.28 [a*]
TIBC (µg/dl)	314.0 ± 40.33	314.7 ± 61.51	283.0 ± 58.18 [a**b*]	290.5 ± 54.38 [a*b*]
UIBC (µg/dl)	226.9 ± 49.71	246.2 ± 70.55	217.7 ± 59.38 [b*]	217.1 ± 64.96 [b*]
Transferrin (µg/dl)	220.7 ± 29.41	215.4 ± 50.60	197.7 ± 40.90 [a**]	202.99 ± 40.31
S.Trans (%)	28.1 ± 9.55	21.9 ± 8.65 [a**]	23.4 ± 9.86 [a*]	26.5 ± 12.44
Ferritin (ng/ml)	111.2 ± 33.43	150.3 ± 53.51[a**]	132.4 ± 50.45 [a*]	128.1 ± 35.32

*p<0.05, **p<0.01
[a] significant difference between C with A1, A2 and A3
[b] significant difference between A1with A2 and A3

The differences between males and females regarding iron and ferritin levels are presented in figures 1 and 2, respectively. The result indicated a statistically significant difference in iron and ferritin levels between males and females in all groups (C, A1, A2 and A3), showing that iron and ferritin levels in females were lower than in males.

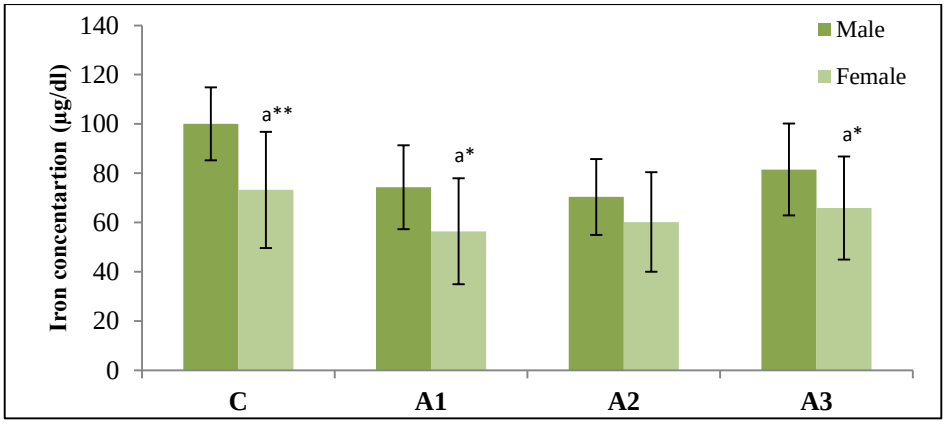

Figure 1: Serum iron level in males and females in patient and control groups
***p<0.05, **p<0.01; [a] significant difference between males and females in the same group**

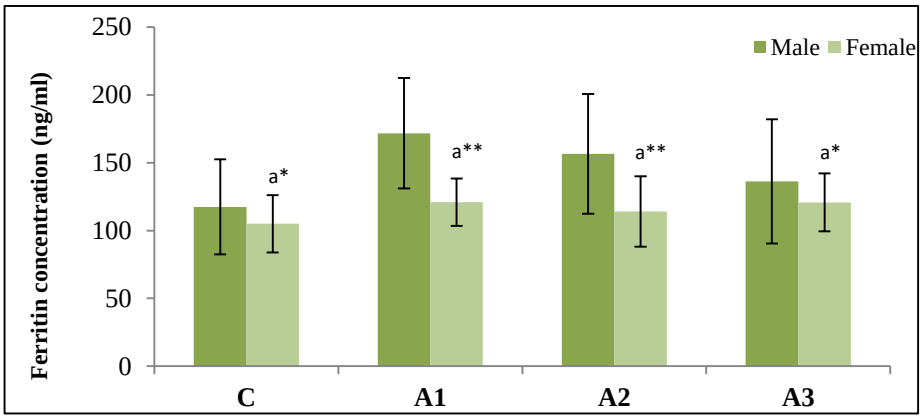

Figure 2: Serum ferritin level in males and females in patient and control groups
***p<0.05, **p<0.01; [a] significant difference between males and females in the same group**

The activity of CAT in the A1, A2, and A3 groups was significantly lower than in the control group, as shown in figure 3.

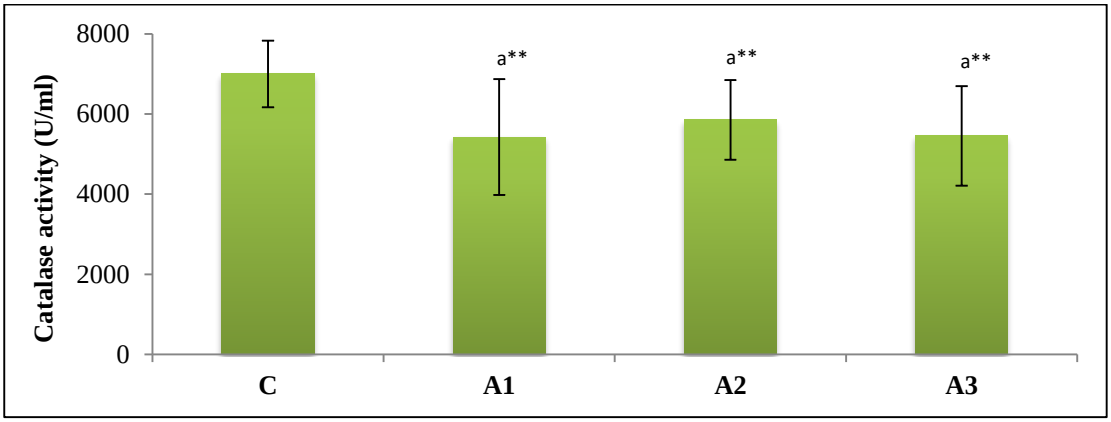

Figure 3: Serum catalase activity (U/ml) in all study groups
***p*<0.05, ****p*<0.01 ; [a] significant difference between C with A1, A2 and A3**

The correlation between all variables in the patient groups A1, A2, and A3 are illustrated in Tables 4, 5, and 6, respectively. In the A1 group, a highly significant positive correlation was found between (FBG with HbA1c), (iron with S.Trans), (TIBC with UIBC), (UIBC with transferrin) and (TIBC and transferrin). Also, a highly significant negative correlation was observed between S.Trans with TIBC, UIBC and transferrin. A significant negative correlation was reported between iron and UIBC, while a significant positive correlation was found between Iron and CAT activity.

Table 4: Pearson correlation coefficients between the biochemical parameters in the A1 group

Parameters	HbA1c	Iron	TIBC	UIBC	Transferrin	S.Trans	Ferritin	CAT
FBG	0.701**	0.273	0.127	0.007	0.124	0.154	-0.023	-0.056
HbA1c		0.086	-0.074	-0.093	-0.019	0.068	-0.007	-0.008
Iron			0.046	-0.375*	-0.040	0.818**	0.084	0.359*
TIBC				0.872**	0.883**	-0.415**	-0.200	-0.044
UIBC					0.796**	-0.706**	-0.202	-0.127
Transferrin						-0.440**	-0.140	-0.014
S.trans							0.226	0.120
Ferritin								0.141

*p< 0.05; ** p<0.01

Table 5, shows clearly that there is a highly significant positive correlation between (FBG and HbA1c), (iron and S.Trans), (TIBC and UIBC), (TIBC and transferrin) and (UIBC and transferrin). A highly significant negative correlation was observed between UIBC and S.Trans, while a significant positive correlation was noticed between iron and CAT activity in the A2 group

Table 5: Pearson correlation coefficients between the biochemical parameters in the A2 group

Parameters	HbA1c	Iron	TIBC	UIBC	Transferrin	S.Trans	Ferritin	CAT
FBG	0.490**	-0.015	-0.055	-0.048	-0.068	-0.020	-0.241	0.170
HbA1c		0.059	-0.002	-0.024	-0.031	0.049	-0.041	-0.023
Iron			0.241	-0.238	0.240	0.880**	0.168	0.330*
TIBC				0.883**	0.999**	-0.201	-0.262	0.125
UIBC					0.882**	-0.624**	-0.320	-0.038
Transferrin						-0.202	-0.262	0.125
S.trans							0.309	0.245
Ferritin								0.053

*p< 0.05; ** p<0.01

A highly significant positive correlation was found between (iron with S.Trans), (TIBC with UIBC), (TIBC with transferrin) and (UIBC with transferrin). A highly significant negative correlation was found between (iron with UIBC) and S.Trans with TIBC, UIBC and transferrin. A significant positive correlation was noticed between iron and CAT activity in the A3 group.

Table 6: Pearson correlation coefficients between the biochemical parameters in the A3 group

Parameters	HbA1c	Iron	TIBC	UIBC	Transferrin	S.Trans	Ferritin	CAT
FBG	0.118	0.063	-0.269	-0.254	-0.204	0.189	-0.410	-0.117
HbA1c		-0.072	0.296	0.282	0.329	-0.169	-0.085	-0.036
Iron			-0.105	-0.554**	-0.131	0.864**	0.178	0.301*
TIBC				0.886**	0.980**	-0.567**	0.041	-0.335
UIBC					0.882**	-0.878**	-0.049	-0.187
Transferrin						-0.575**	-0.055	-0.317
S.trans							0.125	-0.004
Ferritin								-0.144

*p< 0.05; ** p<0.01

Discussion:

The most common non-communicable disease in the world that may lead to death is DM. Reports indicate that over 50% of DM cases worldwide are undiagnosed (32). The global prevalence of diabetes in 2019 is estimated to be 9.3% (463 million people). By 2030, it will increase to 10.2% (578 million), and by 2045, it will reach 10.9% (700 million) cases (33). The IDF reports that T2DM was the most common type of diabetes among people in 2011 (5), accounting for 90% of all diabetes cases worldwide (34). Iraq has an extremely high prevalence of diabetes, with one in ten persons suffering from T2DM (35). Thus, diabetes is a serious public health problem for Iraqis due to its high prevalence rate, growing incidence rate, and financial burden.

The FBG level for T2DM patients in the current study was higher than the control; consistence with other studies (36,37). One of the most effective indicators of the complications risk, including kidney and cardiovascular diseases, is the duration of diabetes. It was found that the incidence of severe hyperglycemia rises with age and disease duration (38). The blood glucose level and the red blood cells' lifetime affect the level of HbA1c in the blood (39). Based on HbA1c and FBG concentrations, our study revealed substantial changes in glycemic control in the three diabetic study groups. There is a chance that the illness will worsen as it becomes more chronic, consistent with earlier studies (40). The current study showed that T2DM patients had significantly higher HbA1c levels than healthy controls, with an exceptionally high level in those with the longest duration of DM. This is consistent with a prior study by Ito et al., who reported that HbA1c values rise with longer disease duration, both in older and younger diabetics (41). The higher FBG levels were linked to the increase in HbA1c levels in A1 and A2 groups, in the current study. Zhu et al. (42) also found a positive correlation between FBG and HbA1c. The American Diabetes Association (ADA) recommended HbA1c levels of 5.7-6.4% for prediabetes diagnosis and 6.5% or higher for diabetes progression (43).

According to earlier studies, T2DM and iron metabolism are intricately related. Iron levels have been shown to influence glucose metabolism, and glucose metabolism influences several iron metabolic processes. Iron and the metabolism of glucose have a reciprocal interaction. Further factors that affect and amplify these interrelated processes are oxidative stress and inflammatory cytokines (44). One of the vital trace elements for the human body is Iron. Three to five grams of Iron are found in the body, and the body mainly controls iron levels through absorption. Body dysfunction may result from either an excess or a deficiency of Iron (45). The results of the present study showed that healthy controls had significantly higher iron levels than T2DM patients in all three groups, but it remains within the normal range. These findings are in agreement with those of previous studies (46,47). The decrease in serum iron levels in people who have T2DM can be caused by several factors, including the metabolic disruptions in T2DM which may result in the "iron resistance" phenotype, which can cause signals that regulate iron homeostasis to become dysregulated (48). T2DM patients may encounter iron deficiency anemia, which can lead to lower serum iron levels (49). These results contrast other studies that showed higher iron levels in T2DM cases than in healthy controls (50-52). The current study pointed to the possible role of iron in the etiology of T2DM and the numerous difficulties induced by free iron in T2DM patients.

Ferritin is the main intracellular iron storage protein and has been identified as a biomarker of inflammation and body iron storage (53). The current study showed that serum ferritin in T2DM patients is significantly higher than in controls in consistence with previous studies (19,54,55). However, its level has no correlation with blood sugar or HbA1c in diabetic patients. These findings agreed with the results of Thilipkumar et al. (56).

Ferritin as an acute-phase reactant which may indicate inflammation. Delayed clearance of glycosylated ferritin in diabetics may result in elevated ferritin levels, which may represent elevated iron stores; which are a few possible explanations for elevated ferritin levels in T2DM patients (57,58). The ferritin level was exceptionally high in the A1 group in the current study in agreement with an earlier study that reported a substantial correlation between serum ferritin level and newly diagnosed diabetes (59). Memon et al. (60) reported that T2DM patients with poor glycemic control more frequently had elevated serum ferritin levels compared to patients with good glycemic control. This observation can explain the findings that the long duration of the disease had lower ferritin levels compared to the short disease duration, which may be due to the patient's good glycemic control. Serum ferritin levels are frequently correlated with insulin resistance indicators, such as higher blood glucose and insulin levels. On the other hand, some studies are increasingly recognizing that serum iron affects glucose metabolism, even without severe iron overload or lack of iron (61). Therefore, high iron stores can predict diabetes development, according to previous epidemiological studies. Iron converts reactive free radicals into highly reactive ones. As the serum ferritin level increases, it affects insulin synthesis and secretion by the pancreas and interferes with the insulin-extraction capacity of the liver. Deposition of iron in muscles leads to muscle damage and decreases glucose uptake (62). Also, the glycation of transferrin stimulates ferritin synthesis by decreasing its capacity to bind ferrous iron and increasing the quantity of free iron (24).

The most significant molecule for delivering iron into cells is serum transferrin, a glycoprotein with two iron-binding domains. Transferrin is primarily produced in the liver and performs various tasks, including intracellular iron transport, iron transport across the intestinal mucosa, and non-specific defiance against microbes through the chelation of free iron (30,63). Our study showed that T2DM patients had a significantly lower serum transferrin concentration than healthy controls in the A2 group only. Transferrin is highly correlated with TIBC in all duration groups. T2DM incidence and a low soluble transferrin receptor-to-ferritin ratio level were observed by Arija et al. (64), but there was no correlation with the soluble transferrin receptor. The direct relationship between serum transferrin and T2DM has been the subject of very few investigations. Serum transferrin is prone to leaking from glomeruli due to its molecular weight and negative charges. Consequently, tracking urine transferrin levels may help assess the development of diabetic complications like diabetic nephropathy early on (45). Total iron-binding capacity (TIBC) is a total quantity of iron measurement that blood proteins are capable of binding, which aids in assessing the body capacity to bind and move iron through the blood. It equals UIBC plus the serum iron level. While low levels may signal iron overload, high TIBC levels may indicate iron deficiency (48). In iron-deficient conditions, the relative transferrin content compared to iron content increases, and thus, the TIBC values are high. For this reason, the increase in TIBC levels in this study may be due to the decrease in iron levels in T2DM patients.

The concentration of iron and ferritin levels in male T2DM is higher than in females. Manikandan et al. (24) reported that the differences between sexes may be because most females are anemic due to physiological processes like pregnancy and menstruation. This result is consistent with Han et al. (65) and Al Akl et al. (66) studies, which found a statistically significant positive connection between blood ferritin levels and diabetes, metabolic syndrome, and obesity in male patients rather than female patients.

It has been demonstrated that H_2O_2 acts as an oxidant and damages the β-cell interrupting the signaling pathway of insulin production. A previous study reported that a four-fold increase in the H_2O_2 concentration was observed in T2DM patients than in healthy individuals, and the observation was corroborated with observations of low CAT activity in the β-cells of hyperglycemic mice models. Thus, the lack of CAT, which is responsible for degrading the H_2O_2 to water and oxygen, can contribute to the development of DM (24). The present study found that CAT activity was lower in all T2DM study groups than in healthy controls. Prior studies reported lower plasma iron concentrations and CAT activity in T2DM, consistent with our findings (8,67). Oxidation plays an important role in different T2DM complications. Because of the CAT low levels or activity, cells may have higher concentrations of hydrogen peroxide which leads to oxidative stress conditions leading to the progression of different complication types (24).

Our results indicated a significant positive correlation between CAT activity and iron concentration in all patient groups. One of the T2DM risk factors is increasing oxidative stress with a reduction in total antioxidant capacity (TAC) reported by Mahmood (17), in contrast to increased iron levels. One of the reasons for decreased CAT activity in T2DM is genetic factors; reduced blood CAT activity brought on by the many CAT mutations found in diabetic individuals may result in higher blood and tissue amounts of hydrogen peroxide. The oxidation-sensitive pancreatic ß-cells may be harmed by these elevated hydrogen peroxide levels, which would reduce insulin synthesis (67). Since CAT needs iron for its catalytic activity, the decrease in CAT activity in our research might be caused by a reduction in iron levels. According to these findings, CAT deficiency may be a risk factor for T2DM. However, applying the findings to all T2DM patients is not suitable. To better understand the association between iron levels and CAT activity

in T2DM patients and the applicability of using this correlation as a predictor for both the onset and severity of diabetes, additional research with a larger sample size and collecting samples from different regions of Iraq is required.

Conclusions:

Iron plays a significant role in the pathogenesis and complications of T2DM patients. Increases in ferritin levels might be a risk factor for developing T2DM. The observed association between lower iron levels and reduced catalase activity in T2DM is intriguing and can serve as a future predictor for the onset and severity of diabetes, warranting further investigation.

Authors' declaration:

We confirm that all the Figures and Tables in the manuscript are ours. Besides, the figures and images, which are not ours, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in (College of Science, University of Baghdad) according to the code number (Ref. CSEC/0123/0012, Date: 27.01.2023).

Conflicts of Interest: None

Authors' Contribution:

Study conception & design: (Saba Z. Hussein & Esraa H. Oleiwi). Literature search: (Esraa H. Oleiwi). Data acquisition: (Esraa H. Oleiwi). Data analysis & interpretation: (Esraa H. Oleiwi). Manuscript preparation: (Saba Z. Hussein & Esraa H. Oleiwi)). Manuscript editing & review: (Saba Z. Hussein & Esraa H. Oleiwi). Funding acquisition: (Esraa H. Oleiwi).

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استكشاف العلاقة بين حالة الحديد ونشاط الكاتالايز لدى المرضى المصابين بداء السكري من النوع الثاني

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الخلاصة:
خلفية البحث: الكاتالايز هو انزيم مضاد للاكسدة موجود في جميع الكائنات الحية، وهو مسؤول عن تحليل بيروكسيد الهيدروجين، وهو نوع من أنواع المركبات الضارة المعروفة باسم أنواع الأوكسجين التفاعلية (ROS)، إلى أوكسجين وماء غير الضارين. يعد هذا الإنزيم ضروريا" لحماية الخلايا من الأضرار التأكسدية التي يسببها ROS لدى الأفراد المصابين بداء السكري من النوع الثاني T2DM. يتزايد انتشار مرض السكري من النوع الثاني (T2DM) بسرعة، ويشكل تحديًا كبيرًا للصحة العامة في جميع أنحاء العالم. يمكن أن يعطل T2DM تنظيم الحديد في مجرى الدم، مما يؤدي إلى إنتاج أنواع الأوكسجين التفاعلية والتي يمكن أن تلحق الضرر بالخلايا.
الهدف: تبحث الدراسة الحالية في العلاقة بين حالة الحديد ونشاط الكاتالايز لدى المرضى الذين يعانون من T2DM، وامكانية استخامه كمتنبئ لبداية وشدة المرض.
المرضى وطرق العمل: تضمنت هذه الدراسة 150 مشاركا، 50 من الأفراد الأصحاء كانو بمثابة مجموعة ضابطة (C) و100 شخص تم تشخيص إصابتهم بداء السكري من النوع الثاني T2DM وتم تقسيمهم إلى ثلاث مجموعات بناءً على مدة مرضهم: المجموعة A1 (ن = 38؛ أقل من 5 سنوات)، A2 (ن = 37؛ 5-10 سنوات) وA3 (ن = 25؛ اكثر من 10 سنوات). تم اخذ العينات من مستشفى الكندي التعليمي في بغداد خلال الفترة من تشرين الاول 2022 الى نهاية كانون الثاني 2023. قامت الدراسة بتقييم علامات الدم المختلفة لدى جميع المشاركين، بما في ذلك: نسبة الجلوكوز في الدم الصائم (FBG)، خضاب الدم السكري A1c (HbA1c)، الحديد، إجمالي سعة ربط الحديد (TIBC)، سعة ربط الحديد غير المشبعة (UIBC)، الفيريتين، الترانسفيرين، وتشبع الدم بالترانسفيرين (S.Trans) ونشاط الكاتلاز (CAT).
النتائج: بالمقارنة مع المجموعة الضابطة، أظهر المرضى الذين يعانون من T2DM: مستويات أعلى بشكل ملحوظ من FBG، وHbA1c، وFeritin، ولا سيما مستويات أقل من الحديد، وTIBC، وUIBC، وS.Trans. ومن المثير للإهتمام أن المجموعة A2 فقط كانت لديها مستويات أقل بكثير من الترانسفيرين مقارنة بالمجموعة الضابطة. لوحظ إنخفاض كبير في نشاط الكاتلاز في جميع فئات المرضى. بالإضافة إلى ذلك، لوحظ وجود علاقة إيجابية بين مستويات الحديد ونشاط الكاتالايز في جميع مجموعات المرضى.
الإستنتاج: إن إرتفاع مستويات الفيريتين في الدم قد يكون عامل خطر لحدوث النوع الثاني من داء السكري. بالإضافة إلى ذلك، فإن العلاقة الملحوظة بين إنخفاض مستويات الحديد وإنخفاض نشاط الكاتالايز لدى المرضى الذين يعانون من النوع الثاني من داء السكري أمر مثير للاهتمام. من المحتمل أن يكون هذا الإرتباط بمثابة مؤشر مستقبلي لبداية مرض السكري وشدته، مما يستدعي إجراء مزيد من البحث.
الكلمات المفتاحية: داء السكري من النوع الثاني، الحديد، الفيريتين، الترانسفيرين، نشاط الكاتالايز

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Research Article

Uncovering Factors Contributing to Poor Asthma Control among Asthmatic Patients in Erbil City - Kurdistan Region

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Abstract

Background: Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation and variable expiratory airflow limitation.

Objective: The objective of this study was to uncover factors contributing to poor asthma control in Erbil City, Iraq.

Methods: To assess the asthma control in Erbil City a cross-sectional observational study was conducted on200 patients with asthma from the 1st, June to 31st, December 2023. Demographic, clinical characteristics, triggers, comorbidities, inhaler problems, causes of visit and state of asthma control were evaluated using Global Initiative for Asthma 2023 as the assessment tool questionnaire. Asthma control level was divided into three level controlled, partly controlled and uncontrolled.

Results: In this study 200 patients with asthma (108 females and 92 males) were studied.
The mean age ± SD of the patients was 35.61 ± 17.182 years and the female to male ratio was nearly 1:1.Asthma control was very poor as only 24 patients (12%) were controlled. Neither sex nor age group has statistically significant association with asthma control. Factors that significantly associated with asthma control included improper inhaler technique (45.5%), fear of addiction (29%) improper inhaler prescription (27.5%), coat (23%), device type (22.5%), infections (21%), indoor and outdoor exposure(18%), tobacco smoking (17%), allergic rhinitis (28.5%), gastroesophageal reflux disease (21.5%) and short-acting beta agonist alone therapy 104(52%). Other factors were non-significantly associated with asthma control such as emotional stress (9.5%), food allergy (8.5%), obesity(15%), atopic dermatitis (6%), obstructive sleep apnea (15%), and pregnancy (5.5%).

Conclusion: The current study concluded that factors associated with uncontrolled asthma were improper inhaler technique, fear of addiction, improper inhaler prescription, cost, device type, infections, indoor and outdoor exposure, tobacco smoking, allergic rhinitis, Gastro-esophageal Reflux Disease and short-acting beta agonist alone therapy.

Keywords: Asthma; Control; Comorbidities; Inhaler; Triggers.

Introduction:

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of respiratory symptoms, such as wheezing, shortness of breath, chest tightness, and cough, that vary over time and in intensity, together with variable expiratory airflow limitation (1).

Asthma is one of the common chronic respiratory disorders affecting 1–29% of the population in different countries (2).

The asthma prevalence in the Middle East ranges from 4.4% to 7.6%3while in Iraq according to some national studies is below 9% in adults (4,5), which is lower than other parts of the world namely Europe and North America. Asthma as a disease regarded as a controllable disease and the aim of almost all guidelines including Global Initiative for Asthma (GINA) guidelines for asthma management is to offer a good and normal healthy life(1). Control of asthma is defined as the level of control in which the various features such as symptoms, and good quality of life with reducing the future adverse events have been removed or reduced by treatment (6).

In 2019, globally estimated that over 260 million people are living with poorly controlled asthma with a high frequency of disabilities and premature deaths especially in low- and middle-income countries(7)Asthma control evaluation according to a study (Characterization of Severe Asthma Worldwide) was done in several countries including the United Kingdom, United States, Italy and South Korea, and a Web-based Database registry from Australia, Singapore, and New Zealand from 2014 to 2017. According to that study 57.2% of patients had poorly controlled asthma (8). In Middle East countries still there is no dependable data on asthma control although there are some data from several studies like the ESMAA study in which only a small percentage of asthmatics were controlled for example in Jordan (14.8%), Iraq (17.5%), Kuwait (42.6%), and Qatar (41.1%) were controlled (9). In Turkey around 90% of adults with asthma were uncontrolled (10), in

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Saudi Arabia 68.1% of asthmatics were uncontrolled (11).

Asthma control is determined by the interaction between the patients' comorbidities, underlying disease processes, environment, genetic background, psychosocial factors, and the treatment that they are taking (1).

Uncontrolled asthma has negative impacts on quality of life12, and increases risk of exacerbations (13).

There are several tools for evaluation of asthma control, one of the important tools is GINA defined criteria for assessment of asthma control1. GINA assessment tool criteria measure symptom control in the past 4 weeks.

Asthma control levels are divided into three levels according to GINA criteria, first is well-controlled (all symptoms' criteria are absent), second is partly-controlled (one to two criteria are present), and uncontrolled (three to four criteria are present) (1).

Risk factors or causes of uncontrolled asthma are numerous including; Short-acting beta agonist (SABA) overdose (> one canister per month) (14), improper inhaler technique (up to 80% of community patients) (15), poor medication adherence (16), allergic rhinitis (17) Gastro-esophageal Reflux Disease (GERD)
(18), obesity (19), confirmed food allergy (20), atopic dermatitis (21), obstructive sleep apnea (22), pregnancy (23), tobacco smoking (24), psychological problems (25), and indoor and outdoor exposure (26).

Among patients with uncontrolled asthma around 3.7% of them have severe asthma despite good inhaler technique and adherence (27).

There are insufficient dependable clinical data and large studies about the level of asthma control in Iraq, therefore; the current study aimed to uncover factors contributing to uncontrolled asthma in Erbil City, Iraq.

Materials and Methods:

Study design: This cross-sectional observational study was conducted to assess asthma control
in Erbil City. The study was conducted on 200 patients who were diagnosed previously with asthma in a private clinic and Rizgary Teaching Hospital from 1st, June to 31st, December 2023.

Patient data were collected through questionnaire filled by doctors during hospital or private clinic visits.

Demographic, clinical characteristics, triggers, comorbidities, inhaler problems, causes of visit, and the state of asthma control using the GINA 2023 assessment tool questionnaire.

Study population: The age group which was diagnosed with asthma was 12 to 81 years old. These patients were presented to a hospital or outpatient private clinic. Any patient with other chronic respiratory disease other than asthma was excluded from the study.

Assessments: Asthma control levels were divided into three levels according to GINA criteria (controlled, partly-controlled and uncontrolled).The GINA criteria include (day time symptoms > twice/week, any night waking, short acting beta receptor agonist reliever for symptoms > twice/week, and any activity limitation due to asthma), and risk factors for poor asthma outcomes (risk factors for exacerbation).

Inhalational Device Assessment Tool score (IDAT) was used for assessing inhaler technique which is composed of five scores from good inhaler technique (score 4-5) to poor inhaler technique (score 1-2).

Authors' declaration

The study was conducted by ethical principles that have their origin in the Declaration of Helsinki. The study was approved by the Hawler Medical University Ethics Committee. Consent and permission were taken from the patients at the first visit.

Statistical Analysis

The statistical analysis was performed using Statistical Package for the Social Sciences version 23 (SPSS 23, IBM Company, and Chicago, USA). The Chi-squared test was used, but when the Chi-squared test was inappropriate then Fisher's exact test was used. $P \leq 0.05$ was considered statistically significant.

Results:

The mean age ± SD of the patients was 35.61 ± 17.182 years (ranging from 15 to 81 years), and the female-to-male ratio was 1.17:1. Most of the patients were between ages 15 to 34 years old (51.5%) as shown in figure 1.

DEMOGRAPHIC CHARACTERISTICS OF STUDY PATIENTS WERE SHOWN IN TABLE 1; ASTHMA CONTROL WAS VERY POOR, ONLY 24 PATIENTS (12%) WERE CONTROLLED ACCORDING TO GINA GUIDELINES.

Table 2 showed factors affecting asthma control in study patients; in the study, neither sex nor age group has a statistically significant (*P*-value > 0.05) association with asthma control.

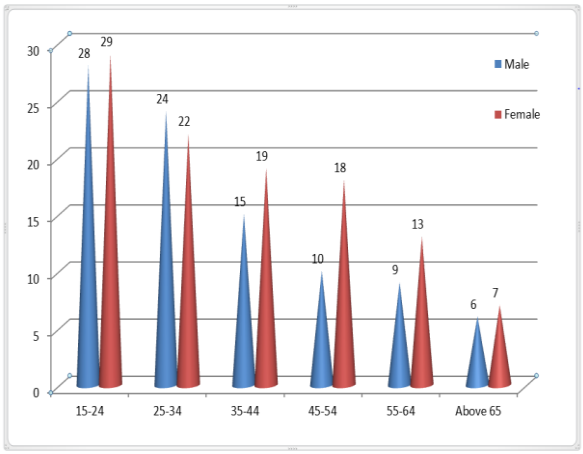
Among inhaler related causes of uncontrolled asthma, there were statistically significant, inhaler related factors including improper inhaler technique (45.5%, *P*-value = 0.001) which is assessed by the Inhalational Device Assessment Tool (IDAT), fear of addiction (29%, *P*-value = 0.003) improper inhaler prescription (27.5%, *P*-value = 0.001)), coast (23%, *P*-value = 0.005) and device type (22.5%, *P*-value = 0.015).

Triggers had an important effect on asthma control, some of them were statistically significant like infections (21%, *P* -value = 0.007), indoor and outdoor exposure (18%, *p*-value = 0.018), and tobacco smock (17%, *p*-value = 0.039) while other triggers like emotional stress (9.5%, *p*-value = 0.223) and food allergy (8.5%, *p*-value = 0.278) were statistically not significant.

Some of the comorbidities were statistically significant (*P* -value < 0.05) associated with asthma control, like allergic rhinitis (28.5% , *p*-value = 0.016) and GERD (21.5% , *p*-value = 0.018) while other comorbidities have no statistically significant association with asthma control like obesity(15% , p-value = 0.284), Atopic dermatitis (6% , *p*-value = 0.323), obstructive sleep apnea (15% , *p*-value = 0.089), psychological problem (7% , *p*-value = 0.277), and pregnancy (5.5% , *p*-value = 0.952).

Different factors affecting asthma control in the same patient, about 139 (69.5%) of patients with non-controlled asthma have at least two risk factors.

Table 3 showed the effect of inhaler medication type on asthma control. According to our study, SABA alone therapy 104(52%, p-value =0.001) has statistically significant negative impact on asthma control.



P VAUE = 0.791

FIGURE 1: SEX AND AGE-GROUP DISTRIBUTION AMONG STUDIED PATIENTS.

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF STUDY PATIENTS

Characteristic	Category	No. (%)
Sex	Male	92 (46
	Female	108 (54)
Age Group/ year	15-24	57(28.5)
	25-34	46 (23)
	35-44	34 (17)
	45-54	28 (14)
	55-64	22 (11)
	Above 65	13 (6.5)
Asthma control status	Well-controlled asthma	24 (12)
	Partly-controlled asthma	90 (45)
	Uncontrolled asthma	86 (43)
BMI*	Low	16 (8)
	Normal	72 (36)
	Over weight	68 (34)
	Obese	44 (22)
Smoking Status	Current smoker	34 (17)
	Ex-smoker	36 (17.5)
	Never smoked	130 (55)
Family history	Asthma	88 (44)
	Allergic rhinitis	92 (46)
	Eczema	30 (15)
Pregnancy	Pregnant	11 (5.5)

*BMI: body mass index

Table 2: Different factors affecting asthma Control in study patients.

Factor		Asthma Control: Well-controlled asthma No. (%)	Partly-controlled asthma No. (%)	Uncontrolled asthma No. (%)	Total No. (%)	*P*-value
Sex	Male	14 (58.3)	43 (47.8)	35 (40.7)	92 (46)	
	Female	10 (41.7)	47 (52.2)	51 (59.3)	108(54)	0.278
Inhaler related	Technique	0 (0.0)	43 (47.8)	48 (55.8)	91(45.5)	0.001
	Fear of addiction	0 (0.0)	27 (30)	31 (36)	58 (29)	0.003
	Prescription	0 (0.0)	22 (24.4)	33 (38.4)	55 (27.5)	0.001
	Coast	0 (0.0)	19 (21.1)	27 (31.4)	46 (23)	0.005
	Device type	0 (0.0)	21 (23.3)	24(27.9)	45 (22.5)	0.015
Trigger	Infection	0 (0.0)	17 (18.9)	25 (29.1)	42 (21)	0.007
	Indoor and outdoor exposure	0 (0.0)	13 (14.4)	23 (26.7)	36 (18)	0.018
	Tobacco smock	0 (0.0)	15 (16.7)	19 (22.1)	34 (17)	0.039
	Emotional stress	0 (0.0)	9 (10)	10 (11.6)	19 (9.5)	0.223
	Food allergy	0 (0.0)	9 (10)	8 (11.8)	17 (8.5)	0.278
Comorbidities	Allergic rhinitis	1 (4.2)	27 (30)	29 (33.7)	57(28.5)	0.016
	GERD	0 (0.0)	20 (22.2)	23 (26.7)	43(21.5)	0.018
	Obesity	1 (4.2)	15 (16.7)	14(16.3)	30(15)	0.284
	Atopic dermatitis	0 (0.0)	5 (5.6)	7 (8.1)	12(6)	0.323
	OSA	0 (0.0)	15 (16.7)	15 (17.4)	30(15)	0.089
	Psychological	0 (0.0)	6 (6.7)	8 (9.4)	14(7)	0.277
	Pregnancy	1 (4.2)	5 (5.6)	5 (5.8)	11(5.5)	0.952
No. of risk factors*	One risk factor	3 (12.5)	28(31.1)	1(1.2)	32 (16)	0.001
	≥ two risk factor	0 (0.0)	62 (68.9)	63 (89.5)	139 (69.5)	
	No risk factor	21(87.5)	0 (0.0)	8 (9.4)	8 (9.3)	

OSA: obstructive sleep apnea, GERD**:** Gastro-esophageal reflux disease

Table 3: Effect of inhaler medications type on asthma control

Inhaler type	Asthma Control: Well controlled asthma No. (%)	Partly controlled asthma No. (%)	Uncontrolled asthma (%)	Total No. (%)	P-value
SABA* alone	1 (4.2)	52 (57.8)	31 (59.3)	104(52)	
LABA**+ICS***	16 (66.7)	29 (32.2)	25 (29.1)	70 (35)	0.001
LABA+ICS+SABA	7 (29.2)	5 (5.6)	7 (8.1)	19 (9.5)	
LABA+ICS+LAMA****	0 (0.0)	4 (4.4)	7 (3.5)	7 (3.5)	

SABA*: short-acting agonist, LABA**: long-acting beta agonist, ICS***: inhaled corticosteroid, LAMA****: long-acting antimuscarinic agent.

Discussion

This study was done on 200 patients with bronchial asthma, the mean age of the patients was 35.61 ± 17.182 years (ranging from 7 to 81 years), and the female to male ratio was; 1.17:1.

According to our study, although bronchial asthma is more common among females, through an unknown mechanism, it was statistically not significant. These results were similar to other studies regarding the increasing prevalence of asthma among females, but in those studies, the results were statistically significant (1,2,3).

IN CURRENT STUDY, ASTHMA CONTROL WAS VERY POOR IN WHICH ONLY 24 PATIENTS (12%) WERE CONTROLLED BY USING GINA GUIDELINES.

Globally one of well-known important causes of uncontrolled asthma is related to inhaler causes. In our study the most common factors affecting asthma control were inhaler-related factors, inhaler-related factors including improper inhaler technique (45.5%, p-value = 0.001) which is assessed by the Inhalational Device Assessment Tool (IDAT), fear of addiction (29%) improper inhaler prescription (27.5%)), coast (23%) and device type (22.5%), all these factors were statistically significant. Our results were similar to previous studies which were done on inhaler-relatedcauses of uncontrolled asthma like improper inhaler technique (18,4,5), fear of inhaler addiction (6), device type (7), and cost of the inhaler (8).

In our study, triggers like infections (21%), indoor and outdoor exposure(18%), and tobacco smoking (17%) while other triggers like emotional stress (9.5%) and food allergy (8.5%) were among the causes of uncontrolled asthma but statistically not significant, similar results with statistically significant triggers were found in other studies (23,27,25,9,10).

Comorbidities had a significant impact on asthma control. In our study, allergic rhinitis had a statistically significant impact on asthma control (28.5%); therefore good control of concomitant allergic rhinitis may improve asthma control as seen in other studies (17). Gastro-esophageal reflux disease is another comorbidity that had significant on asthma control (21.5%) according to another study demonstrated bidirectional relationship with asthma control (18), higher BMI scores independent of sex, age and severity of asthma (19), study had a statistically significant association with asthma control while in our study, despite of the presence of an association between asthma control and obesity, but statically non-significant (15%)

Atopic dermatitis and asthma are commonly associated with each other, whether they are of the same disease spectrum or comorbidities and their effect on disease still not clear as seen in other studies (21). In our study, there was an association between atopic dermatitis and asthma control but statically non-significant (6%). Obstructive sleep apnea is significantly associated with asthma control22, but in our study not significant (15%), regarding the association with pregnancy, (5.5%) were statistically non-significant, not like other studies (23).

Different factors including multiple triggers, and comorbidities affect asthma control in the same patient, about 139 (69.5%) of the patients with non-controlled asthma have at least two risk factors which were statistically significant, similar results were obtained in other studies (11,12).

Inhaler medications type had significant effect on asthma control, according to our study, inhaled short-acting beta agonist (SABA) alone therapy 104(52%) had a statistically significant negative impact on asthma control as seen in other studies (16, 14), while in inhaled corticosteroids (ICS) + inhaled long-acting beta-agonist (LABA), especially formoterol, had a statistically significant positive impact on asthma control as seen in other studies (xxviii).

Conclusion

In our study, the most important statistically significant factors affecting asthma control were improper inhaler technique, fear of addiction, improper inhaler prescription, coast, device type, infections, indoor and outdoor exposure, tobacco smock, allergic rhinitis, gastro-esophageal reflux, and SABA alone therapy). However, other factors, although they were associated with asthma control but statistically non-significant like obesity, obstructive sleep apnea, psychological and pregnancy. Interestingly 69.5% of patients with non-controlled asthma had at least two risk factors.

Authors' contribution:

We confirm that all the Figures and Tables in the manuscript are ours. Authors sign on ethical considerations the local ethical committee in the College of Medicine; Hawler Medical College approved the project according to code number (2 on 30/10/2023).

Conflicts of Interest: None

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الكشف عن العوامل التي تساهم في ضعف السيطرة على الربو: مجموعة مستقبلية

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الخلاصة:

خلاصة الخلفية: الربو هو مرض غير متجانس، ويتميز عادة بالتهاب مجرى الهواء المزمن وتقييد تدفق الهواء الزفير المتغير
الهدف: كان الهدف من هذه الدراسة هو الكشف عن العوامل التي تساهم في ضعف السيطرة على الربو في مدينة أربيل في العراق.
الطُرق: تم إجراء هذه الدراسة الرصدية المقطعية على 200 مريض مصاب بالربو في الفترة من 1 يونيو 2023 إلى 31 ديسمبر 2023 لتقييم السيطرة على الربو في مدينة أربيل. الخصائص الديموغرافية والسريرية والمحفزات والأمراض المصاحبة ومشاكل الاستنشاق وأسباب الزيارة وحالة السيطرة على الربو باستخدام استبيان أداة تقييم GINA 2023 .ينقسم مستوى التحكم في الربو إلى ثلاثة مستويات يمكن التحكم فيها، ومستوى يمكن التحكم فيه جزئيًا، وغير يمكن التحكم فيه.
النتائج: تمت في هذه الدراسة دراسة 200 مريض مصاب بالربو (108 إناث و 92 ذكر (كان متوسط العمر ± SD للمرضى 35.61 ± 17.182 سنة (يتراوح من 15 إلى 81 سنة)، ونسبة الإناث إلى الذكور هي 1.17:1. السيطرة على الربو سيئة للغاية وتم التحكم في 24 مريضًا فقط (12٪. (ليس للجنس ولا للفئة العمرية علاقة ذات دلالة إحصائية مع السيطرة على الربو. تشمل العوامل ذات الأهمية الإحصائية المرتبطة بالسيطرة على الربو تقنية الاستنشاق غير الصحيحة (45.5%)، والخوف من الإدمان (29%)، ووصفات الاستنشاق غير الصحيحة (27.5%)، والساحل (23%)، ونوع الجهاز (22.5%)، والالتهابات (21 (% ، والتعرض الداخلي والخارجي (18%)، ودخان التبغ (17%)، والتهاب الأنف التحسسي ((28.5%)، والارتجاع المعدي المريئي (21.5(% علاج SABA وحده 104 (52%)، في حين أن العوامل الأخرى المرتبطة بالسيطرة على الربو ولكنها ليست ذات دلالة إحصائية مثل الإجهاد العاطفي (9.5%) وحساسية الطعام (8.5%)، والسمنة (15%)، والتهاب الجلد التأتبي (6%) ، انقطاع التنفس الانسدادي أثناء النوم (15%)، والحمل (5.5%).
الاستنتاج: من العوامل ذات الأهمية الإحصائية المرتبطة بسوء التحكم في الربو هي أن الربو الذي يتم التحكم فيه بشكل سيئ مرتبط بأجهزة الاستنشاق (تقنية الاستنشاق غير المناسبة، وصفة الاستنشاق غير المناسبة، وعدم الالتزام بجهاز الاستنشاق) والمحفزات (العدوى، والحساسية، والدخان، والتلوث) والأمراض المصاحبة (التهاب الجيوب الأنفية، والارتجاع المعدي المريئي.
الكلمات المفتاحية: المثيرات الربو، السيطرة، الاستنشاق، الأمراض المصاحبة.

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Research Article

Long-Term Effects of Scopolamine on Brain Tissue of Mice

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Abstract:

Background: Scopolamine is an anticholinergic drug that disrupts cholinergic transmission in the central nervous system as well as causes cognitive abnormalities and pathological hallmarks that are similar to those seen in Alzheimer's Disease. Therefore, it is used for induction of Alzheimer's Disease in animal models.

Objective: to investigate the effects of long-term induction with scopolamine on the brain tissue of mice.

Methods: Seventy adult mice were divided into 2 equal groups: The first group was the normal control group received distilled water only. The second one was the Alzheimer's Disease induction group received intraperitoneal scopolamine (1mg/kg) for 14 days only after that distilled water was given for the next 6 months. Ten mice were isolated from each group at zero time, after 2 weeks of induction, after 3-month and after 6 months and subjected to the behavioral tests then sacrificed for determination of biochemical factors (including brain-derived neurotrophic factor, total antioxidant status, malondialdehyde, and amyloid β). Data were analyzed using *t*-tests, and ANOVA. All values expressed as Mean±SD and *P* value <0.05 were considered significant.

Result: Scopolamine produced brain histopathological changes similar to those of human Alzheimer's disease. However, it does not produce further statistically significant differences in behavioral tests and biochemical markers during the total period of study.

Conclusion: scopolamine produces brain tissue changes that persist for a long period and it can be used for long-term study of Alzheimer's disease.

Keywords: Alzheimer's disease**;** Antioxidant; Cognitive function; Oxidative stress; Scopolamine**.**

Introduction

Scopolamine (SCM) is an anticholinergic drug that disrupts cholinergic transmission in the central nervous system (CNS) and demonstrates competitive antagonism at muscarinic acetylcholine receptors (mAChRs) (1).

As it easily crosses the blood-brain barrier, scopolamine is frequently utilized in neuroscience research to induce cognitive impairments, similar to those seen in Alzheimer's Disease (AD), in experimental animals (2). These animal models are commonly used to test medications for possible therapeutic usefulness in people with AD-type dementia (1, 3).

Moreover, it is used with different doses in different studies. Some of these studies used it as a single dose and others as multiple doses. Therefore, it is unclear if SCM can induce different intensities and durations of amnesia after single or repeated doses (4).

In addition, many studies indicated that SCM increases the deposition of A*β*, the level of reactive oxygen species, and reduces antioxidant concentration leading to lipid peroxidation that induces oxidative stress.

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Also, it decreases the brain-derived neurotrophic factor (BDNF) and cAMP-response element binding protein (CREB) expression in the brain which are hallmarks of AD disease and ultimately cause memory impairment and synaptic dysfunction (3, 5). Previous studies concerned with the use of SCM for induction of AD in animal models did that over a short induction period. Therefore, the current study aimed to investigate the long-term effects of scopolamine on cognitive and memory functions as well as the pathological hallmark of Alzheimer's disease in mice.

Methods

An experimental study was conducted from 1st, July 2022 to 1st, June 2023.

The current study involved ninety adult female mice (4–8 weeks old) weighing (20–25g) were purchased from the Al-Razi center, Ministry of Industry and Minerals, Baghdad, Iraq. The mice were kept in the experimental area for 2 weeks for the habituation phase and housed as ten mice per cage at an appropriate temperature (25°C) and humidity (30% ± 10%), with a standard 12-hour light/dark cycle and free access to water and standard food (high protein feed and milk powder).

In the beginning, twenty mice were included in a pilot study to detect the effective dose and duration of treatment for Scopolamine Intraperitoneal (IP/SCM) to induce AD (scopolamine hydrochloride, 1mg/kg, was dissolved in distilled water for injection and given IP to induce AD in mice (6). The results of the pilot study showed that IP/SCM (1mg/kg) for 14 days induced AD effectively. Then, seventy adult mice were divided into 2 equal groups; the first group was the normal group that received distilled water only during the total period of the study and was considered a control group, and the second one was the induction untreated Alzheimer group that received IP/SCM (1 mg/kg) for 14 days only after that distilled water was given for the next 6 months.
Mice were subjected to behavioral tests (Barnes Maze (7), Novel Object Recognition (8), and Y-Maze Tests (9). Then, these mice were anesthetized and sacrificed to isolate brain tissue for further determination of biochemical markers such as BDNF, TAS, MDA, and 1-42 *β*-amyloid peptide. This procedure was repeated 3 and 6 months after induction.

Data were analyzed using *t*-tests (paired and unpaired) and ANOVA. All values expressed as Mean±SD and *P* value <0.05 were considered significant (10).

Results

Histopathological changes

Results from the current study revealed that histopathological sections of brain tissue from mice induced with SCM for 2 weeks showed microscopically changes (Figures 1 & 2)

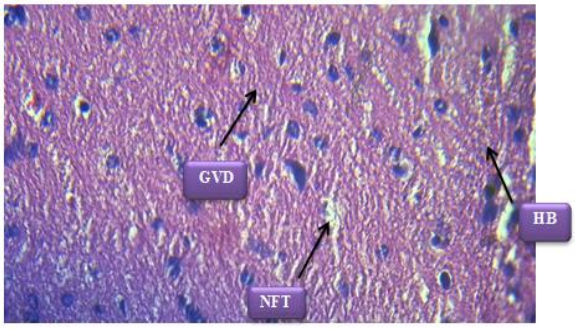

Figure (1): Histological section of mice AD brain tissue after induction with scopolamine for 2 weeks (current study) showing numerous NFT, HB, and GVD (H&E stain) (40X).

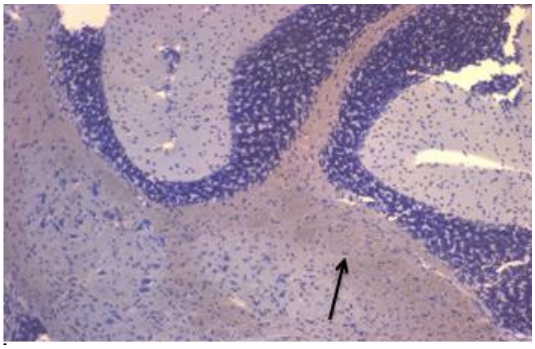

Figure (2): Histological section of mice AD brain tissue after induction with scopolamine for 2 weeks (current study) showing multifocal severe deposition of amyloid beta plaques (orange-red color) (arrow) (Congo red stain) (10X).

Also, findings indicated the validity of AD animal model created in the current study. Similar to those reported in human AD brain tissue (Figures 3, 4, and 5)

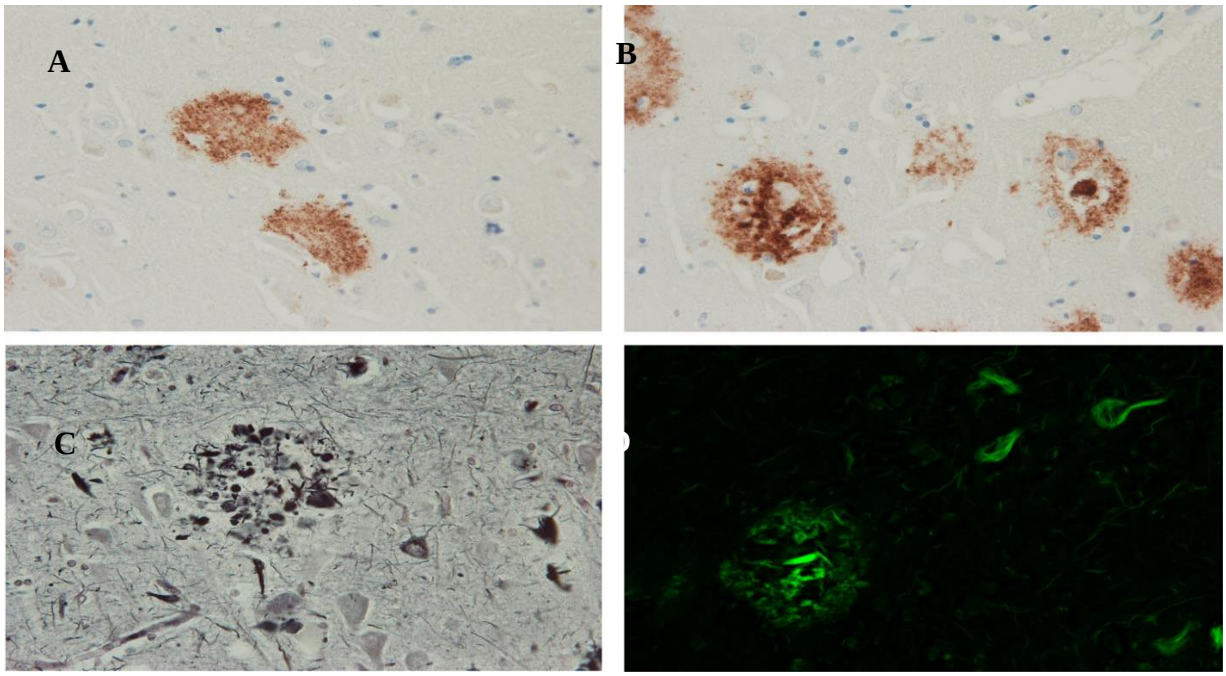

Figure (3): Histological section of human AD brain tissue showing the presence of amyloid *β* Senile Plaques by using antibodies directed against Aβ peptides (A&B), Bielchowsky silver staining (C) or Thioflavin S staining (D) (DeTure & Dickson, 2019).

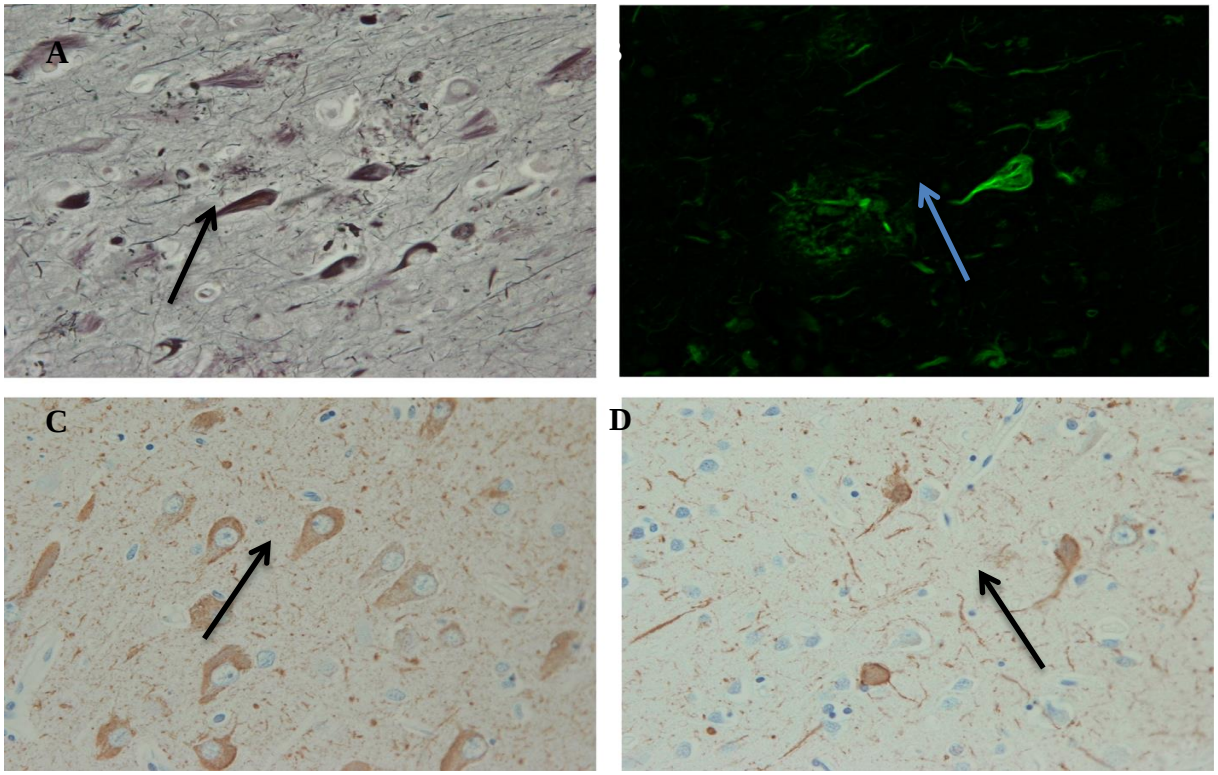

Figure (4): Histological section of human AD brain tissue showing the presence of Neurofibrillary Tangles by using Silverstaining (A), Thioflavin S (B), and tau immunohistochemistry (C, D). (DeTure & Dickson, 2019).

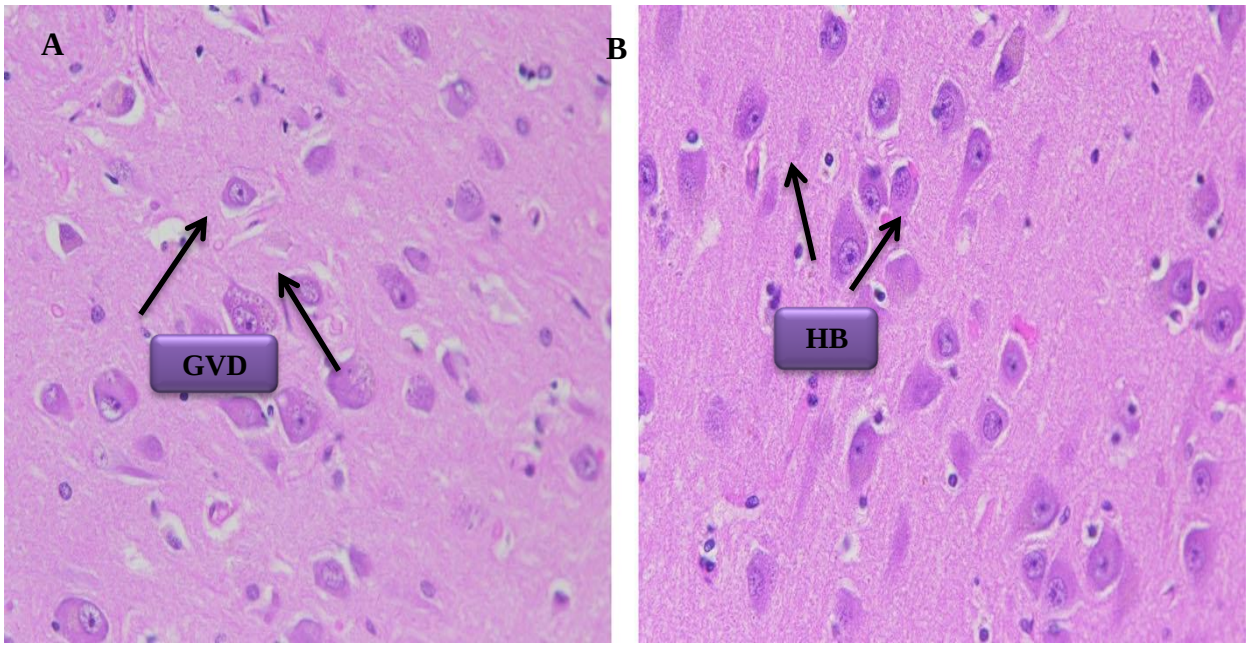

Figure (5): Histological section of human AD brain tissue showing the presence of Granulovacuolar Degeneration (acid phosphatase histochemistry) (A) and Hirano Bodies (H&E stain) (B) (DeTure & Dickson, 2019).

Behavioral tests and biochemical markers along the total period of the study for normal control groups: The current study revealed that there were no statistically significant differences in behavioral tests (Barnes Maze Test, Novel Object Recognition Test, and Y-Maze Test) or biochemical markers (Amyloid *β*, BDNF, MDA, and TAS) in the normal control groups throughout the entire duration of study (6 months) (Tables 1& 2, respectively).

Table (1): Behavioral tests for normal groups at zero time, after 3 months, and after 6 months of treatment (Y-maze Test, Novel Object Recognition Test, and Barnes Maze Test)

Behavioral Test			Mean ± SD			*P* value
			Nor. 0T (n=10)	Nor. 3M (n=10)	Nor. 6M (n=10)	
Y- Maze Test			79.346±3.774	77.554±4.816	77.384±2.215	0.681
NOR Test			80.70±4.446	76.506± 2.073	78.153±6.057	0.341
Barnes Maze test	Acquisition training	Primary latency(s)	45.7±6.056	41.5±4.428	42±5.657	0.196
		Primary error	16±3.367	17±4.522	20±5.888	0.210
		Primary hole distance	8.2±2.699	9.3±2.750	8.875±1.458	0.603
	Probe trial	Primary latency(s)	15.8±3.564	16.6±2.881	16.2±3.899	0.936
		Primary error	6±1.581	5.8±1.924	4.4±1.342	0.278
		Primary hole distance	4.4±1.140	6.2±1.789	8±2.916	0.055
	Reversal learning	Primary latency(s)	50.555±7.859	54.142±11.889	51.4±12.176	0.715
		Primary error	13.111±3.919	10.8±2.932	12±2.748	0.231
		Primary hole distance	10.555±2.455	11.062±2.909	10.2±2.529	0.722

Nor. 0 T=normal group at zero time, Nor. 3M = normal group after 3 months of treatment, Nor. 6M = normal group after 6 months of treatment, s = second, SD = Standard deviation, NOR = Novel Object Recognition Test.

Tables (2): Mean levels of biochemical markers for normal control groups at zero time, normal after 3 months, and normal after 6 months of treatment

Marker	Mean ± SD			*P*- value
	Nor. 0 T (n=6)	Nor. 3 M (n=6)	Nor. 6 M (n=6)	
BDNF (ng/mL)	3.062 ± 0.966	4.092 ± 1.134	4.026 ± 1.125	0.354
Amyloid β (ug/L)	9.930 ± 0.567	12.207 ± 1.683	11.694 ± 1.846	0.076
TAS (pg/mL)	35.192± 3.672	37.378 ± 1.02	41.887 ± 10.994	0.096
MDA (nmol/mL)	0.621 ± 0.018	0.613 ± 0.05	0.594 ± 0.117	0.845

Nor. 0T=normal group at zero time, Nor. 3M = normal group after 3 months of treatment, Nor. 6M = normal group after 6 months of treatment, BDNF= Brain derived neurotrophic factor, TAS = Total antioxidant status, MDA = Malondialdehyde, SD = Standard deviation.

Behavioral tests and biochemical markers for AD induction groups over the total period of the study: Current study revealed that there were no statistically significant differences in behavioral tests (Barnes Maze Test, Novel Object Recognition Test, and Y-Maze Test) or biochemical markers (Amyloid β, BDNF, MDA, and TAS) during the total period of the study for the AD-induction groups (using SCM) after 2 weeks, 3 months, and 6 months (Tables3 and 4, respectively).

Table (3): Behavioral tests for AD induction groups after 2 weeks, 3 months, and 6 months of treatment

Behavioral Test			Mean ± SD			*P*- value
			Induction (SCM 2W) (n=10)	Induction (SCM 3M) (n=10)	Induction (SCM 6M) (n=10)	
Y- Maze Test			46.44±5.185	48.424±3.704	51.204±2.224	0.751
NOR Test			45.744±2.196	47.268±1.846	46.31±2.957	0.605
Barnes Maze test	Acquisition training	Primary latency (s)	158.667±26.585	157.25±21.57	148.111±29.118	0.598
		Primary error	37.833±9.637	38±8.569	36.555±5.659	0.912
		Primary hole distance	17.166±1.642	17.733±1.869	18±2.397	0.597
	Probe trial	Primary latency (s)	58.5±1.914	57.5±3.507	57±5.196	0.846
		Primary error	19±3	22.833±3.544	20.2±2.280	0.137
		Primary hole distance	16.2±2.863	17.667±2.250	15.6±2.701	0.416
	Reversal learning	Primary latency (s)	143.625±17.508	146.8±25.952	165.75±16.568	0.092
		Primary error	34±7.406	31±6.96	30.75±7.648	0.611
		Primary hole distance	16.5±3.070	17.4±2.633	17.75±2.187	0.627

SCM 2W = after 2 weeks of induction by scopolamine, SCM 3M= after 3 months of treatment in the scopolamine induction group, SCM 6M= after 6 months of treatment in the scopolamine induction group, s = second, SD = Standard deviation, NOR = Novel Object Recognition Test.

Table (4): A comparison of the mean levels of biochemical markers in the induction groups throughout the study

Marker	Mean±SD			*P* value
	Induction (SCM 2 W) (n=6)	Induction (SCM 3M) (n=6)	Induction (SCM 6 M) (n=6)	
BDNF (ng/mL)	0.686 ± 0.137	0.624 ± 0.213	0.643 ± 0.161	0.832
Amyloid *β* (ug/L)	57.227± 8.553	61.827± 10.059	49.122± 11.292	0.062
TAS (pg/mL)	10.123 ± 2.949	11.336 ± 1.042	9.450 ± 3.092	0.438
MDA (nmol/mL)	2.764 ± 0.344	2.560 ± 0.377	3.012 ± 0.763	0.354

SCM 2W = after 2 weeks of induction with scopolamine, SCM 3M= after 3 months of treatment in the scopolamine induction group, SCM 6M= after 6 months of treatment in the scopolamine induction group, BDNF= Brain-derived neurotrophic factor, TAS = Total antioxidant status, MDA = Malondialdehyde, SD = Standard deviation.

Comparison of behavioral tests between the AD-induction group after 2 weeks and the normal control group at zero time

Table (5): Comparison between the AD induction group and normal control group at zero time for behavioral (Y-maze, Novel Object Recognition, and Barnes Maze) tests

Behavioral Test			Mean±SD		*P*-value
			Nor. 0T (n=10)	Induction (SCM 2W) (n=10)	
Y maze test			79.346±3.773	46.44±5.185	<0.001
NOR Test			80.70±4.446	45.744±2.196	<0.001
Barnes Maze test	Acquisition training	Primary latency (s)	45.7±6.056	158.666±26.585	<0.001
		Primary error	16±3.366	37.833±9.637	<0.001
		Primary hole distance	8.2±2.699	17.166±1.642	<0.001
	Probe trial	Primary latency (s)	15.8±3.563	58.5±1.914	<0.001
		Primary error	6±1.581	19±3	<0.001
		Primary hole distance	4.4±1.14	16.2±2.863	<0.001
	Reversal learning	Primary latency (s)	50.555±7.859	143.625±17.508	<0.001
		Primary error	13.111±3.919	34±7.406	<0.001
		Primary hole distance	10.555±2.455	16.5±3.07	<0.001

SCM 2W = after 2 weeks of induction by scopolamine, Nor. 0T=normal group at zero time, s = second, SD = Standard deviation, NOR = Novel Object Recognition Test.

Comparison of Biochemical markers between the AD induction group after 2 weeks and the Normal control group at zero time: The current study indicated that there was a highly significant elevation in the mean level of amyloid *β* and MDA, while there was a significant reduction in the mean level of BDNF and TAS in the AD induction group after 2 weeks in comparison with the normal control group at zero time (Table 6).

Table (6): Comparison of mean levels of biochemical markers between AD induction groups and normal group at zero time

Marker	Mean±SD		*P*-value
	Nor. 0 T (n=6)	Induction (SCM 2W) (n=6)	
BDNF(ng/mL)	3.062 ± 0.966	0.686 ± 0.137	0.006
Amyloid *β* (ug/L)	9.930 ± 0.567	57.227± 8.553	<0.001
TAS (pg/mL)	35.192± 3.672	10.123 ± 2.949	<0.001
MDA (nmol/ml)	0.621 ± 0.018	2.764 ± 0.344	0.003

SCM 2W = after 2 weeks of induction by scopolamine, Nor. 0T=normal group at zero time, SD = Standard deviation, BDNF= Brain derived neurotrophic factor, TAS = Total antioxidant status, MDA = Malondialdehyde.

Discussion

Validity of the animal model of AD created in the current study: In the current study, SCM caused accumulation of amyloid (A*β) plaques*, NFT, GVD, and HB by histopathological examination, which is similar to AD of human brain tissue (11).

The similarities between human and mouse AD brain tissues obtained in the current study support the validity of the animal model created in the current study and, therefore, suggest that its results can be applied to humans with AD (12). Behavioral tests and biochemical markers for normal control groups: The age of the young mice enrolled in the current study at zero time was (1-2 months), while after 3 months of therapy was (4-5 months), and after 6 months of therapy was (7-8 months). In this study, the cognitive and memory functions of normal control mice were preserved over the total period of the study by measuring behavioral tests including the Barnes maze test, Novel Object Recognition (NOR) Test, and Y-maze test. Thus, these data excluded any effect of aging on the result of the current study (13). These results were in agreement with the results of some previous studies that assessed age-related memory and cognitive function in multiple age groups of normal mice (14-17). Aging is associated with cognitive decline and may be linked to minimal neuronal loss (13). However, mature CNS neurons at a young age are very resistant to cell death. Therefore, neuronal cell death is limited to the adult brain (18).

Additionally, the present study observed that there were no differences in the level of A*β* in the brains of normal mice over the total period of the study and these results agreed with those from previous studies

that measured the level of Aβ in the brains of normal mice (19, 20). Furthermore, for different ages of mice (young, middle-aged, and elderly), the level of the β -secretase (BACE1) enzyme that is necessary for the synthesis of Aβ is consistent (19). Consequently, the Aβ level during the young age is consistent. The present study found that there are no differences in the levels of BDNF in young normal mice of different ages throughout the study. These findings agreed and contrasted those of some previous studies (21-24). The BDNF gene plays a crucial role in neuronal generation, function, and memory. In addition, neuronal BDNF mRNA expression of the hippocampus and cortex is unaffected by aging in normal mice, hence the level of BDNF remains consistent over time (25). Regarding oxidative stress level, the current study has shown that there have been no statistically significant changes in the concentration of MDA and TAS among young normal mice of varied ages. One of the earliest studies has compared the activity of antioxidant enzymes and MDA levels among normal mice of different ages and discovered a contradicter result in which GPx and copper-zinc superoxide dismutase CuZn/SOD activities are higher in 18 and 28 months old mice than younger, 2 month old, mice, while manganese superoxide dismutase (MnSOD), GRD activities, and MDA level do not change with aging (26).

Oxidative damage is considered one of the predominant mechanisms of cellular and tissue damage in aging (27). The oxidative damage during aging occurs due to neuroinflammation, and increased expression of pro-inflammatory factors (28). In the healthy brain of young mice, microglia are "resting" and dormant, which eliminates the impact of neuroinflammation on young mice's normal brain function (29). Thus, OS in young mice is kept at a low level.

Biochemical markers and behavioral tests for AD-induction group over the total period of the study: The current study revealed that the effects of SCM on cognitive and memory function as well as biochemical markers (Amyloid β, BDNF, MDA, and TAS) were consistent throughout the study (6 months). These results suggested that SCM has a prolonged effect, which will strengthen the impact of the study's medications and these findings have not been reported in other previous studies because all other studies focused on the short-term effects of SCM.

Scopolamine causes cholinergic neuronal damage in the hippocampus by enhancing DNA damage and inhibiting the mRNA expression of many genes encoding neuronal factors that are crucial for cell survival as well as increasing oxidative stress by enhancing lipid peroxidation and decreasing the antioxidant system capacity (30). Additionally, SCM increases Aβ deposition which further enhances oxidative stress (31). Furthermore, SCM interferes with the expression of neurofilaments, which are essential for axonal transport in neurons (32). The loss of cholinergic function in the hippocampus is associated with serious cognitive impairments (32) which may last for a long time.

On the other hand, results obtained from this study found that SCM interfered with, and subsequently caused impairment of, learning and short-term as well as long-term memories as evidenced by conducting Barnes-maze, NOR, and Y-maze tests.

It has been demonstrated that SCM can impair memory and cognitive functions in mice by administering single or multiple injections at various concentrations through assessing many behavioral tests (33-36).

Aykac et al. (2018) found that administering SCM (1mg/kg) IP for 14 days dramatically raised MDA levels, lowered GSH levels, decreased BDNF expression, and lowered short- and long-term memory (36). In addition, Anand et al. (2022) concluded that SCM single injection (2mg/kg) increased oxidative stress in the hippocampus by increasing thiobarbituric acid-reactive substances (TBARS), which reflect lipid peroxidation and decreased GSH as well as CAT levels (37). Also, a recent study conducted by Cheedella *e*t al. (2023) found that SCM (5mg/kg) for 7 days reduced CAT activity and H&E-stained histological sections of the brain showed severe blood capillary congestion with perivascular edema (scars), along with edema and deposition of amyloid plaques in the hippocampus when compared with normal mice (31).

However, a study done by Ban et al. (2020) found conflicting results regarding the impact of SCM on oxidative stress such as that CAT activity was unaffected after SCM injection in mice compared to normal mice (34). Additionally, Lee et al. (2010) found that SCM significantly increased SOD and GSH-Px antioxidant enzyme activities (38).

Despite that, it is well-known that the non-selective muscarinic acetylcholine receptor antagonist scopolamine (SCM) prevents cholinergic signals from traveling through the brain. This SCM-induced dysregulation of cholinergic activity and increased activity of acetylcholinesterase in the hippocampus interferes with mouse learning and memory functions (3, 32). One of the mechanisms causing scopolamine-induced amnesia is oxidative stress through increasing the levels of malondialdehyde, a marker of lipid peroxidation, and lowers the activity of many antioxidant enzymes (4). Additionally, there is a relationship between Aβ and oxidative stress, because prooxidants elevate Aβ formation and Aβ generates oxidative stress (39). Moreover, the degree of synapse loss and cognitive deficits do correlate well with the amounts of soluble Aβ in the brain (19).

Conclusion

In light of results reported by the current study, scopolamine produces histopathological changes in mice brain tissue similar to those reported in human AD brain tissue. In addition, the effects of scopolamine on memory and cognitive functions as well as on pathological hallmark of AD persist for a long period (about 6 months).

Authors' Declarations

Mohammed AH AL- Zobaidy is an Editorial board member but did not participate in the peer review process other than as an author.

We hereby confirm that all the Figures and Tables in the manuscript are ours. The project was approved by the local ethical committee at the Department of Pharmacology/ College of Medicine, University of Baghdad, Baghdad, Iraq, with reference number (PHARMACOMED) in U.vB 23.13 (Appendix I).

Conflict of interest: None

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Authors' contribution

Study conception & design Mohammed AH JAbarah AL- Zobaidy. Literature search & Manuscript preparation are done by Neven Nihal Hana Istifo. Data acquisition, Data analysis & interpretation are done by Neven Nihal Hana Istifo and Kasim Sakran Abass. Manuscript editing & review are done by Mohammed AH JAbarah AL- Zobaidy.

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الآثار الطويلة المدى للسكوبولامين على أنسجة المخ لدى الفئران

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الخلاصة

خلفية البحث: السكوبولامين هو دواء مضاد للكولين يعطل انتقال الكوليني في الجهاز العصبي المركزي كما أنه يسبب تشوهات إدراكية وعلامات مرضية مشابهة لتلك التي تظهر في مرض الزهايمر. ولذلك، يتم استخدامه لتحريض مرض الزهايمر في النماذج الحيوانية.

الأهداف: كان الهدف من الدراسة الحالية هو دراسة آثار التحريض طويل المدى مع السكوبولامين على أنسجة المخ لدى الفئران.

طرق العمل: تم تقسيم سبعين فأراً بالغاً إلى مجموعتين متساويتين: المجموعة الأولى كانت مجموعة طبيعية ومراقبة تلقت الماء المقطر فقط. أما المجموعة الثانية فكانت مجموعة تحريض مرض الزهايمر حيث تلقت السكوبولامين داخل الصفاق (1 ملجم / كجم) لمدة 14 يومًا فقط بعد ذلك تم إعطاء الماء المقطر لمدة 6 أشهر التالية. تم عزل عشرة فئران من كل مجموعة في وقت الصفر، بعد أسبوعين من التحريض، وبعد 3 أشهر وبعد 6 أشهر، وتم إخضاعها للاختبارات السلوكية ثم تم تشريحها لتحديد العوامل البيوكيميائية (بما في ذلك عامل التغذية العصبية المشتق من الدماغ، وحالة مضادات الأكسدة الكلية، والمالونديالدهيد). والأميلويد β). وقد تم تحليل البيانات باستخدام اختبارات t، وANOVA. مع اعتبار جميع القيم المعبر عنها كقيمة متوسط ± SD وقيمة $P < 0.05$ ذات دلالة احصائية.

النتائج: أنتج السكوبولامين تغيرات نسيجية مرضية في الدماغ مشابهة لتلك التي تحدث في مرض الزهايمر البشري. ومع ذلك، لم تنتج فروق ذات دلالة إحصائية أخرى في الاختبار السلوكي، والعلامات البيوكيميائية، خلال الفترة الإجمالية للدراسة.

الاستنتاجات: يُحدث السكوبولامين تغيرات في أنسجة المخ والتي تستمر لفترة طويلة ويمكن استخدامه لدراسة مرض الزهايمر على المدى الطويل.

الكلمات المفتاحية: الاجهاد التاكسدي؛ الوظيفة المعرفية؛ سكوبولامين؛ مرض الزهايمر؛ مضادات الأكسدة.

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Research Article

Exogenous Insulin Effect on the Initiation of Breast Cancer in Type 2 Diabetes Mellitus Patients

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Abstract

Background: Growing evidence has indicated that women with type 2 diabetes mellitus are at an increased risk of developing breast cancer. However, as a major adjuvant treatment, the influence of hormone therapy such as exogenous insulin on type 2 diabetes mellitus in primary breast-cancer remains controversial.

Objective: To explore the relationship between different types of hypoglycemic medications for diabetes and the factors that may contribute to the initiation of breast cancer.

Methods: The study included 80 blood samples taken from 80 females recruited from Ibn Al-Bitar Center for Cardiac Surgery Hospital in Baghdad, Iraq, between November 2022 and February 2023. They were within an age range of 40 to 70 years. They were divided into three groups according to the treatment strategy and duration of disease as follows: Group 1: (20) patients who take insulin only, Group 2: (20) patients who take metformin with insulin, and G 3: (40) patients on metformin only for less than one year. An enzymatic oxidation technique was used to test the following biochemical parameters for all research groups: Total cholesterol, triglycerides, fasting blood glucose, and high-density lipoprotein cholesterol. Using the Friedewald formula, the very low-density lipoprotein cholesterol and low-density lipoprotein cholesterol were determined. Using an enzymatic process, Insulin levels and Insulin like growth factor-1(IGF-1), Estrogen receptor alpha (ERα) levels, and breast cancer susceptibility protein 1 (BRCA1) were measured with an enzyme-linked immunosorbent assay (ELISA). Glycated hemoglobin (HbA1c) level was measured with a sandwich immunodetection method. Finally, The Homeostasis Model Assessment for insulin resistance (HOMA-IR) was calculated according to the specific formula.

Results: The values for HbA1C%, FBS, and lipid profile showed non-significant differences when compared between study groups. While BRCA-1, estrogen receptor alpha (ERα), insulin, insulin growth factor IGF-1, and HOMA IR All showed significant differences among all groups.

Conclusion: Taking metformin only for less than one year seemed to contribute to higher levels of BRCA-1.

Keywords: BRCA1 protein; Breast cancer; Estrogen receptor alpha; Insulin; Type 2 diabetes mellitus.

Introduction

Diabetes mellitus refers to a multi-etiological metabolic condition characterized by chronic hyperglycemia, lipid and protein metabolism disorders arising from insulin secretion, insulin action, or both. Diabetic patients may suffer from several symptoms, such as excessive thirst, frequent urination, blurry vision, and decreased weight (1). Diabetes can develop to a high level of sugar keto acids, which may be associated with complications and disorders of the large blood vessels that cause several diseases, including stroke, and other disorders (2). Understanding the classical insulin signaling system is crucial to comprehend the pathophysiology and pathophysiological events of type 2 diabetes. The primary hormone for controlling blood sugar is insulin, and in most cases, a healthy balance between insulin secretion and action is what keeps blood sugar levels normal (3). Breast cancer is the most common kind of cancer Worldwide, and the main reason for cancer-related death. Since breast cancer is a complex disease, the exact mechanism by which it starts is unknown. Nonetheless, the pathogenesis of breast cancer involves a complex interaction between hereditary, environmental, and lifestyle variables (4). Made up of two disulfide bridges connecting the α and β chains as a dimer and a third intrachain disulfide bridge in the α chain, insulin is an endocrine peptide hormone consisting of 51 amino acids. Pancreatic beta cells secrete insulin, which is necessary for maintaining healthy levels of lipids and glucose. Along with helping to maintain glucose homeostasis, insulin also supports some other cellular functions, such as the regulation of glycogen production, lipid metabolism, DNA synthesis, gene transcription, amino acid transport, protein synthesis, and degradation (5). Insulin-like growth factor-1(IGF-1) is a polypeptide

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of 70 residues that is divided into A through D domains. The A-C domains are present in proinsulin, but the A and B Domains are the only ones present in mature insulin. Growth hormone controls the majority of the liver's secretion of circulating IGF-1, but various organs can express IGF-1 in an autocrine/paracrine way (6). Insulin-like growth factors are proteins that serve a variety of purposes, including regulating cell differentiation and transformation as well as promoting cell motility, inhibiting apoptosis, and stimulating cell proliferation. Several studies have revealed that malignancies, such as prostate, pre- and postmenopausal breast, lung, thyroid, and colorectal cancers, are linked to elevated levels of IGF-1 (7). One of the main features of type 2 diabetes and obesity is insulin intolerance. Over the past ten years, endoplasmic reticulum (ER) stress has become a significant player in this field, as numerous recent studies have demonstrated its involvement in the initiation of insulin resistance (IR). In addition to acting indirectly by encouraging lipid accumulation, ER stress appears to operate directly as a negative modulator of the insulin signaling pathway (8). Thus the current study tried to explore the relationship between different types of hypoglycemic medications for diabetes and the factors that may contribute to the initiation of breast cancer.

Patients and Methods

The study sample

The participants in the current study were recruited from Ibn AL-Bitar Center for Cardiac Surgery Hospital, and the laboratory procedures were carried out in the College of Science for Women / University of Baghdad. The women included in the study were those with type 2 diabetes who did not have breast cancer. The study included (80) women with an age range of (40-70) years. They were divided into three groups according to the treatment strategy and duration of disease as follows: Group 1: (20) patients who take insulin only, Group 2: (20) patients who take metformin with insulin, and G 3: (40) patients on metformin only for less than one year. A sample of seven milliliters of blood sample was collected from each participant and divided into two tubes; an EDTA tube for HbA1c (Boditech I-chroma, Korea), and a gel tube for BRCA-1, estrogen receptor alpha, insulin, insulin growth factor (ELISA - My Sunlong, China), FBS and lipid profile (measured manually by using a Biosystem Spine kit). The tubes were left for 20-25 minutes at a room temperature of 25 ºC, and were then centrifuged at 2000-3000 rpm for 10 minutes. The separated serum was kept in deep freeze at- 20°C.

Exclusion criteria: Cases with Covid-19, pregnant women, patients with breast cancer, and smokers.

Statistical Analysis

The statistical analysis was done using version 26 of the SPSS program. The group description was presented as medians and mean ± SE. The ANOVA test used to study the differences between the means, in addition to linear regression to study the relationships between variables. The probability value used to determine statistical significance was ($p \leq 0.05$).

Results

Table 1 shows the mean and median age and duration of disease (years) for the participants in the three study groups

Table (1): Mean and median age and disease duration of the study groups

Parameters	Study Groups		
	(1) Insulin only No. (20)	(2) Metformin + insulin No. (20)	(3) Metformin only < 1 year No. (40)
Age (year)	64.4 ± 1.19 (66)	60.2 ± 1.90 (61)	52.3 ± 1.00 (53)
Disease duration (years)	10.00±0.001 (10)	10.00±0.001 (10)	1.6 ± 0.08 (2)

Table 2 shows that the differences in the mean values of FBS, HbA1c%, cholesterol, TG, HDL-C, LDL-C and VLDL-C for the three study groups were not significant. Groups 1 had the highest mean value for FBS and the lowest for cholesterol; group 2 had the highest mean value for cholesterol, TG, LDL and VLDL and the lowest mean value for HDL; and group 3 had the highest mean value for HDL and the lowest mean value for FBS, HbA1C, TG, LDL and VLDL

Table (2): Mean and median values for blood glucose and lipids in the study groups

Parameters	Study Groups			P-value
	(1) Insulin only No. (20)	(2) metformin + insulin No. (20)	(3) Metformin only < 1 year - No. (40)	
FBS -(mg/dL)	234.6±23.52 [a] (219)	222.0±21.24 [a] (195)	187.1±14.49 [a] (159)	0.302
HbA1c%	9.1±0.450 [a] (8.71)	9.1±0.396 [a] (8.70)	7.8±0.211 [a] (7.35)	0.153
Cholesterol (mg/dL)	178.5±11.48 [a] (185.50)	196.3±14.05 [a] (184.5)	180.4±9.43 [a] (180)	0.223
TG (mg/dL)	172.4±13.91 [a] (176)	179.3±14.63 [a] (161.5)	165.5±14.84 [a] (146)	0.984
HDL-C (mg/dL)	36.6±1.92 [a] (36.50)	36.4±1.99 [a] (36.51)	40.7±1.26 [a] (40)	0.129
LDL-C (mg/dL)	107.6±10.61 [a] (110.50)	123.7±13.23 [a] (110)	106.6±9.072 [a] (106)	0.134
VLDL-C (mg/dL)	34.4±2.76 [a] (35)	35.9±2.92 [a] (32.5)	33.1±2.97 [a] (29)	0.982

Table 3 shows highly significant differences among the three study groups in terms of the mean values of parameters relevant to breast cancer and insulin. Group 1 had the highest mean value for IGF-1 and HOMA IR and the lowest mean value for BRCA-1; group 2 had the highest mean value for Erα and insulin; and group 3 had the highest mean value for a

nd the lowest mean value for ‹BRCA-1 and the lowest mean value for Erα, IGF-1, insulin and HOMA IR.

Table (3): Mean and median values for breast cancer and insulin related variables in the study groups

Parameters	Study Groups			P-value
	(1) Insulin only No. (20)	(2) metformin + insulin No. (20)	(3) Metformin only < 1 year - No. (40)	
‹BRCA-1 (ng/ml)	3.1±0.76 [bc] (3.12)	3.3±0.19 [c] (3.01)	3.5±0.19 [c] (3.96)	0.0001*
Estrogen Receptor Alpha (ERα) (pg/ml)	446.6±18.62 [b] (437.7)	473.1±28.67 [b] (458.50)	404.7±15.15 [ab] (442.90)	0.0001*
IGF-1 (ng/ml)	7.5±0.36 [c] (7.34)	7.1±0.46 [bc] (6.49)	6.6±0.45 [bc] (7.13)	0.0001*
Insulin (mU/L)	2.0±0.06 [bc] (2.04)	2.3±0.16 [c] (2.18)	1.8±0.07 [ab] (1.90)	0.0001*
HOMA IR	1.2±0.12 [b] (0.98)	1.2±0.11 [b] (1.10)	0.8±0.07 [ab] (0.69)	0.005*

* *a, b, c are Significant variants that denoted by different small letters, and non-significant variations are denoted by identical small letters*

Discussion:

Type 2 diabetes mellitus is a metabolic disorder marked by persistently elevated blood sugar levels. Gender and age have an impact on diabetes management. Due to their many medical disorders involving the heart and kidneys, women and people over 60 years of age are more affected, which restricts and hinders medical treatment (9). Potent hormone insulin stimulates many signaling pathways, some of which are essential to the biology of cancer. Insulin can either directly or indirectly increase the risk of cancer by influencing the levels of other modulators, including hormones, adipokines, and the insulin-like growth factor-1 (IGF-1) (10).The higher level of BRCA-1 in the third group than other groups in the current study is in agreement with Samuel et al (11) who found that BRCA-1 protein levels increase in patients taking metformin for less than one year because metformin can activate the AMP-activated protein kinase (AMPK) pathway, which is a signaling pathway that regulates cell growth and metabolism. The AMPK pathway has been shown to increase BRCA-1 gene

expression. Metformin may also reduce BRCA-1 protein degradation. Metformin can inhibit the activity of the proteasome, which is a complex that degrades proteins. This could lead to an increase in BRCA-1 protein levels. The result agrees with Boucher et al., who noticed a slight increase in the concentration of BRCA-1 protein in the first group. From various studies, it has been found that insulin can increase the level of BRCA-1 protein (12). This effect may be happening through the insulin receptor signaling pathway. Insulin stimulates a series of signal molecules through binding with its receptor which ultimately enhances expression of BRCA-1 gene (12). Again, insulin and metformin might have complementary effects on levels of BRCA-1 protein. As such, the joint influence created by these two medications outweighs their separate accomplishments (13).

The current study agrees with other studies which have shown that high levels of ERα are found in patients taking insulin for more than a decade, in their breast and endometrial tissue. This might be due to the fact that cells grow after being stimulated by insulin and ERα serves as a growth factor receptor (14). Other studies have found that those patients who have higher levels of ERα in their breast and

endometrial tissue are at risk if they develop cancer. This is because estrogen can promote cancer cell growth. In addition, elevated expression of ERα among patients taking metformin and insulin may carry numerous clinical implications. For instance, since ERα is a target for breast cancer therapy, these hormone therapies can then be better suited for patients whose levels of such proteins go up. Moreover, there are various other physiological processes including cardiovascular health, cognition as well as bone metabolism where ERα plays a role (15). Thus, alterations in the levels of ERα can have more far-reaching effects on the overall wellbeing of individuals who do use metformin along with insulin.

Conclusion:

Taking metformin only for less than one year seemed to contribute to higher levels of BRCA-1. The ERα signaling by IR and IGF1 is amplified by elevated insulin and IGF-1 levels in females with T2DM. These observations and predictions imply that diabetes, and specifically T2DM, may contain mechanisms for the formation or production of cancer through the use of T2DM medications that affect ERα and IGF-1 levels.

Authors' Declaration: We confirm that all the Figures and Tables in the manuscript are ours. Besides, the Figures and images, which are not ours, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's Approval-Ethical Clearance: The project was approved by the local ethical committee in (College of Science, University of Baghdad) (On October 27, 2022).

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Authors' Contributions: Hanan H. Hassan & Perry H. SaifUllah: Study conception, Hanan H. Hassan & Perry H. SaifUllah Study design. Hanan H. Hassan Acquisition of data. Hanan H. Hassan: Analysis, Interpretation of data, Supervision, Validation, Visualization, Writing- reviewing and editing of manuscript. Hanan H. Hassan & Perry H. SaifUllah: Study conception, Investigation, Methodology, Acquisition of data analysis, Interpretation of data, Writing-drafting of manuscript.

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تأثير الأنسولين الخارجي على بدء سرطان الثدي في مرضى السكري من النوع 2

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الخلاصة
الخلفية: تشير الأدلة المتزايدة إلى أن النساء المصابات بداء السكري من النوع الثاني أكثر عرضة للإصابة بسرطان الثدي. ومع ذلك، كعلاج مساعد رئيسي، فإن تأثير العلاج الهرموني مثل الإنسولين الخارجي على داء السكري من النوع الثاني في سرطان الثدي الأولي لا يزال مثيرا للجدل.
الهدف: علاقة الأنواع المختلفة من أدوية السكري بالعوامل التي قد تسهم في بدء الإصابة بسرطان الثدي
المرضى والمنهجية: شملت الدراسة 80 عينة مأخوذة من 80 سيدة تراوحت أعمارهن بين 40 إلى 70 عاما. تم تقسيمهن إلى ثلاث مجموعات حسب استراتيجية العلاج ومدة المرض على النحو التالي: المجموعة الاولى: (20) مريضة يتناولن الأنسولين فقط، المجموعة الثانية: (20) مريضة يتناولن الميتفورمين مع الإنسولين، والمجموعة الثالثة: (40) مريضة يتناولن الميتفورمين فقط لمدة تقل عن عام، وتم جمعهن من مستشفى مركز إبن البيطار لجراحة القلب في بغداد، العراق، بين تشرين الأول 2022 وشباط 2023. تم قياس بعض المعلمات البيوكيميائية لجميع مجموعات الدراسة والتي تشمل: الجلوكوز الصائم والكوليسترول الكلي والدهون الثلاثية والكوليسترول الدهني عالي الكثافة بطريقة الأكسدة الأنزيمية. وباستخدام معادلة فرايدإيوالد، تم تحديد كوليسترول البروتين الدهني منخفض الكثافة وكوليسترول البروتين الدهني منخفض الكثافة. باستخدام عملية إنزيمية، تم قياس مستويات الإنسولين وعامل النمو الشبيه بالإنسولين (IGF-1)، ومستويات مستقبلات هرمون الأستروجين ألفا (ERα)، وبروتين قابلية الإصابة بسرطان الثدي (BRCA-1) باستخدام مقايسة الإمتصاص المناعي المرتبط بالإنزيم (ELISA). تم قياس مستوى الهيموجلوبين السكري (HbA1c) باستخدام طريقة الكشف المناعي للساندويتش. وأخيرا، تم حساب تقييم نموذج التوازن لمقاومة الإنسولين (HOMA-IR) وفقا للصيغة المحددة
النتائج: أظهرت نتائج المجموعات المتعلقة بـ HbA1C%، FBS، وملف الدهون إختلافات غير معنوية عند مقارنتها بين مجموعات الدراسة. في حين أظهرت كل من، BRCA-1، ومستقبلات هرمون الإستروجين ألفا (ERα)، والإنسولين، وعامل النمو الشبيه بالإنسولين IGF-1، ونموذج التوازن لمقاومة الإنسولين HOMA IR ، أهمية بين جميع المجموعات
الإستنتاج: يبدو أن تناول الميتفورمين لمدة تقل عن عام واحد فقط يساهم في ارتفاع مستويات بروتين قابلية الإصابة بسرطان الثدي BRCA-1.
الكلمات المفتاحية: بروتين BRCA-1، سرطان الثدي، مستقبلات هرمون الإستروجين ألفا، الإنسولين، داء السكري من النوع الثاني

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Research Article

Isolation and Identification of Phenolic Compounds in Guava leaves and Assessment of their Cytotoxic Effects against AMJ-13 and MCF-7 Breast Cancer Cell Lines

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Abstract:

Background: *Psidium guajava*, commonly known as guava, is a tropical tree prized for its nutritious fruit and medicinal properties. This member of the Myrtacease family is rich in phytochemicals, which are natural compounds with potential health benefits. Studies have shown that guava leaves and fruits possess various pharmacological activities, including anti-cancer properties against several cancer cell lines.

Objectives: This study aimed to isolate and identify phenolic compounds in guava leaves and assess their cytotoxic activity against AMJ-13 and MCF-7 cell lines by ethyl acetate fraction of guava leaves grown in Iraq.

Methods: The researchers will employ High-performance liquid chromatography and Fourier transform infrared techniques to analyze the guava leaf extract. Subsequently, preparative High-performance liquid chromatography was used to isolate the specific phenolic compounds of interest, and a colorimetric MTT reduction assay, was conducted using guava extract to assess their effects on human cancer cell lines. Analysis using GraphPad Prism software version 6.

Results: The researchers successfully isolated pure samples of caffeic acid, luteolin, and Gallic acid, which are all flavonoids, from the guava leaf extract using preparative High-performance liquid chromatography. These isolated phenolic compounds were from ethyl acetate fraction (F3). This enriched fraction was tested for its cytotoxic activity against the Iraqi AMJ-13 and human MCF7 breast cancer cell lines. The results showed a decrease in cell viability, indicating the fraction's potential anti-cancer properties. The fraction was more effective against the Iraqi AMJ-13 cells with an IC50 value of 414.3 µg /ml, compared to the human MCF7 cells with an IC50 value of 698.3 µg /ml.

Conclusion: The analytical techniques used in this study, like HPLC and FTIR, provided a detailed profile of the phenolic compounds present in guava leaves. This information, combined with the cytotoxic tests, suggests that guava leaves have the potential to kill cancer cells in a concentration-dependent manner.

Keywords: Caffeic acid; Gallic acid; Guava; Luteolin; Ultrasonic-assisted extraction.

Introduction:

Worldwide, the use of traditional medicines has a long history and encompasses an easily accessible and affordable source of treatment (1-3). Many people who live in developed countries depend on traditional medicine (4-6). *Psidium guajava* grows in tropical and subtropical areas of the world and contains several bioactive compounds. It belongs to the family Myrtaceae (7,8). All parts, including the fruits, leaves, and barks have been traditionally used as folkloric herbal medicines and appear in many medicinal uses(9,10). Many previous studies reported many phytochemical constituents in guava leaves that have many pharmacological activities and medicinal properties Various pharmacological reports have verified the capacity of this plant to reveal hepatoprotection, antigenotoxic, anti-bacterial, cytotoxic activity against cancer cell lines, anticough, anti-diabetic and anti-inflammatory events(11-14). Guava has a high content of antioxidant compounds, guava leaves have a higher content of gallic acid, ellagic derivatives quercetin, resveratrol, daidzin, guaijaverin, avicularine, hyperin, morin, chlorogenic acid, rutin, vanillic acid, p-hydroxyl benzoic acid, syringic acid, myricetin, naringenin, and apigenin(15,16). Many previous studies approved the *in vitro* and *in vivo* anti-cancer activities of different fractions and extracts from all guava parts and especially leaves against human breast cancer cell line (17-19). This study focuses on the isolation of phenolic compounds, from *Psidium guajava* (guava) leaves newly cultivated in Iraq and the assessment of the cytotoxic activity of guava leaf ethyl acetate fraction against Iraqi breast cancer cell line and human breast cancer cell line.

Thus, this study was conducted to evaluate the cytotoxic effect of the phenolic compounds of guava leaves on AMJ-13 and MCF-7 cell lines.

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Material and method

preparation of plant material **:** In spring 2023, *Psidium guajava* leaves were collected from Musayyib city and first grown in Iraq. After the

collection, the plant specimens underwent identification and authentication conducted by expert Dr. Sukaena Abass from the College of Sciences. The plant components were thoroughly dried in the shade, finely ground, and stored to use for further extraction and analysis (20). Extraction of guava leaves: Approximately 50 grams of finely ground *Psidium guajava* leaves underwent extraction using an ultrasonic bath sonicator, capitalizing on the cavitation principle to enhance the release of plant compounds. The ultrasonic waves, operating at 40 KHz, dissolved 50 grams of leaf powder in 750 ml of 70% ethanol at 30°C for 40 minutes (21). The crude ethanol extract underwent filtration and concentration under condensed pressure using a rotary evaporator. The dried extract obtained was liquified in 500 ml of distilled water and underwent successive partitioning with 500 ml aliquots of petroleum ether, ethyl acetate, chloroform, and n-butanol. This partitioning process was repeated three times for each solvent in a separatory funnel. All fractions, except n-butanol, underwent drying over anhydrous sodium sulfate, followed by filtration and subsequent evaporation with a rotary evaporator until complete dryness (22).

Preliminary phytochemical assessment: Initial chemical examinations were carried out to decide the incidence or lack of polyphenolic compounds in Iraqi *Psidium guajava* achieved for extracting leaves ethyl acetate fractions (F3) and butanol fraction (F4). On examination done by Alkaline reagent tests, a few amounts of extracts plus drops of sodium hydroxide, and a yellow color appeared. Still, it appeared colorless by adding drops of diluted HCL (23).

Analysis of Guava Fractions by high-performance liquid chromatography and isolation of phenolic compounds by Preparative high-performance liquid chromatography: A qualitative check through HPLC for the (F3) and (F4) fractions of leaves, comparing them to caffeic acid, luteolin, and Gallic acid standards, Sample preparation involved dissolving (F3) and (F4) fractions in methanol. This solution underwent filtration using a filter membrane (0.4 µm) before inoculation into the HPLC column. Matching between samples and standards was achieved based on observed retention times (24). The conditions for the analysis of the (F3) and (F4) fractions by HPLC were conducted using a stationary phase on Knuaer, Germany C18 column (5 µm particle size, 250 x 4.6 mm) with a Dionex Ultimate 3000 liquid chromatograph. The mobile phase consisted of a 1% aqueous acetic acid solution and acetonitrile utilizing gradient elution. The rate of flow was maintained at 0.7 ml/min. The column was thermally regulated at 28°C, and an inoculation volume of 20 µl was employed. The gradient elution proceeded linearly, transitioning from 10% to 40% of the component (25,26). Phytochemical ingredients in plant extracts are often present in small quantities, necessitating a sensitive instrument for recognition and isolation. In this study, Preparative high-performance liquid chromatography was employed for the isolation of phenolic compounds, Preparative high-performance liquid chromatography apparatus specification, and conditions as in previous studies (27,28).

Examination by Fourier transform infrared (FT-IR),

FT-IR spectra of isolated compounds were documented in the FTIR spectrometer (Shimadzu)in the range of wave number 500 to 4000 cm–1 (29).

Cell Line Maintenance

The method of work was conducted for cell line maintenance according to Freshney RI (30).

MTT assay

The F3 fraction cytotoxicity effect on Iraqi breast cancer cell line AMJ-13 and human breast cancer cell line MCF-7 was determined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay based on the detection of mitochondrial dehydrogenase activity in living cells, first day of poring the cells in a 96-well microplate by counting cells using trypan blue, about 1 x 104 cells were cultured in each well. After the cells were cultivated, the 96-well plate was sited in an incubator at 37 ℃ for 24 hours until 60% of the well surface was filled, otherwise, more time was needed (31).

On the second day, treatment of AMJ-13and MCF-7cell lines with different concentrations (31.2, 62.5, 125, 250, 500, 1000µg/ml), (25, 50, 100, 250, 500, 1000 µg/ml), of guava leaves F3 for 72 h after emptying the supernatant of each well by the sampler, 100 µl of every dilution was added to the wells. The pouring pattern was drawn and eight wells were considered for each dilution. On the third day adding MTT dye 24 hours later, the medium was removed and MTT solution 100 µl (0.5 mg/ml) was added to the plates in the shady and put in the incubator for 4 hours (32). Then, the top medium of the wells was detached with a sampler, and DMSO 100 µl was added to the wells, and placed on a shaker for 20 minutes (at this stage, the container should be hidden so as not to be exposed to light). Finally, the intensity of the resulting color was read by a (DNM-9602G) microplate reader at 570 nm (33).

Statistical analysis

Data were analyzed using GraphPad Prism version 6 and presented as mean ± SD of triplicate measurements. In addition, data were compared using an unpaired *t*-test, and the significance levels were considered at $P < 0.0001$.

Results

Extraction by Ultrasonic bath sonicator

The selected method for leaf extraction was based on ultrasound-assisted extraction (UAE), resulting in a higher percentage yield of approximately 20 %w/w from 50g of guava leaves according to the following formula (34,35): **percentage yield =weight of crude (g)/weight of plant material (g) x100**. This method relies on the principle of cavitation, which enhances the release of compounds from the plant material. The conditions for UAE included ultrasonic waves at 40 KHz, utilizing 50g of leaf powder in 750 ml of 70% ethanol at 30°C for 40 minutes. Subsequently, the crude ethanol extract underwent filtration and

concentration under reduced pressure using a rotary evaporator.

Preliminary phytochemical investigation: Chemical tests were done on the guava leaves and showed the results were positive indicating the presence of phenolic compounds in F3 and F4 fractions.

Analysis of Guava Fractions by High-performance liquid chromatography

Analysis of the F3 and F4 fractions was conducted for leaves using HPLC, as depicted in figure 1.

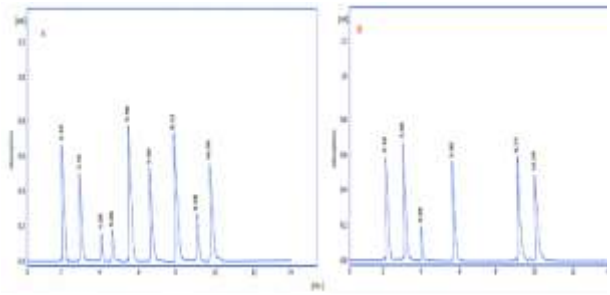


Figure (1): HPLC of guava leaves F3 fraction. HPLC of guava leaves F4 fraction

HPLC examination remarkably close retention time value of caffeic acid standard, luteolin standard, and gallic acid standard matched to the isolated A3, A8, and A2 compounds isolated by PHPLC as depicted in figures 2- 7.

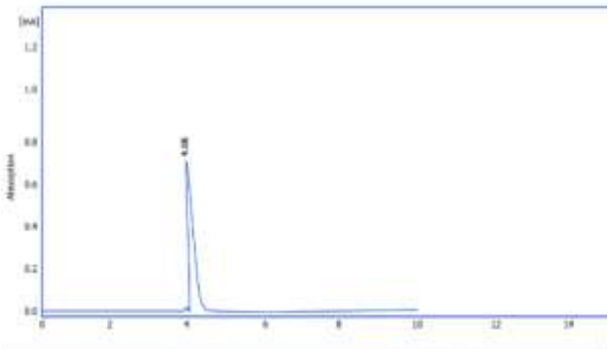


Figure (2): PHPLC chromatogram of isolated A3 compound

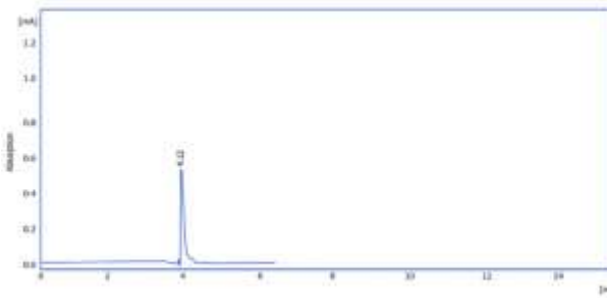


Figure (3): HPLC chromatogram of caffeic acid standard

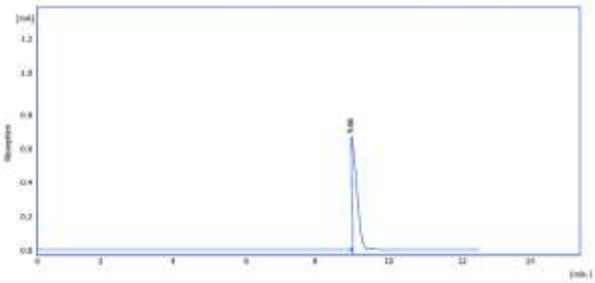


Figure (4): PHPLC chromatogram of isolated A8 compound

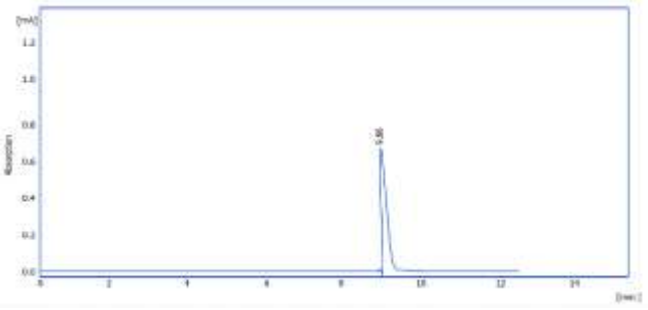


Figure (5): HPLC chromatogram of luteolin standard

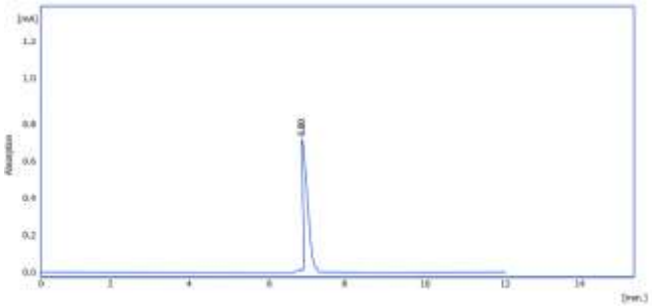


Figure (6): PHPLC chromatogram of isolated A2 compound

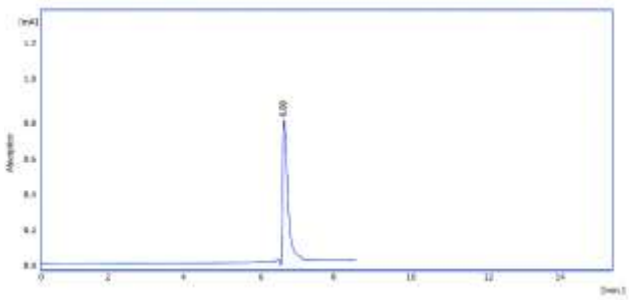


Figure (7): HPLC chromatogram of Gallic acid standard

Analysis by FT-IR

Fourier transform infrared spectroscopy analysis of Compound A3 as in Figure 8, A8 as in Figure 9, and IR spectra of Compound A2 in Figure 10. IR spectroscopy analysis of Compound A3 revealed the presence of 3346.5 bands referring to phenolic O-H stretching band,2972 referring to C-H stretching of the benzene ring,2887 referring to asymmetric and symmetric stretching of CH2,1649 referring to aromatic C=O stretching vibration band,1454 refer to O-H bending of carboxylic acid,881 refer to C-H bending fingerprint of aromatic(out of plane) so IR

spectra of A3 matching with IR spectra of caffeic acid standard [36]. In addition, A8 IR spectra revealed the presence of the 3381 band refers to O-H stretching of phenol, the 2904 band refers to C-H stretching of phenol, the 1651 band refers to C=C stretching vibration,1166 refers to C-O-C stretching, 1029 refers to C-O-H stretching so IR spectra of A8 matching with IR spectra of luteolin standard [37]. A2 IR spectra revealed the presence336.93 band refer to the O-H stretching broad band of phenol,3284 bands refer to carboxylic acid O-H stretching band,1618 bands refer to the C=O stretching of carboxylic acid,1541.1 refers to Aromatic C=C stretching bands,1450 band refers to O-H bending of phenyl O-H,1203 and 1099 bands refer to C-H bending fingerprint of aromatic (in-plane), 867, 663 and 790 bands refer to C-H bending fingerprint of aromatic (out of plane) So IR spectra of A2 matching with IR spectra of gallic acid standards [38].

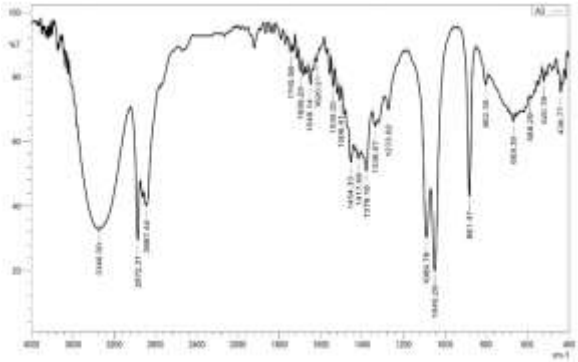


Figure (8): FTIR spectrum of isolated A3 compound

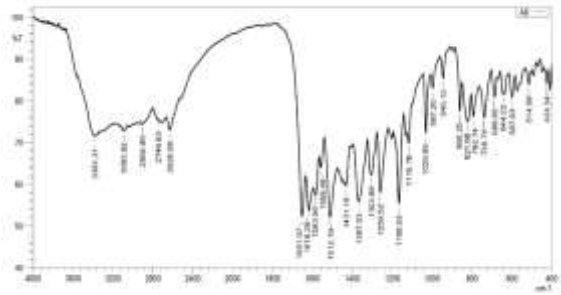


Figure (9): FTIR spectrum of isolated A8 compound

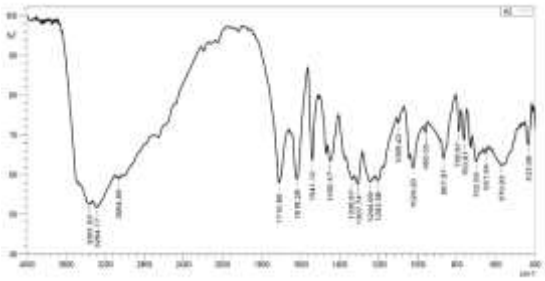


Figure (10): FTIR spectrum of isolated A2 compound

MTT assay

The anticancer effect of guava leaves F3 fraction against AMJ-13 cancer cell line was weighed by MTT assay, and AMJ-13 was exposed to sequential concentrations (31.2, 62.5,125, 250, 500, 1000µg/ml) of guava leaf F3 for 72 h to assess its effects on the viability of cell line depending on a concentration-dependent mode as shown in figure11. Guava leaf exhibited a decrease in cell viability (%) with IC_{50} values of 414.3 µg /ml as in figure 12. Also, guava leaves F3 made morphological alterations and cell loss as shown in figure 13. The anticancer effect of guava leaves F3 against the MCF-7 human cancer cell line was weighed by MTT assay.MCF-7was exposed to sequential concentrations (25 µg/ml, 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml) of the guava extract to assess its effects on the viability of cell line as shown in figure14. The decrease in MCF7cell viability (%) by guava F3 is presented in table 1. All concentrations of guava leaf extract compared to control significantly inhibited cell lines ($P < 0.0001$). Guava F3 also made morphological alterations and cell loss as shown in figure15.

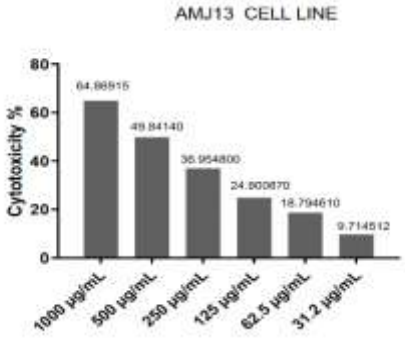


Figure (11): Cytotoxic effect of F3 fraction guava leaves on AMJ13 cell line

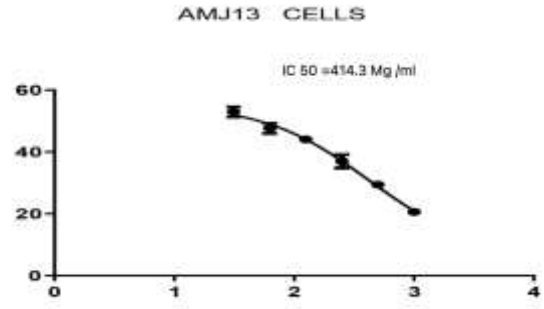


Figure (12): IC_{50} of F3 guava leaves on AMJ13 cell line

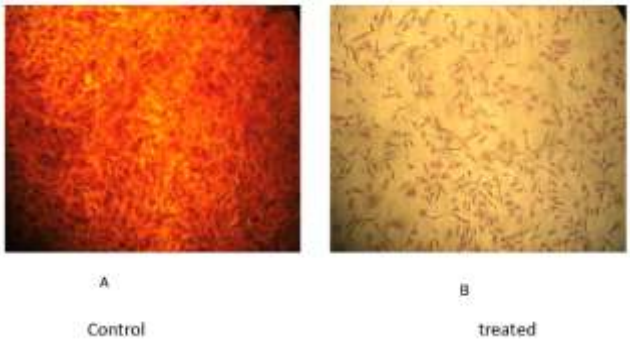


Figure (13): Morphology of untreated AMJ13(A). Morphology of AMJ13 cell line after treatment with F3 fraction of Guava leaves (B). Hematoxylin and Eosin staining, under magnification power 100x.

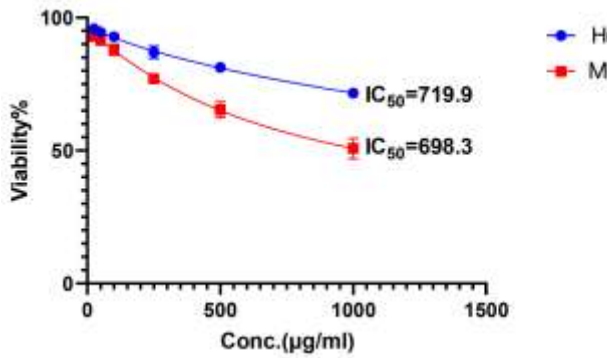
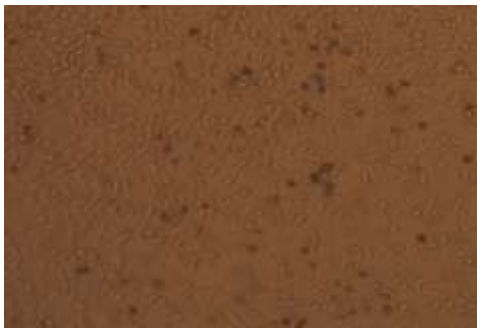
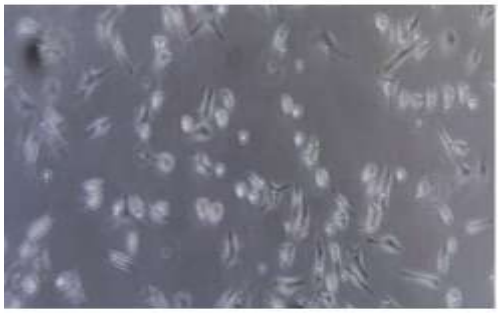


Figure (14): IC_{50} of guava F3 fraction on MCF7

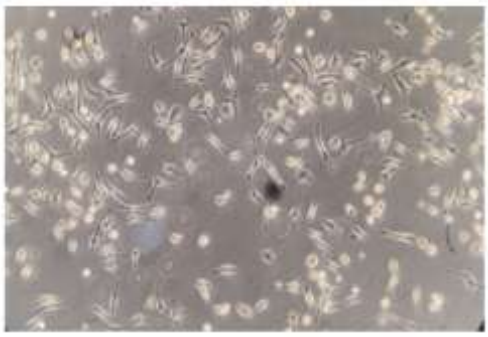
A



B



C



D

Figure (15). Morphology of control(A). Morphology of MCF7 cell line after management with guava leaves F3 at conc. 1000 µg/ml(B). Morphology of HDFn before management with guava leaves F3(C). Morphology of HDFn after treatment with guava leaves F3(D). Hematoxylin and Eosin staining, under magnification power 100x.

Table (1): Cytotoxic effect of guava leaves F3 on MCF7 and HdFn cells after 72 hours incubation at 37ºC

Guava leaves extract concentration (µg mL)	Viable cell counts of MCF7cell line (Mean ± S.D)	Viable cell counts of HdFn cell line (Mean ± S.D)
1000	50.77167±3.743934	71.64333±1.837285
500	65.39367±3.042849	81.28867±1.528013
250	77.04467±1.831309	87.037±2.311917
100	87.92467±2.087783	92.86267±1.274761
50	91.47367±1.164728	94.52167±0.98406
25	92.97833±1.226403	95.83367±0.30634

Discussion

Extraction of guava leaves

This study used an ultrasonic bath sonicator a rapid and effective extraction technique that uses ultrasound to generate rapid movement of solvents, resulting in a higher mass transfer speed and acceleration of extraction resulting in a higher percentage yield of approximately 20 % w/w from 50g of guava leaves. Compared to other traditional methods of extraction like maceration and Soxhlet this method is more economical, eco-friendly, and convenient for getting good extraction results as remembered by previous studies (39). So, this method is used for guava leaf extraction to get a higher percentage yield because better conditions are obtained, the ultrasonic waves at 40 KHz, utilizing 50g of leaf powder in 750 ml of 70% ethanol at 30°C for 40 minutes.

Preliminary phytochemical investigation

An alkaline reagent test was used as a preliminary phytochemical investigation to decide the incidence or lack of polyphenolic compounds in Iraqi *Psidium guajava* the results were positive where a yellow color appeared and it appeared colorless by adding drops of diluted HCL indicating the presence of phenolic compound in F3 and F4 fractions. These results are consistent with the results of previous studies to discover the presence of phenolic compounds by using an alkaline reagent test (40). Therefore, the results were positive because guava leaves contain a large number of phenolic compounds and many flavonoids were isolated from guava leaves such as ellagic derivatives, quercetin, resveratrol, daidzin, guaijaverin, avicularine, hyperin, morin, chlorogenic acid, rutin, vanillic acid, p-hydroxyl benzoic acid, syringic acid, myricetin, naringenin, and apigenin as previously reported (15,16). Analysis

of Guava Fractions by High-performance liquid chromatography. This analytical method is a practical way to determine the presence of a wide range of substances contained in the extract of plant leaves. The retention time of isolated compound A3 was 4.08, the retention time of the caffeic acid standard was 4.12, and retention time of the isolated compound A8 was 9.66 and the retention time of the luteolin standard was 9.66. Also, the retention time of the isolated compound A2 was 6.08, and the retention time of the gallic acid standard was 6.08 as shown in figures from 1 to 7. Retention time is the amount of time a compound spends on the column after it has been injected, if a sample contains several compounds, each compound in the sample will spend a different amount of time on the column according to its chemical composition (40). In this study, HPLC examination indicates a remarkably close retention time value of caffeic acid standard, luteolin standard, and gallic acid standard matched to the isolated A3, A8, and A2 compounds isolated by PHPLC, a single peak in HPLC chromatogram usually correlates to a single compound and thus can be used to obtain important information about individual constituents of crude extracts compared with standard compounds as shown in figures from 1 to 7.

FTIR spectra

IR spectroscopy analysis of compound A3 revealed the presence of many major peaks at 3346.5 bands referring to phenolic O-H stretching band,2972 referring to C-H stretching of the benzene, 2887 referring to asymmetric and symmetric stretching of CH2, 1649 referring to aromatic C=O stretching vibration band,1454 refer to O-H bending of carboxylic acid. Also, IR spectroscopy of the A8 compound revealed the presence of many major peaks at3381 band refers to O-H stretching of phenol, the 2904 band refers to C-H stretching of phenol, the 1651 band refers to C=C stretching vibration,1166 refers to C-O-C stretching, 1029 refers to C-O-H stretching. While IR spectroscopy of A2 showed the presence of many major peaks at 3361.93 referring to the O-H stretching broad band of phenol,3284 bands refer to carboxylic acid O-H stretching band,1618 bands refer to the C=O stretching of carboxylic acid. Compared to previous studies most of the major peaks appear the same for caffeic acid, luteolin, and gallic acid tested by FTIR (36-38). So, these functional groups present in the spectroscopy of A3, in turn, indicate the presence of phenolic acid by the presence of major peaks at 3346.5 bands referring to the phenolic O-H stretching band this indicates that this isolated compound belongs to the phenolic compounds, IR spectroscopy of A8 showed the presence of functional groups related to phenol, benzene ring and carbonyl indicate that isolated compound is flavonoid compound as compared with literature studies (37). The FTIR spectroscopic technique of A2 confirms the presence of functional groups in isolated compound A2 that were respectively related to phenol, carbonyl, and benzene, of the phenolic acid compound.

The MTT assay: It is a sensitive and reliable indicator of cellular metabolic activity and is preferred over the other methods measuring this end-point like the ATP and 3H-thymidine incorporation assay, The MTT assay relies on the reduction of MTT, a yellow water-soluble tetrazolium dye, primarily by the mitochondrial dehydrogenases, to purple colored formazan crystals (41). The MTT assay was used to determine the cytotoxicity against AMJ-13 and MCF-7 cancer cell lines, and the results showed that the F3 had a dose-dependent inhibitory activity against AMJ-13 cell growth, with cytotoxicity % of 9.7%, 18.7%, 24.9%, 36.95%, 49.8%, and 64.8% after exposure to 31.2, 62.5, 125, 250, 500 and 1000 µg /mL, respectively, all concentrations of guava leaf extract compared to control significantly inhibited cell lines ($P < 0.0001$). also showed that the F3 had a dose-dependent inhibitory activity against MCF-7 cell growth shown in table 1, a cytotoxicity test was also carried out on normal cells (HdFn cells) to determine the selectivity of the fraction, The purpose of this selectivity value is to determine the level of safety of an anticancer compound against normal cells so that it can be further developed as a chemopreventive agent, F3 fraction noncytotoxic to HDFn, with an IC50 value719.9 µg/mL substantially surpassing concentrations of 100 µg/mL(42). The parameter used for the cytotoxic test was the IC50 value indicating the concentration value that results in the inhibition of cell proliferation by 50% and the potential toxicity of a compound to cells. The IC50 value can show the potential of a compound as being cytotoxic. As the IC50 value increases, the compound toxicity decreases. The results of a cytotoxicity test on the target organ provide direct information about changes that occur specifically in cell functions. The anticancer effect of guava leaves F3 against AMJ-13 and MCF-7 cancer cell lines exhibited a decrease in cell viability (%) with IC50 values of 414.3 µg /ml against AMJ-13 and IC50 values of 698.3 µg /ml against MCF-7. Compared with previous studies the n-hexane fraction has higher activity and selectivity to cancer cells MCF-7 than the ethyl acetate fraction(F3) with IC50 4.8 µg /ml (43). Also, Abdel-Aal *et al.* (2022), showed that the cytotoxic effect of guava leaf extract on MCF7 presented by IC_{50} is 97.5 used 52, 97, 193, and 500µg/ml respectively (44). Other results indicated that the α-humulene compound isolated from the essential oil of guava leaves was active on MCF-7 cells with an IC_{50} value of 0.082 mg/mL (45). In the present investigation, the cytotoxic activity of F3 is more effective toward AMJ13 than MCF-7with cytotoxicity %64.869 at 1000 µg /ml, to the best of our knowledge, this study is the first to biologically evaluate the guava F3 using the *in vitro* cytotoxic potential of this plant toward AMJ-13 cell line, which suggests the anticancerous activity of plant extract can probably be attributed to the phenolic compounds, as determined by the MTT cytotoxicity assay. The cytotoxic activity of the guava leaf F3 against AMJ-13 and MCF-7 might be attributed to its

ability to provoke cytotoxic and apoptotic responses within cancer cells. It was observed that the extract hindered the cell cycle progression at the G1 phase, leading to cell death (46). The implicated signaling mechanisms entail the inhibition of the AKT/mammalian target of rapamycin (mTOR) ribosomal p70 S6 kinase (S6K1) and the mitogen-activated protein kinase (MAPK) activation pathways. It is noteworthy that an elevation in AKT signaling via the mTOR pathway has been documented in diverse carcinoma cell lines (47-49).

Study limitations

In this study, we presented a preliminary study for the identification of some phenolic compounds found in guava leaves and studied the effect of ethyl acetate fraction on some cancer cell lines. One of the limitations of the study is the small number of newly planted trees, which resulted in the obtaining of small quantities of leaves that did not help us to conduct further research procedures such as extracting essential oils from the leaves and knowing their effectiveness against cancer cell lines. Also, the small quantity of leaves did not allow us to isolate larger quantities of phenolic compounds and study their effects, each compound separately, against cancer cell lines.

Conclusion

Isolation of phenolic compounds (caffeic acid, luteolin, gallic acid) from guava leaves proved successful, employing an extraction method that utilized an ultrasonic bath sonicator to enhance the percentage of the yield. The identification of these phenolic compounds was carried out through techniques of HPLC and FTIR spectroscopy. The cytotoxic activity of the guava leaves F3 was evaluated on specific cell lines (AMJ-13 and MCF-7) which exhibited a decrease in cell viability (%) in a concentration-dependent mode.

Authors' Declaration: Conflicts of interest: None. We hereby confirm that all the Figures and Tables in the manuscript are ours. The project was approved by the University of Baghdad /College of Pharmacy.
Conflict of Interest: None
Funding: None

Author contributions:

Study conception & design: Enas J. Kadhim. Literature search: Ashwaq T. Kareem, Data acquisition: Ashwaq T. Kareem. Data analysis & interpretation: Ashwaq T. Kareem. Manuscript preparation: Ashwaq T. Kareem. Manuscript editing & review: Enas J. Kadhim.

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عزل وتوصيف المركبات الفينولية في أوراق الجوافة وتقييم النشاط السام للخلايا ضد خلايا سرطان الثدي العراقي وسرطان الثدي البشري

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الخلاصة
الخلفية:Psidium guajava المعروفة باسم الجوافة، هي شجرة استوائية تقدر بثمارها المغذية وخصائصها الطبية. هذا العضو من عائلة Myrtacease غني بالمواد الكيميائية النباتية، وهي مركبات طبيعية ذات فوائد صحية محتملة. أظهرت الدراسات أن أوراق وثمار الجوافة تمتلك أنشطة دوائية مختلفة، بما في ذلك خصائص مضادة للسرطان ضد العديد من خطوط الخلايا السرطانية.
الاهداف: تبحث هذه الدراسة في عزل المركبات الفينولية لأوراق الجوافة المزروعة في العراق واختبار النشاط السام للخلايا (القدرة على قتل الخلايا السرطانية) ضد خطين من خلايا سرطان الثدي: خط الخلايا AMJ-13 العراقي وخط الخلايا البشرية MCF7 الراسخ لجزء الاثيل اسيتيت.
الطريقه: سيستخدم الباحثون تقنية قوية تسمى التحليل اللوني السائل عالي الأداء (HPLC) ومطياف الأشعة تحت الحمراء لتحويل فورييه (FTIR) لتحليل مستخلص أوراق الجوافة ولمعرفة هيكلية المركبات. وفي وقت لاحق، سيتم استخدام HPLC التحضيري (PHPLC) تقوم هذه الطريقة بفصل المكونات المختلفة داخل المستخلص، وهو شكل متخصص من HPLC، لعزل المركبات الفينولية المحددة ذات الاهتمام.
النتائج: نجح الباحثون في عزل عينات نقية من حمض الكافيك، واللوتيولين، والكالك اسد، وجميعها مركبات فينولية من مستخلص أوراق الجوافة باستخدام HPLC التحضيري. ثم تم استخدام جزء الاثيل اسيتيت المعزولة منه المركبات الفينولية لمعرفة نشاطه السام للخلايا ضد كل من خطوط خلايا سرطان الثدي العراقية AMJ-13 وMCF7 البشرية. وأظهرت النتائج انخفاضا في حيوية الخلية، مما يشير إلى خصائص المستخلص المحتملة المضادة للسرطان. كان المستخلص أكثر فعالية ضد خلايا AMJ-13 العراقية بقيمة IC50 البالغة 414.3 ميكروغرام / مل، مقارنة بخلايا MCF7 البشرية بقيمة IC50 البالغة 698.3 ميكروغرام / مل.
الاستنتاج: قدمت هذه التقنيات التحليلية المستخدمة في هذه الدراسة، مثل HPLC,FTIR ملفا تفصيليا للمركبات الفينولية الموجودة في أوراق الجوافة. تشير هذه المعلومات، بالإضافة إلى اختبارات السمية الخلوية، إلى أن أوراق الجوافة لديها القدرة على قتل الخلايا السرطانية بطريقة تعتمد على التركيز.
الكلمات المفتاحية: استخلاص بالموجات فوق الصوتية، جوافة حامض الكافييك كالك اسد,لتيولين.

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Research Article

Comparative Adhesion of *Pseudomonas aeruginosa* to Human Oral Mucosal Epithelial Cells and Polystyrene Surfaces

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Abstract

Background: The adhesion of bacteria to different surfaces reflects their ability to cause infectious diseases. The distinction between the *Pseudomonas aeruginosa* adhesion to biotic and abiotic surfaces is not clear in the literature.

Objectives: To shed light on the extent of similarities and differences between *P. aeruginosa types* in terms of their ability to adhere to different surfaces.

Methods: Ten isolates of *P. aeruginosa* were isolated from 100 wound and burn swabs. The samples were collected from Baghdad Teaching Hospital, and Al-Yarmouk Teaching Hospital, Baghdad, Iraq (from September 1st to December 24th 2023). The isolates were identified using biochemical and phenotypic tests in addition to VITIK-II technology. The susceptibility to antibiotics was estimated using the disc diffusion method. The microtiter-plate assay was used to measure the biofilm formation. The Human oral mucosal epithelial cells (OMECs) were used to estimate the adhesion of *P. aeruginosa* isolates. Plate and direct bacterial count were used for counting the bacteria adhered to human OMECs.

Results: Norfloxacin showed the highest antibacterial effect, while all isolates were resistant to Amoxicillin and Cefixime. Of all isolates that formed biofilm, P. aeruginosa 2 (Pa2) formed the highest biofilm, followed by Pa6, while the lowest biofilm formation was seen in Pa4. Pa6 showed the highest ability to attach to OMECs followed by Pa2.

Conclusion: The adherence and biofilm formation of *P. aeruginosa* isolates that are resistant to most antibiotics depend on the type of surface to which they adhere.

Keywords: Anti-Bacterial Agents; Antibiotics Resistant; Biofilm; *Pseudomonas aeruginosa;* Pseudomonas Infection

Introduction

Bacterial isolates that cause infectious diseases and are resistant to a wide spectrum of antibiotics, as well as overcoming high concentrations of detergents are considered a serious challenge facing doctors in curing hospital bacterial infections (1). *Pseudomonas aeruginosa* bacteria is the bacterial species that causes nosocomial infections due to its resistance to antibiotics in addition to detergents. *P. aeruginosa* is known to be the causative agent of different infectious diseases, affecting wounds and burns. These infections pose a challenge for professionals tasked with managing cases of *P. aeruginosa-infected* wounds and burns (2). Antimicrobial resistance has an impact on health and economic outcomes because it increases the chance of bacterial infectious disease outbreaks which would negatively impact the health services, ultimately increasing the cost of health care (3).

Human oral mucosal epithelial cells (OMECs) are believed to be a major set of players interacting with bacteria and immune systems (4). In addition, they are an important biological barrier that prevents microorganisms from entering deep tissues and defends the host against chemicals. The adhesion of bacteria to human epithelial cells is critical for bacterial invasiveness of the host (5).

The bacterial cells can attach to a wide range of biotic as well as abiotic surfaces. Different surfaces tend to prompt different responses from the bacterial cells to create the interaction between the bacterial cells and surfaces, yielding the attachment of bacteria to these surfaces (6). As a result, bacteria express appendages and produce adhesion proteins that are directed toward specific ligands or chemical characteristics on the surface (7). Adhesion may be affected by the physicochemical properties of the surface, such as charge, hydrophobic balance, and mechanical strength (6). Bacterial adhesion involves a complex combination of environmental, bacterial and material traits (8). Specific bacterial appendages, non-specific interactions (e.g. van der Waals forces and electrostatic interactions), and surface mechanical induction contribute to adhesion (9). Understanding the mechanisms of bacterial adhesion to surfaces is crucial for addressing biofilm formation, biofouling, and the development of antimicrobial surface technologies. The current study aims to clarify whether *P. aeruginosa* isolates adhesion to biotic surfaces is similar to their adhesion to abiotic surfaces.

Materials and methods

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Isolation and Identification: One hundred wound swabs were obtained aseptically from patients suffering from infected wounds. The samples were collected from Baghdad Teaching Hospital, and Al-Yarmouk Teaching Hospital, Baghdad, Iraq (from September 1st to December 24th 2023). The patients had given their written consent to participate in the study. They had not received antibiotics 3 days prior to the sample collection. The swabs were cultured immediately onto the appropriate media (MacConkey agar, blood agar, and Cetrimide agar (Himedia, India)). The Petri dishes were incubated for 48 hours at 37ºC. Biochemical tests such as oxidase and catalase tests were done. The morphological features of bacterial cells were determined by staining with Gram stain. The VITEK 2 DensiCheck instrument, fluorescence system (bioMe´rieux) (ID-GNB card) was used to identify the isolates (10)

Preparation of bacterial standard inoculum: The identified isolates of *P. aeruginosa* (Pa1-Pa10) were grown in Nutrient broth (Himedia, India) at 37ºC for 24 hours. The bacterial cells were washed three times with phosphate buffer saline (PBS (0.01 M, pH 7.1). The final bacterial counts (10^8 c CFU/ml) were prepared in either Muller Hinton Agar (MHA) for the experiment of disc diffusion method measurement or Tryptic Soya broth (TSB) for the experiment of bacterial adhesion and biofilm formation (11).

Disc Diffusion Method: This method was implemented for antimicrobial susceptibility testing. Briefly, standard inoculums of bacterial isolates of *P. aeruginosa* (10^8 CFU/ml) were spread onto Mueller-Hinton agar (MHA) plates. The plates were used for the sensitivity test. Standard commercial antibiotic discs (six discs were put on each plate). The standard antibiotic discs were Ticarcillin (TCC), Cefixime (CFM), Vancomycin (VAN), Erythromycin (ERY), Oxacillin (OXS), Bacitracin (BAC), Amikacin (AMK), Trimethoprim (TMP), Meropenem (MER), Cefamandole (FAM), Novobiocin (NOV), Imipenem (IPM), Amoxicillin (AMX), Cefadroxil (CFR), Levofloxacin (LEV), streptomycin (STR), Norfloxacin (NOR) for all isolates of *P. aeruginosa*. The plate was then incubated for 18 hours at 37°C. The scale was used to measure the inhibition zones (12, 13). The results were interpreted as resistance (R), Intermediate (I), and sensitive (S) according to the CLSI guideline (13).

Isolation of human oral mucosal epithelial cells (OMECs): The human OMECs were isolated from four healthy volunteers (2 males and 2 females, aged 35 to 47 years). They were isolated from the oral mucosa by gently scraping the inner surface of the mouth using sterile woody sticks. The standard method of Ali & Zgair was followed to prepare the standard number of human OMECs (10^5 cells/ml) in sterile PBS (0.01 M, pH 7.1). The trypan blue stain was used to check the number of viable cells. The viability of human OMECs prepared was 91% (4).

Bacterial adhesion to Human OMECs: The method of Al-Mutalib and Zgair, (2023) was followed to measure the number of bacterial cells that adhered to human OMECs *in vitro* (14). Briefly, 100 µl of $5x10^5$ cells/ml of human OMECs that were suspended in PBS (0.01 M, pH 7.1), 10 µl of 10^8 CFU./ml of bacterial cells prepared in TSB, and 90 µl of sterile MHB were put into endotoxin-free micro-centrifuge tubes (2 ml tube, NEST Scientific USA). The tubes were washed four times with the PBS after incubating them for 2 hours at 37°C to remove non-adherent bacteria. 100 µl of the final volume of 200 µl was lysed with 100 µl of PBS–0.5% Triton×100 (Sigma-Aldrich), diluted tenfold and plated onto nutrient agar plates to enumerate the viable bacteria that adhered onto OMECs. The remaining 100 µl was smeared onto glass slides and stained with Leishman's stain. The slides were examined using a light microscope (CH-Olympus, Japan), and images were taken by a smartphone camera above the eyepiece of the microscope. The number of adhered bacteria to one human OMEC calculated by the average of the number of adhered bacteria on 20 human OMEC at different places on the stained slide.

Biofilm Formation: Two hundred µl of sterile Tryptic soy broth (TSB) (HiMedia, India), and 5 µl of 10^8 CFU/ml (corresponding to the 0.26 at 600 nm) of *P. aeruginosa* isolates (cultured previously into TSB) were added into the sterile flat-bottom polystyrene microtiter plates wells (Sigma-Aldrich). The plates were then incubated at 37°C for 24 hours. Subsequently, the non-attached bacterial cells were removed by aspirating the TSB, and then the wells were washed five times with sterile distilled water. The formed biofilm was dried and fixed at 61ºC for 35 minutes. Following this, 220 µl of Hucker crystal violet (0.45%) was added to each well and incubated for 10 minutes at room temperature. After five washes with distilled water and a drying period of 35 minutes at 37°C (Incubator, Memmert, Germany), 220 µl of acetone: ethanol (30:70) was added to the wells. The absorbance was measured at a wavelength of 570 nm using the BioTek 800 microplate reader (Agilent, USA) (14).

Statistical analyses

Origin v. 8 software (OriginLab, Nothampton, USA) was used to do the statistical analysis. The values were expressed as means ± standard error (M ±SE). The Student t-test was used to calculate the group difference. The Pearson's correlation coefficient was used to explore the correlations between variables. A value of *P* less than 0.05 was considered statistically significant.

Results

Bacterial isolates: In the present study ten isolates were isolated and identified (Pa1-Pa10). These isolates were used in the further experiments of the present study.

Antimicrobial susceptibility test: In the present study, the inhibitory zone made by different antibiotic discs against *P. aeruginosa* clinical isolates (Pa1-Pa10) is shown in Table 1. The highest antibacterial effect was seen with Norfloxacin (12 - 35 mm, only Pa 1 was resistant to this antibiotic). All isolates were

resistant to Amoxicillin and Cefixime. Only one isolate was sensitive to Ticarcillin, Erythromycin, Oxacillin, Amikacin, Trimethoprim, Imipenem, and Cefadroxil. There is a high level of resistance to traditional antibiotics.

Table 1: **The diameter of the inhibition zone (mm) around the different antibiotic discs for *P. aeruginosa* isolates (Pa1 – Pa10)**

Isolates	TCC	CFM	VAN	ERY	OXS	BAC	AMK	TMP	MER	FAM	NOV	IPM	AMX	CFR	LEV	STR	NOR
Pa1	0.0 (R)	0.0 (R)	18 (S)	0.0 (R)	0.0 (R)	8 (I)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	25 (S)	0.0 (R)
Pa2	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	8 (I)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	12 (S)
Pa3	0.0 (R)	0.0 (R)	20 (S)	0.0 (R)	0.0 (R)	9 (S)	24 (S)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	8 (I)	16 (S)	15 (S)	30 (S)
Pa4	0.0 (R)	0.0 (R)	19 (S)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	30 (S)	18 (S)	33 (S)
Pa5	0.0 (R)	0.0 (R)	23 (S)	0.0 (R)	0.0 (R)	8 (I)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	10 (S)	0.0 (R)	0.0 (R)	0.0 (R)	30 (S)	19 (S)	30 (S)
Pa6	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	13 (S)	0.0 (R)	0.0 (R)	0.0 (R)	32 (S)	0.0 (R)	0.0 (R)	20 (S)	16 (S)	27 (S)
Pa7	0.0 (R)	0.0 (R)	0.0 (R)	15 (S)	20 (S)	13 (S)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	21 (S)	0.0 (R)	0.0 (R)	0.0 (R)	24 (S)	18 (S)	35 (S)
Pa8	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	11 (S)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	21 (S)	17 (S)	30 (S)
Pa9	0.0 (R)	0.0 (R)	19 (S)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	19 (S)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	18 (S)	0.0 (R)	27 (S)
Pa10	10 (S)	0.0 (R)	7 (I)	0.0 (R)	0.0 (R)	10 (S)	0.0 (R)	0.0 (R)	0.0 (R)	0.0 (R)	8 (I)	0.0 (R)	0.0 (R)	0.0 (R)	21 (S)	16 (S)	25 (S)

S: sensitive; I: Intermediate; R: resistant

Biofilm formation: All isolates were able to form a biofilm. The highest biofilm production was found in isolate Pa2, followed by Pa6, while the lowest biofilm formation was found in isolate Pa4 (Figuer1).

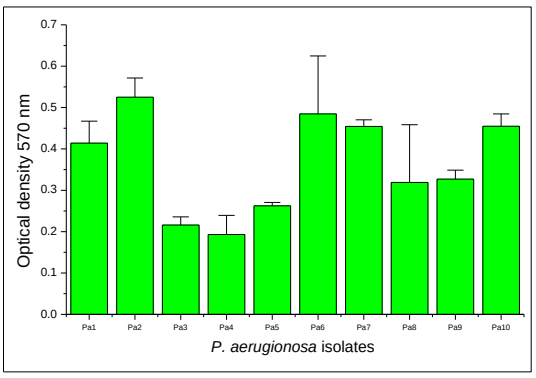

Figure 1: The average biofilm formation of *P. aeruginosa* isolates form infected wounds

Adherence of *P. aeruginosa* to Human OMECs: The adhesion of three isolates of *P. aeruginosa* (Pa2, Pa6, and Pa10, which showed the highest ability to form biofilm) to human OMECs (biotic surface) was tested. Figure 2 shows that the maximum bacterial adherence was seen in isolate Pa6 ($P<0.05$) as compared with Pa 2 and Pa10. A slight difference was seen between the number of bacterial adhesions of Pa 2 and Pa 10 (Figure 2a). When the Leishman's stain technique was used for calculating the number of total bacterial cells adhered to the surface of human OMECs, the highest number of adhered bacteria was seen in isolate Pa 6 followed by Pa10 ($P<0.05$).

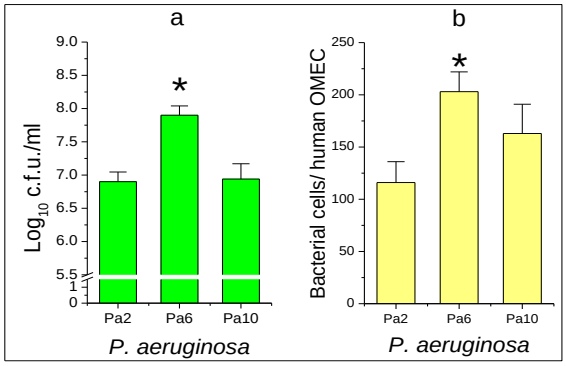

Figure 2: Viable *P. aeruginosa* count (c.f.u./ml) that adhered to human OMECs calculated using plate count method (a)

The asterisk indicates the significant difference from Pa2 and Pa10: The microphotographs of isolate Pa2 attached to the OMEC is shown in Figure 3a. The epithelial cell (Squamous epithelium, OMEC, not exposed to bacterial cells and treated with PBS), appear as large flattened cell with cytoplasm and a small and ellipsoid nucleus. The highest number of adhered *P. aeruginosa* to human OMECs is shown in Figure (3)

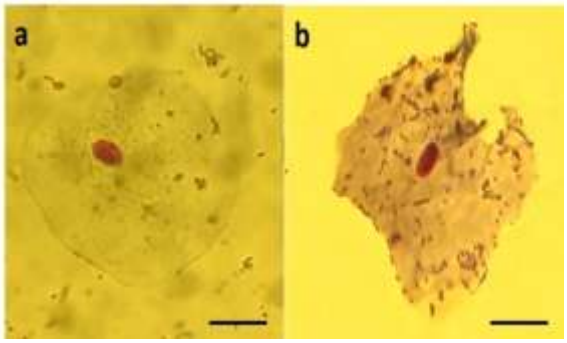

Figure (3): Photomicrographs of human OMEC stained with Leishman's stain and examined under light microscope. a: Human OMEC not treated with *P. aeruginosa*. b: Human OMEC treated with *P. aeruginosa* showing the bacteria adhered to the epithelial surface. The scale bar is 40 µm.

Discussion

The attachment of opportunistic pathogenic bacteria to biotic surfaces such as epithelial cells or abiotic surfaces such as medical plastic devices represents the initial stage in the establishment of biofilms or the invasion of host cells (15). These occasions offer to safeguard the bacteria from the host body's immune system and induce persistent infection. The adhesion of bacterial cells to the mucosal epithelial cells is an essential stage in the procedure of infection, especially in situations of dental infections. The adhesion of bacterial cells to host cells is a diverse procedure that incorporates different mechanisms and factors. An extensive understanding of these communications is essential to designing reliable methods for treating bacterial infections (5).

In the current study, the *P. aeruginosa* isolates showed resistance to a wide spectrum of antibiotics that are routinely used in treating the wound bacterial infection. The study found that *P. aeruginosa* isolates adhered to human OMECs and to polystyrene microtiter plates with high efficiency. The highest bacterial adhesion and formation of a biofilm on polystyrene was seen in the case of Pa 2, while this isolate did not show the highest ability to adhere to living surfaces (human OMECs). The studies addressing the same topic are scanty in the available literature. Previous studies have shown the biofilm formation of *P. aeruginosa* to polystyrene microtiter plate and the adhesion of *P. aeruginosa* to different kinds of human epithelial cells (but not to oral mucosal epithelial cells) (16, 17). However, there was no previous studies addressing the ability of the same isolates of *P. aeruginosa* to adhere to human OMEC and to form biofilm to polystyrene, which endorses the novelty of the current study.

P. aeruginosa appendages, such as flagella, pili, and fimbriae, are involved in their adherence to different types of surfaces (18). These appendages assist in the adhesion of *P. aeruginosa* to semi-solid surface areas along with the change from a motile state of bacteria to biofilm form (19). The appendages serve as added-cellular frameworks that aid in the preliminary accessory of the bacteria adherence to biotic surfaces (20). Additionally, the appendages add to the development of bacteria, like supplying mechanical security and mediating microbial adhesion to surface areas. The bacterial matrix, which consists of polysaccharides, develops a natural three-dimensional network that links and immobilizes microbial cells to form the network of microenvironments of biofilm (20). The role of appendages in adherence to various types of surfaces is not completely comprehended, but the appendages are understood to be crucial for the development as well as determination of *P. aeruginosa* biofilm. The current research showed that the capability of the isolates to adhere differs depending on the surface areas, and they are not just as reliable in adhesion to abiotic as well as biotic surface areas, which validates that the adhesion varies depending on the type of surface areas to which they adhere.

Conclusion

P. aeruginosa isolated from infected wounds showed a high resistance to antibiotics, with adherence to abiotic surface and forming biofilms on polystyrene and adherence to OMECs. The adherence and biofilm formation of *P. aeruginosa* isolates that are resistant to most antibiotics depend on the type of surface to which they adhere.

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Funding: None.

Authors' contribution

Marwa M. Talib: Conceptualization; Data curation; Investigation; Methodology; Project administration; Roles/Writing -original draft; Supervision; Validation.

Jenan A. Ghafil: Roles/Writing -original draft; Visualization; Writing -review & editing; Formal analysis; Resources.

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الإلتصاق المقارن لبكتيريا *Pseudomonas aeruginosa* بالخلايا الظهارية المخاطية للفم البشري وأسطح البوليسترين

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الخلاصة

الخلفية: إن إلتصاق البكتيريا بالأسطح الحية وغير الحية يعكس قدرتها على التسبب في الأمراض المعدية. إن التمييز بين قدرة بكتيريا *Pseudomonas aeruginosa* على الالتصاق بالأسطح الحية وغير الحية ليس واضحا بشكل تام في الأدبيات الطبية.

الأهداف: تهدف الدراسة الحالية إلى تسليط الضوء على مدى أوجه التشابه والإختلاف بين أنواع *P. aeruginosa* من حيث قدرتها على الإلتصاق بالأسطح الحية وغير الحية.

المنهجية: تم عزل عشر عزلات من بكتيريا *P. aeruginosa* من 100 مسحة من الجروح. تم تشخيص العزلات باستخدام الإختبارات الكيموحيوية والمظهرية بالإضافة إلى تقنية VITIK-II. وتم تقدير الإستجابة للمضادات الحيوية باستخدام طريقة إنتشار القرص. تم استخدام صفائح المعيارية الدقيقة-الطيفي لقياس تكوين الأغشية الحيوية على ألواح الصفائح الدقيقة البوليسترينية. تم استخدام الظهارة المخاطية للفم البشري (OMECs) لتقييم إلتصاق عزلات *P. aeruginosa* وتم استخدام عدد البكتريا على الأطباق بعد تخفيفها والعد البكتيري الكلي المباشر لحساب البكتيريا الملتصقة بـ OMECsفي المختبر.

النتائج: أظهر النورفلوكساسين أعلى تأثير مضاد للجراثيم بينما لوحظ أقل تأثير مضاد للجراثيم عند استخدام أموكسيسيلين وسيفيكسيم (جميع العزلات كانت مقاومة). شكلت جميع العزلات من Pa2 أعلى غشاء حيوي يليه Pa6، في حين شوهد أقل تكوين للبيوفيلم في حالة .Pa4 بينما أظهر Pa 6 أعلى قدرة على الإرتباط بـ OMECs البشرية، يليه. .Pa2

الإستنتاج: أن عزلات *P. aeruginosa* مقاومة لمعظم المضادات الحيوية وأن قدرتها على الإلتصاق وتكوين الأغشية الحيوية تعتمد على نوع السطح.

الكلمات المفتاحية: الإلتصاق، الحساسية للمضادات الحيوية، تكوين الأغشية الحيوية، [، الزائفة الزنجارية (*Pseudomonas aeruginosa*).

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Research Article

Evaluation of Interleukin-31 Serum Levels in Patients with Chronic Kidney Disease on Hemodialysis with and without Uremic Pruritus

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Abstract:

Background: Interleukin-31 has been linked with developing and maintaining pruritus in various dermatological and non-dermatological diseases.

Objectives: To evaluate interleukin-31 serum levels in hemodialysis patients with and without uremic pruritus as a potential contributor.

Methods: This cross-sectional study involved ninety adult chronic kidney disease patients on hemodialysis. All of the enrolled patients were on a three-times/week hemodialysis regimen. Patients were divided into two groups of 45 patients each. Group 1 involved those with pruritus and Group 2 involved those free of pruritus; based on the itching severity scale (ISS). Serum levels of interleukin-31, intact parathormone, urea, creatinine, and calcium were assessed before the hemodialysis session. Serum interleukin-31 levels were also assessed after the hemodialysis session. The statistical analysis was performed using the Statistical Package for Social Science (SPSS) .The median and interquartile range (IQR) were used to present data on the continuous variables. Mann-Whitney test was used to compare the differences between medians of the two groups, and Wilikson test was used to compare the differences between medians of IL-31 before and after hemodialysis. Spearman's correlation was employed to assess the correlation among the studied variables. A P-value less than 0.05 was considered significant.

Results: In the pre-dialysis samples, serum levels of interleukin-31 in patients with uremic pruritus were not significantly different from those in patients without uremic pruritus [1361.55 (741.96) pg/mL and 1395.75(624.75) pg/mL, respectively; P=0.36]. However, patients with uremic pruritus had higher serum creatinine levels than patients without uremic pruritus [9.8(5.5) mg/dL and 8.15(3.18) mg/dL, respectively; P=0.02]. The two groups had no significant differences in intact parathormone, calcium, or urea serum levels. In both of the study groups, serum interleukin-31 levels in patients with and without uremic pruritus were significantly reduced by hemodialysis. In the post-dialysis samples, serum interleukin-31 levels in patients with uremic pruritus were not statistically different from those in patients without uremic pruritus [864 (164.58) pg/mL and 879.7(84.19) pg/mL, respectively; P=0.83].

Conclusions: The association of serum interleukin-31 levels with uremic pruritus in chronic kidney disease CKD patients on hemodialysis need further verification.

Keywords: Chronic kidney disease**;** Hemodialysis**;** Itching; InterlukinL-31; Uremic Pruritus.

Introduction:

Chronic kidney disease (CKD) is one of the leading public global health problems. The estimated worldwide prevalence of CKD is more than 10%, affecting about 843.6 million individuals in 2017; and patients with end-stage renal disease (ESRD) needing renal replacement therapy, including hemodialysis, peritoneal dialysis, and kidney transplantation, is estimated between 4.902 and 7.083 million(1). Numerous etiological factors have been associated with the development of CKD(2,3). Generally, ESRD patients on hemodialysis have a lower quality of life compared to the general population; this has been attributed to several factors including, physical limitation, psychological distress, sleep disturbances, comorbidities, and medication-related issues(4). Uremic pruritus (UP) also called CKD-associated pruritus is one of the most common complications of CKD. It is a prevalent, unpleasant, yet seldom fatal symptom. Over 70% of hemodialysis patients had pruritus, with 40% reporting at least moderate itching. The pruritus significantly lowers the quality of life for many people. The high proportion of pruritus that happens at night may cause sleep disruption. 60% of cases involved patients with significant itching and frequent sleep problems(5). The causes of UP are currently not fully obvious. Furthermore, The knowledge regarding patients receiving hemodialysis

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about UP is limited(6). Some pathogenetic factors such as histamine, calcium, magnesium, immunological dysregulation, neuropathy, and opioid imbalance have been proposed as potential contributors(7). Interleukin-31 (IL-31) is a cytokine that plays a crucial role in skin inflammation and has been implicated in pruritus. Several studies have proposed a connection between IL-31 and the pathogenesis of various dermatological and non-dermatological diseases; thus IL-31 has been linked with the development and maintenance of pruritus(8). Chronic pruritus with no apparent cause is one of the disorders where IL-31 has been linked to this type of pruritus(9). In addition, IL-31 has an obvious role in itching related to liver problems, especially those involving cholestasis(10). The possible mechanism for itching induced by IL-31 is that; humoral immunity reaction is induced by T-helper-2 cells, involving the secretions of some interleukins such as (IL-4, IL-5, IL-6, IL-10, IL-15, IL-16, and IL-31), then the sensory neurons in the skin were activated by IL-31, leading to itching(11). Finally, the pre-dialysis IL-31 levels were found to be elevated in CKD patients on hemodialysis compared to healthy individuals, and the levels in hemodialysis patients were associated with the development and intensity of UP(12,13). However, Haggag M et al. (2022) reported no association between serum IL-31 levels and UP in hemodialysis patients.

The study aimed to evaluate interleukin-31 serum levels in hemodialysis patients with and without UP.

Materials and Methods:

This cross-sectional study was conducted at the hemodialysis unit of Al-Fallujah Teaching Hospital in Fallujah City, Iraq, from July to November 2023. A total of 90 adult patients with CKD of both sexes [51 males, and 39 females].and were on a hemodialysis regimen three times weekly. The participants were divided into two groups: the first group consisted of 45 patients with UP, and the second group consisted of 45 patients without UP. The grouping of patients was based on the Itch Intensity Scale (ISS), where a score of 0 indicated no pruritus, 1 indicated mild pruritus, 2 indicated moderate pruritus (stressful but not interfering with regular activities or sleep), and 3 indicated severe pruritus (impacting regular activities or sleep) (14).Patients with the following conditions were excluded from the study: skin rash, primary skin disorders, systemic causes of pruritus (including polycythemia vera, chronic liver disease, thyroid and parathyroid diseases, malignancy, and neuropsychiatric disorders), or communication problems, as well as those on antipruritic therapy. Pre- and post-dialysis session blood samples (5 -ml) were collected from each participant. The pre-dialysis serum samples were used to measure the levels of urea, creatinine, calcium, phosphorus, intact parathormone, and IL-31; and the post-dialysis serum samples were used to measure the serum levels of IL-31 only.

All participants were informed about the aim and the expected benefits of the study; verbal consent was obtained from participants before being enrolled in the study.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Science (SPSS), version 26 software. Shapiro-Wilk test was used to check the uniformity of data distribution. The median and interquartile range (IQR) were used to present data on the continuous variables. Mann-Whitney test was used to compare the differences between medians of the two groups, and Wilikson test was used to compare the differences between medians of IL-31 before and after hemodialysis. Categorical variables were expressed as numbers (percent) and the difference was checked by the Chi-square test. Spearman's correlation was employed to assess the correlation among the studied variables. A *P*-value less than 0.05 was considered significant.

Results

Characteristics of patients: The age range for CKD patients without UP, who were recruited in this study, was 18 to 82 years, with a median (IQR) of [56 (24)]. On the other hand, the age range for CKD patients with UP was 18 to 79 years, with a median (IQR) of [53 (24)]. Table (1) shows that there is no age difference between CKD patients with UP and those without (P = 0.580). Of the 45 patients with CKD who did not have uremic pruritus, 27 (60%) were men and 18 (40%) were women; However, of the 45 patients who did have uremic pruritus, 24 (53.3%) were men and 21 (46.7%) were women. Table (1) shows that there is no gender difference (P = 0.523) across the research groups. Regarding the intensity of pruritus, among the 45 patients with CKD who had uremic pruritus, 5 (11.1%) had mild pruritus, 19 (42.2%) had moderate pruritus, and 21 (46.7%) had severe pruritus (P < 0.001; Table 1).

Table 1. Characteristics of patients

Variable	CKD without UP (n=45) No. (%)	CKD with UP (n=45) No. (%)	*P*-value
Age (Years)	[56 (24)]	[53 (24)]	0.580
Gender			
Male	27(60)	24 (53.3)	0.523
Female	18 (40)	21(46.7)	
Severity of pruritus			
Mild	-----	5(11.1)	0.001
Moderate	-----	19(42.2)	
Severe	------	21(46.7)	

Where; n=number; CKD=chronic kidney disease; UP=uremic pruritus

Some Biochemical characteristics: The current study found that there was a statistically negligible difference in the two groups' serum urea, serum Calcium, serum phosphorus, and serum intact

parathormone levels before dialysis. The median (IQR) of these tests is greater in CKD with UP than in those without UP. There were statistically significant differences in the serum Cr levels. (P = 0.02). Table (2).

Table 2. Some biochemical characteristics of patients before hemodialysis

Variable	CKD without UP (N=45) No. (%)	CKD with UP (N=45) No. (%)	*P*-value
Urea (mg/dL)	[127 (50.15)]	[127 (74.75)]	0.75
Creatinine (mg/dL)	[8.15 (3.18)]	[9.8(5.5)]	0.02*
Phosphorus (mmol/L)	[5.3 (1.65)]	[5.8 (3.3)]	0.18
Calcium (mg/dL)	[9 (0.8)]	[9.5 (1.4)]	0.08
Intact Parathormone (Pg/ml)	[15.85 (9.8)]	[17.78 (17.8)]	0.386

Where n=number; CKD=chronic kidney disease; UP=uremic pruritus
**P<0.05 was a significant difference*

Serum Interleukin-31 Level of Patients: In the pre-dialysis session, the current study revealed a statistically non-significant difference between the groups' measured serum IL-31 levels. The median (IQR) of this test is 1395.75(624.75) pg/mL for those without the UP group, while the median (IQR) is 1361.55 (741.96) pg/mL for those with the UP group. (*P=0.36*; Figure 1). In the post-dialysis, the current study revealed a statistically non-significant difference between the groups' measured serum IL-31 levels. The median (IQR) of this test is 879.7(84.19) pg/mL for those without the UP group, while the median (IQR) is 864 (164.58) pg/mL for those with the UP group. (*P*=0.831; Figure 1).

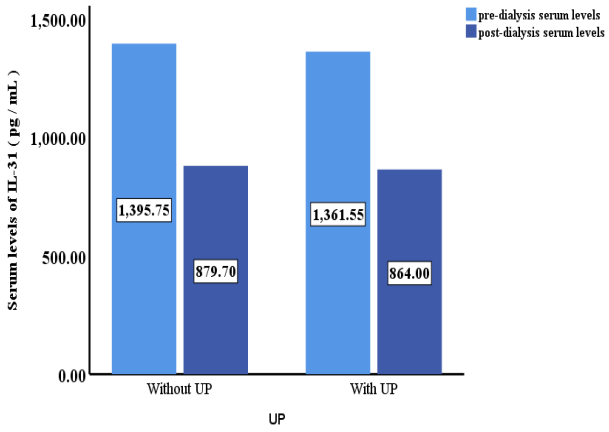

Figure 1: pre- and post- hemodialysis serum interleukin-31 levels of participants

Comparison between the levels of IL-31 before hemodialysis and after hemodialysis: The current study revealed a statistically significant difference between the groups' serum L-31 before hemodialysis and those tests after hemodialysis. The median (IQR) of these tests is higher in the pre-dialysis group than the post-dialysis [1435 (601.61) pg/mL and 873.45 (109.54) pg/mL, respectively; P <0.001].

Correlation studies between IL-31 and variables of all patients in the pre-dialysis session: Table (3) shows the correlations of serum IL-31 levels with the studied variables of the patients before the dialysis session. Regarding the IL-31 level, it showed no significant correlation with age, gender, urea, creatinine, calcium phosphorus, or intact parathormone (P>0.05; Table 3).

Table 3. Correlation of IL-31 with the studied variables of all patients before hemodialysis
**P<0.05 was significant.*

Parameters	Interleukin-31	
	spearman's correlation	*P*-value
Age(year)	0.4	0.71
Gender	-0.16	0.13
Urea (mg/dL)	0.051	0.636
Creatinine (mg/dL)	-0.202	0.057
Phosphorus (mmol/L)	-0.099	0.352
Calcium (mg/dL)	0.154	0.148
Intact parathormone (pg/mL)	-0.41	0.485

Discussion:

A variety of variables have been studied as possible causes of UP in dialysis patients, with varying degrees of outcome.(15,16). Multiple mechanisms have been hypothesized to explain the incomplete understanding of the exact pathophysiology of UP. (17,18).The present study aimed to measure serum levels of IL-31, during pre- and post-dialysis sessions, and to investigate the possible contribution of IL-31 in the pathogenesis of itch in patients on chronic hemodialysis. The impact of age and sex on the development of UP in CKD patients on chronic dialysis was inconsistent in the literature. According to one study, male sex and older age were risk factors for UP.(19). Conversely, another study found that among dialysis patients, UP is linked to younger age and female sex.(20). Other studies predicted that below this age range were susceptible to UP, due to many causes although these age ranges were not included in this study.(21–23).yet, clinical studies observed no association between sex and UP development among CKD patients (24,25).

The severity of pruritus in CKD patients can be mild, moderate, or severe(26).In an Indian study carried out on 120 eligible participants, (55.83%) of them had UP; the majority (73.1%) had mild pruritus, while moderate and severe pruritus was reported in (19.4%) and (7.5%) respectively(27). Another Chinese study

on 148 CKD patients receiving hemodialysis showed that (40.54%) of patients had UP; half of the patients with UP had moderate pruritus; while mild and severe pruritus was reported in (36.7%), and (13.3%) of the patients; respectively(26). Furthermore, a Pakistani clinical trial on 173 male patients on hemodialysis observed that (49.1%) of the patients had UP; (55.3%) of patients with UP had mild pruritus; while moderate and severe pruritus was reported in (34.1%), and (10.6%) of the patients respectively(28). In the present study, a higher percentage (46.2%) of patients with UP had moderate pruritus, followed by severe (42.2%), and mild (11.1%) pruritus. The difference in serum levels of urea, creatinine, phosphorus, calcium, and intact parathormone among CKD patients with and without UP was the subject of several studies, with very variable findings. Hu et al. (2019) reported higher levels of all of these analytes in patients with UP as compared to those without UP(29); Makhlough et al.(2014*)* reported elevated serum levels of blood urea nitrogen and intact parathormone only(30); while Tajbakhsh et al. (2013) reported abnormal serum calcium levels only(25). In the present study, only the serum creatinine levels of the pre-dialysis session samples were significantly higher in patients with UP. Elevation of serum creatinine levels has multiple reasons such as polycystic kidney disease(31). Several studies have proposed a connection between IL-31 and the pathogenesis of various dermatological and non-dermatological diseases; thus IL-31 has been linked with the development and maintenance of pruritus(8,32). The effect of IL-31 is mediated by binding to a heterodimeric receptor consisting of IL-31 receptor A (IL31RA) and Oncostatin M receptor (OSMR)(33). Activation of the IL-31 receptor triggers the activation of various signaling pathways, such as the JAK, STAT, or PI-3 kinase pathways. This subsequently affects a variety of different cell types, including epithelial cells, keratinocytes, peripheral sensory neurons, and the dorsal horn of the spinal cord(34).IL-31 inhibits the normal differentiation of keratinocytes, which are the predominant cells in the outermost layer of the skin. This inhibition of keratinocyte differentiation leads to epidermal thickening and an increase in trans-epidermal water loss(35). Furthermore, it has been observed that the overexpression or increased levels of IL-31 are associated with increased sensory neuronal outgrowth. This suggests a potential role for IL-31 in enhancing nerve fiber density and potentially contributing to the perception of pruritus(36). The role of IL-31 in UP has gained a lot of attention; most of the studies in this regard have reported an association between serum IL-31 levels and the development as well as intensity of UP(12,13). However, Haggag et al. (2022) reported no association between serum IL-31 levels and UP in hemodialysis patients(37). The finding of the latter occurs by the finding of this study; where there was no association between serum IL-31 levels and UP in both pre-and post-dialysis session samples. It is worth mentioning that Ardinata et al. (2021) showed that 6 weeks of acupuncture was associated with a reduction of pruritus dimensions in CKD patients on hemodialysis without altering serum IL-31 levels(38). Therefore, the role of IL-32 in UP needs further clarification. Secondary hyperparathyroidism is an important feature of CKD–mineral and bone disorder and plays an important role in the development of bone disease and vascular calcification. The mechanism responsible for secondary hyperparathyroidism may be that: low levels of vitamin D and hypocalcemia resulted in stimulation of the parathyroid glands to secrete parathormone as a compensatory mechanism for maintenance of hemostasis (39). There were significant differences regarding intact parathormone levels in one clinical trial conducted on CKD patients on hemodialysis(40). The current study showed no significant difference between patients with and without UP regarding serum intact parathormone levels. The disparity of findings regarding prevalence, the severity of UP, and biochemical findings in CKD patients with and without UP among different studies may be attributed to the differences in the inclusion and exclusion criteria, the number of participants, the categorizing criteria of the severity of pruritus, duration since the onset of hemodialysis, and the type of dialysate and the semipermeable membranes used in hemodialysis. One limitation of the present study is the relatively low number of patients in the two study groups which might mask the association of IL-31 with UP. Another limitation of the study is the cross-sectional nature of the study which does not permit testing of the causal relationship between serum IL-31 levels and UP. Furthermore, the effect of diet on the biochemical findings, and the residual renal function difference among participants were not considered because of the lack of cooperation of patients. Another limitation of the current study is the relatively small sample size of patients that were enrolled from a single center may impact the statistical power. hence, a larger-scale, multicenter study is recommended to establish the present study's findings.

The strength points of the present study include controlling for clinical parameters and the measurement of the serum levels of the IL-31 pre- and post-hemodialysis session that enables the examination of the accumulation of this cytokine, as well as, the efficiency and adequacy of the hemodialysis procedure.

Conclusions

The association of serum IL-31 levels and UP in CKD patients on hemodialysis need further verification.

Authors' declaration:
We hereby confirm that all the Figures and Tables in the manuscript are ours. The project was approved by the University of Baghdad's College of Pharmacy's Ethics Committee gave its approval (RECAUBCP2572623 on 25/7/2023). Before the participation agreement was recorded, each participant was given information about the study's goals and anticipated advantages.
Conflicts of Interest: none
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Authors' contributions:
Study conception & design: (Mustafa Sh. Abdulqahar& Ali. A. Kasem). Literature search: (Mustafa Sh. Abdulqahar). Data acquisition: (Mustafa Sh. Abdulqahar). Data analysis & interpretation: (Mustafa Sh. Abdulqahar& Ali. A. Kasem). Manuscript preparation: (Mustafa Sh. Abdulqahar& Ali. A. Kasem). Manuscript editing & review: (Ali. A. Kasem).

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تقييم مستويات مصل إنترلوكين 31 لدى المرضى الذين يعانون من مرض الكلى المزمن على الديلزة الدموية مع أو بدون حكة يوريمية

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الخلفية: تم ربط الإنترلوكين -31 بتطور الحكة والحفاظ عليها في العديد من الأمراض الجلدية وغير الجلدية.
الأهداف: تقييم مستويات المصل انترلوكين-31 في مرضى غسيل الكلى الذين يعانون من أو بدون حكة يوريمية.
طرق البحث: شملت هذه الدراسة المقطعية تسعين مريضا بالغا مصابا بمرض الكلى المزمن يخضعون لغسيل الكلى. كان جميع المرضى المسجلين يخضعون لنظام غسيل الكلى ثلاث مرات أسبوعيا. تم تقسيم المرضى إلى مجموعتين كل منهما 45 مريضا. المجموعة 1 شملت أولئك الذين يعانون من الحكة والمجموعة 2 شملت أولئك الذين لا يعانون من الحكة. بناء على مقياس شدة الحكة. تم تقييم مستويات الإنترلوكين-31، الباراثورمون، واليوريا، والكرياتينين، والكالسيوم قبل جلسة غسيل الكلى. تم أيضا تقييم مستويات الإنترلوكين-31بعد جلسة غسيل الكلى.
النتائج: مصل ما قبل غسيل الكلى غير مختلف بين المرضى غير المصابين بحكة والمصابين بها. [1395.75(624.75) بيكوغرام/مل و1361.55 (741.96) بيكوغرام/مل، على التوالي؛ (قيمة الاحتمال = 0.36) من ناحية أخرى، كان لدى المرضى الذين يعانون من الحكة اليوريمية مستوى كرياتينين في الدم أعلى بكثير [9.8(5.5) ملغم/ديسيلتر] من المرضى الذين لا يعانون منها [8.15(3.18) ملغم/ديسيلتر] (قيمة الاحتمال = 0.02). لم يكن هناك اختلاف كبير في مستويات مصل الباراثورمون أو الكالسيوم أو اليوريا بين المرضى الذين يعانون من حكة أو بدون
كان متوسط قياس مصل الانترلوكين-31في المرضى الذين يعانون من الحكة والمرضى الذين لا يعانون منها مختلفا بشكل كبير، عند 1435 (601.61) بيكوغرام/مل و873.45 (109.54) بيكوغرام/مل، على التوالي، قبل وبعد جلسات غسيل الكلى.).
كان لدى المرضى الذين لا يعانون من الحكة مستوى انترلوكين-31أكبر بشكل غير ملحوظ في عينة مصل ما بعد غسيل الكلى [879.7(84.19) بيكوغرام/مل] من المرضى الذين يعانون من حكة [864 (164.58) بيكوغرام/مل] (الاحتمال = 0831).
الاستنتاج: مستويات المصل انترلوكين 31 لدى مرضى مرض الكلى في المرحلة النهائية الذين يعانون من الحكة اليوريمية ليس لها أي دور في الحكة.
الكلمات المفتاحية: اعتلال الكلية المزمن , ديلزة دموية, ، حكة ,الإنترلوكين 31, الحكة اليوريمية

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Research Article

The Total Antioxidant Capacity and its Relationship with Atherosclerosis Risk Factors in a Sample of Iraqi Individuals with Type 2 Diabetes Mellitus

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Abstract

Background: Diabetes mellitus is significantly related to cardiovascular disease, such as atherosclerosis. Antioxidants are essential in the prevention of atherosclerosis by a variety of mechanisms, which encompass the suppression of free radical production, inhibition of low-density lipoprotein oxidation, and prevention of atherosclerotic plaque formation.

Objectives: This study aims to evaluate the levels of total antioxidant capacity and malondialdehyde as oxidative stress indicators in Iraqi type 2 diabetes mellitus (T2DM) patients and investigate their relationship with atherosclerotic risk factors.

Methods: This case-control study took place between October 2023 to January 2024 at Al-Karkh General Hospital in Baghdad. The study included a total of 130 participants: 70 individuals diagnosed with T2DM and 60 healthy controls were recruited from relatives of patients attending the hospital and hospital employees who did not have T2DM. The two study groups were age-matched. Blood samples from both groups were analyzed to determine the following parameters: Lipid profile, total antioxidant capacity (TAC), Malondialdehyde (MDA) as an oxidative stress marker, glycated hemoglobin (HbA1c), and atherogenic indices (e.g. atherogenic index of plasma).

Results: The mean serum TAC in the T2DM group was significantly lower than the control group (46.9 ± 5.05 U/mL vs. 70.8 ± 4.71 U/mL). This indicates a highly significant difference between the groups. Additionally, in the T2DM group, statistically significant inverse correlations were observed between TAC and most of the measured lipid profile parameters, HbA1c, and atherogenic indices.

Conclusions: Low TAC may be a potential predictor of atherosclerosis in T2DM patients and highlight the relationship between oxidative stress, lipid metabolism, and thermogenesis.

Keywords: Antioxidant; Diabetes Mellitus Type 2; Glycated Hemoglobin; Malondialdehyde; Oxidative stress.

Introduction:

Diabetes mellitus is a persistent metabolic condition that impacts the processing of carbohydrates, fats, and proteins, due to insufficient insulin production, diminished insulin effectiveness, or a mix of both (1). It is identified by elevated blood glucose levels (2). The persistently elevated levels of glucose in the blood cause long-term damage and dysfunction in several organs, including the heart and blood vessels (3). Over the past decade in Iraq, diabetes has increased by 115%, from 19.6 cases per 1000 individuals in 2000 to 42.3 cases per 1000 individuals in 2015, marking a significant epidemic (4). Atherosclerosis is the predominant type of cardiovascular disease (CVD), characterized by the buildup of lipids and inflammation in the major arteries (5). Oxidative stress (OS) arises from an imbalance between free radical generation and antioxidant defense mechanisms (6). OS has been associated with various disorders, such as atherosclerosis, revealing the several routes via which oxidants cause cellular damage (7). Antioxidants are compounds that prevent the oxidation of various molecules through the scavenging of oxidants or the reduction of free radical production (8). The development and advancement of atherosclerotic plaques is promoted by elevated levels of OS (9). Total antioxidant capacity (TAC) is an important indicator of the body's total antioxidant defense system, which is vital in the fight against OS (10), and is the capacity of every antioxidant in plasma to scavenge free radicals (11). The existing epidemiological studies show that CVDs have associations with lower levels of antioxidants and higher levels of oxidants (12). Malondialdehyde (MDA), a result of the cellular oxidation of polyunsaturated fatty acids, is widely used as an oxidative stress biomarker. In T2DM, there has been a significant increase in MDA (13, 14).

The current study aims to evaluate the levels of total antioxidant capacity and malondialdehyde as oxidative stress indicators in Iraqi T2DM patients and investigate their relationship with atherosclerotic risk factors.

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Patients and Methods

This case-control study took place between October 2023 to January 2024 at Al-Karkh General Hospital in Baghdad. After the provision of informed written permission, 130 Iraqi individuals were grouped into: Group 1 (70 individuals with a confirmed diagnosis of T2DM), and Group 2 (60 healthy individuals as controls) were recruited from relatives of patients attending the hospital and hospital employees who did not have T2DM. The two study groups were age-matched. The study was approved by the College of Medicine/ University of Baghdad.

Exclusion criteria were: Type 1 DM, alcoholism, smoking, pregnancy, antioxidant supplement use, CVDs, renal disease, liver diseases, or other medical disorders that might affect the results, in addition to recent surgery.

Serum samples were analyzed for the following biochemical markers: Total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (TG). These parameters were assayed enzymatically on a fully automated Monarch 240 analyzer using Biorex Instruments, United Kingdom. Glycated hemoglobin (HbA1c) was measured by an automated instrument (Cobass C111, Germany). TG/5, the Friedewald formula, was used to determine very low-density lipoprotein (VLDL). Additionally, the atherogenic indices (AIs) were estimated as follows: The atherogenic coefficient (AC) and the atherogenic index of plasma (AIP) were calculated as Log (TG/HDL-C), and TG/HDL, respectively. Total antioxidant capacity (TAC) and MDA were estimated using enzymatic colorimetric and enzyme-linked immunosorbent assay (ELISA) methods, respectively. (Elabscience, USA, and Human, USA).

Statistical analysis:

The study employed SPSS® software version 26.0 on Microsoft Windows for statistical analysis. When comparing the difference between means of variables in the study groups, the student's t-test was used. Pearson's correlation coefficient was calculated to test the correlation between continuous variables. The scatter diagrams were used to show these correlations. Statistical significance was indicated by a P-value of less than 0.05.

Results

The mean age was 57.9 ± 5.85 years in G1, and 53.3 ± 6.16 years in G2. There were 40 and 30 males in G1, 28 females, 32 males and females in G2. G1 had a lower mean TAC level than the controls (G2). Significant differences were found between the mean levels of AIs of the two groups, with G1 showing higher levels than G2. The mean MDA was higher in G1 than G2. The mean values of the lipid profile were significantly higher in G1 (TG, LDL, HDL, and VLDL) than G2. There was no statistically significant difference in the mean TC between the two groups, table (1).

Table (1): Mean± SD values for biochemical parameters in the two study groups

Parameter (Mean ± SD)	Study Groups		P-Value
	T2DM -G1 (n = 70)	Controls - G2 (n = 60)	
HbA1c (%)	8.9 ± 1.55	5.3 ± 0.22	<0.001*
TAC (U/ml)	46.9 ± 5.15	70.8 ± 4.71	<0.001*
MDA (ng/ml)	40.0 ± 9.22	11.2 ± 3.36	<0.001*
TC (mmol/l)	4.8 ± 1.13	4.5 ± 0.93	0.67
TG (mmol /l)	2.8 ± 0.98	1.2 ± 0.52	<0.001*
HDL (mmol /l)	1.2 ± 0.21	1.6 ± 0.30	<0.001*
LDL (mmol /l)	2.9 ± 0.76	2.1 ± 0.45	<0.001*
VLDL (mmol /l)	39.7 ± 10.19	20.5 ± 9.59	<0.001*
AIP	0.3 ± 0.17	0.1 ± 0.18	<0.001*
AC	3.0 ± 0.65	1.8 ± 0.45	<0.001*

The Pearson correlations (r) between TAC and biochemical markers in G1 and G2 are shown in Table (2) and Figures (1 and 2). TAC shows significant negative correlations with HbA1c, AIs, and the majority of lipid profiles components (P < 0.01). As for HDL, the correlation was positive. TC was not significantly correlated.

Table (2): Pearson's correlation coefficient between TAC and biochemical markers in the study groups

Parameters	TAC (U/ml)	
	r	P
HbA1c (%)	- 0.75	<0.01*
MDA (ng/ml)	- 0.63	<0.01*
TG (mmol/L)	- 0.68	<0.01*
TC (mmol /L)	- 0.15	0.099
HDL (mmol /L)	0.59	<0.01*
LDL (mmol /L)	- 0.47	<0.01*
VLDL (mmol /L)	- 0.60	<0.01*
AIP	- 0.69	<0.01*
AC	- 0.64	<0.01*

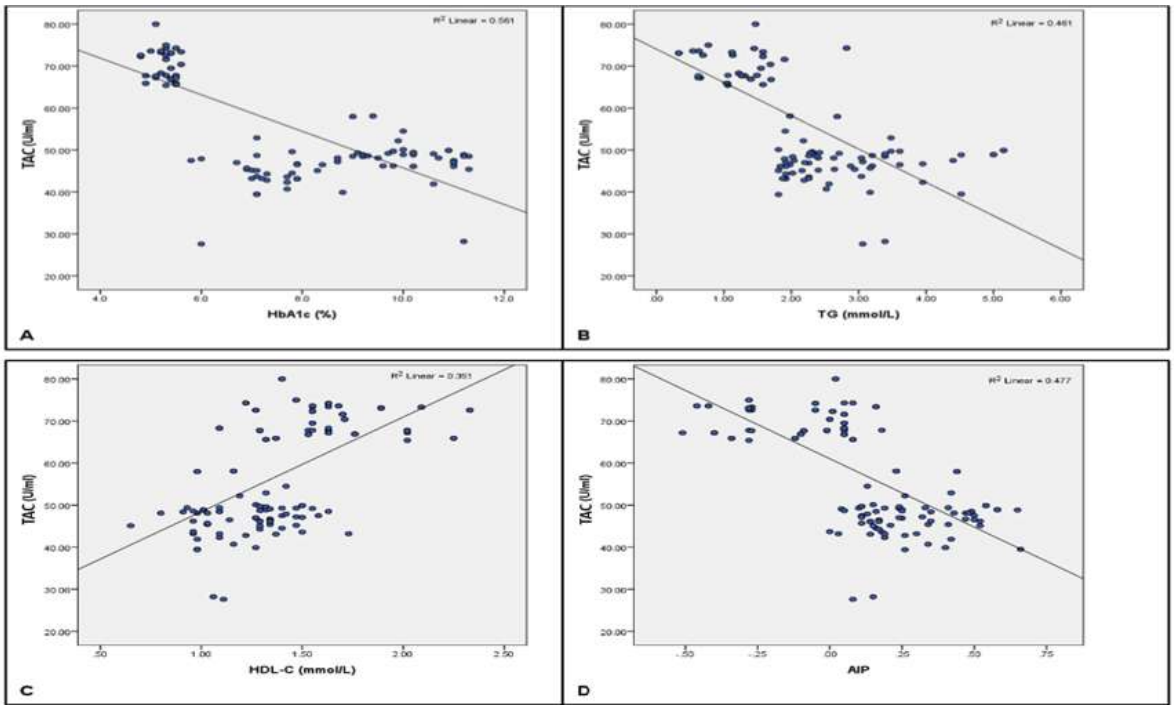

Figure 1: TAC correlations with HDL (r = 0.59), TG (r = - 0.68), HbA1c (r = - 0.75), and AIP (r = - 0.69)

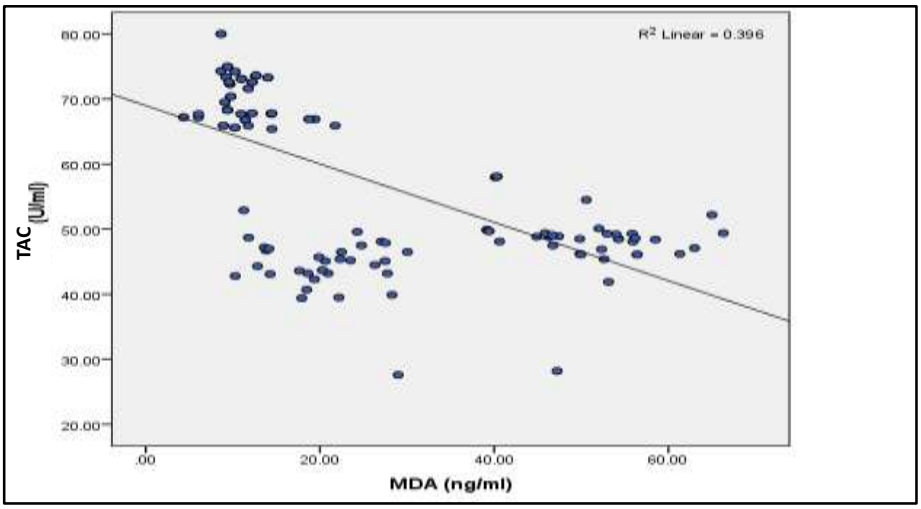

Figure 2: Negative correlation between TAC and MDA (r= - 0.63)

Discussion

The lower TAC and higher MDA in T2DM than the controls in the current study corroborate those of earlier studies by Mousa et al. (15) and Yarube et al. (16) which demonstrated increased OS and reduced antioxidant capacity in individuals with T2DM. Elevated levels of MDA indicate heightened OS among individuals with T2DM, possibly contributing to the development and advancement of atherosclerosis. According to a study by Mehri et al. (17), the TAC in individuals with CVDs was significantly lower than in healthy people. Additional studies have indicated the possibility of a correlation between reduced overall antioxidant capacity and CVDs (18). In addition, Kumar et al., indicated that patients with T2DM have substantially elevated MDA levels (19). Mahreen et al. (20), provided evidence that MDA may serve as a predictive indicator for diabetic complications, as it was found to be substantially elevated in T2DM patients who suffered complications as opposed to those without. These studies collectively support the potential link between reduced TAC, elevated MDA, and atherosclerosis.

The findings of this study align with the common dyslipidemia pattern seen in individuals with T2DM as reported by Mazzone et al. (21). Previous studies have linked metabolic dyslipidemia in T2DM to an increased risk of CVDs and an accelerated development of atherosclerosis (22, 23). The current study corroborates the connection between increased levels of serum TG and the development of T2DM. This finding is consistent with earlier studies that

have shown this relationship in other populations (24, 25). High TG levels are acknowledged as a significant risk factor for CVDs (26).

Patients with T2DM have reduced HDL-C values, which may be attributed to obesity and hypertriglyceridemia (27). The mechanism by which HDL particles obtain free cholesterol from cells has been recognized as the basis for the atheroprotective effects associated with HDL (28, 29).

LDL is especially exposed to oxidation to form oxidized LDL (oxLDL), which plays a role in atherosclerosis development (30). Previously established correlations between elevated LDL-C levels and diabetes align with the results obtained in the current investigation (31). T2DM dysregulates lipoprotein metabolism, leading to elevated hepatic VLDL production and impaired clearance of intestinal chylomicrons and VLDL (32).

The findings of the current study of a significantly higher mean AI value in T2DM than controls can be related to an elevated risk of CVDs by greater atherogenicity in T2DM patients. The AIs marker, which is derived from lipid profiles, may enhance clinical risk assessment for CVDs, according to Acar et al. (33). Our results corroborate those by Fu et al., showing that T2DM patients have higher than normal levels of AIP (34). In addition, HbA1c levels were affirmed to be positively related to AIP, which may suggest that AIP can play a part in evaluating diabetes (35). Moreover, as compared with conventional lipid variables, AIP and AC were found to have a more useful effect on the likelihood of CVDs (36). These results provide evidence for the role that AIs may have in the development of atherosclerosis in T2DM.

Limitation

This case-control study with a moderate sample size (n=130) establishes associations but not causation. The short study duration (October 2023- January 2024) limits assessment of longer-term trends. The study population from one Baghdad hospital may not be generalizable to the entire Iraqi T2DM population or other populations. Additionally, selection bias may be present as the study compared T2DM patients to healthy controls, ideally a group with confirmed atherosclerosis would have been included for a more robust comparison.

Conclusions

The study concluded that individuals with T2DM have lower levels of TAC than healthy controls. This indicates increased oxidative stress, which is linked to atherosclerosis. The study also showed a relationship between low TAC and various risk factors for atherosclerosis, suggesting that low TAC could be a predictor of the atherosclerosis.

Authors' declaration:

We certify that every figure and table in the manuscript is a part of the present study. Additionally, permission has been obtained for the Figures and photos to be republished in conjunction with the text even though they are not related to the current study. Signing the permission based on ethical Considerations-Ethical Approval: According to code number 56, the project was given approval by the local ethics committee at the College of Medicine/University of Baghdad on (26/ 2/ 2024).

Conflicts of Interest: None

Funding: None

Authors' contributions:

Study conception & design: (Halla Gh. Mohamoud). Literature search: (Hasan H. Idan). Data acquisition: (Hasan H. Idan). Data analysis & interpretation:(Hasan H. Idan). Manuscript preparation: (Hasan H. Idan & Halla Gh. Mohamoud). Manuscript editing & review: (Marwa M. Talib).

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القدرة المضادة للأكسدة الكلية وعلاقتها بعوامل خطر تصلب الشرايين لدى الأفراد العراقيين المصابين بداء السكري من النوع الثان

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الخلاصة:
خلفية البحث: يرتبط مرض السكري النوع الثاني بشكل كبير بأمراض القلب والأوعية الدموية، مثل تصلب الشرايين. تعتبر مضادات الأكسدة ضرورية في الوقاية من تصلب الشرايين من خلال مجموعة متنوعة من الآليات، والتي تشمل منع إنتاج الجذور الحرة، وتثبيط أكسدة الدهون البروتينية منخفضة الكثافة، ومنع تكوين الخثرة الدموية داخل الشرايين.
الأهداف: تهدف هذه الدراسة إلى تقييم مستويات إجمالي القدرة المضادة للأكسدة والمالونديالديهايد كمؤشرات للإجهاد التأكسدي لدى مرضى السكري من النوع الثاني العراقيين، وكذلك التحقيق في علاقتهما بعوامل خطر تصلب الشرايين.
طرائق العمل: هذه الدراسة هي دراسة للحالات والعينة الضابطة أجريت في الفترة من أكتوبر 2023 إلى ديسمبر 2023 في مستشفى الكرخ العام بمدينة بغداد. شملت الدراسة 130 مشاركا، منهم 70 مصابا بالسكري من النوع الثاني و60 من الأصحاء. تم إجراء تحاليل لعينات الدم لتحديد المعايير التالية: فحص نسبة الدهون، إجمالي القدرة المضادة للأكسدة والمالونديالديهايد كعلامة للإجهاد التأكسدي، فحص مستوى السكر في الدم حيث تشمل (HbA1c)، ومؤشرات تصلب الشرايين (على سبيل المثال، مؤشر تصلب الشرايين في البلازما).
النتائج: أظهر مرضى السكري من النوع الثاني في العراق انخفاضا في مستويات إجمالي القدرة المضادة للأكسدة في مصل الدم إلى جانب ارتفاع مستوى المالونديالديهايد والعديد من عوامل خطر تصلب الشرايين، بما في ذلك الدهونات الثلاثية والكوليسترول البروتيني الدهني منخفض الكثافة ومؤشرات تصلب الشرايين. وتجدر الإشارة إلى وجود ارتباطات عكسية إحصائيا داخل مجموعة مرضى السكري من النوع الثاني بين إجمالي القدرة المضادة للأكسدة ومعظم نسبة الدهون في الدم، HbA1c، ومؤشرات تصلب الشرايين، باستثناء الدهون البروتينية عالية الكثافة حيث لوحظ وجود ارتباط موجب.
الإستنتاجات: قد يكون انخفاض إجمالي القدرة المضادة للأكسدة مؤشرا محتملا لتصلب الشرايين لدى مرضى داء السكري من النوع الثاني ويسلط الضوء على العلاقة بين الإجهاد التأكسدي واستقلاب الدهون وتصلب الشرايين.
الكلمات المفتاحية: مرضى السكري من النوع الثاني، تصلب الشرايين، مرضى القلب، القدرة المضادة للأكسدة الكلية، الجهد التأكسدي، انزيم مالونديالدهيد، نسبة الدهون في الدم

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Research Article

Assessment of Serum P53 Protein Level in Adult Patients with Acute Myeloid Leukemia in Correlation with Response to Treatment

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Abstract:

Background: Acute myeloid leukemia (AML) is an adult leukemia characterized by rapid proliferation of undifferentiated myeloid precursors, leading to bone marrow (BM) failure and impaired erythropoiesis. The p53 tumor suppressor protein regulates cell division and inhibits tumor development by preventing cell proliferation of altered or damaged DNA. It orchestrates various cellular reactions, including cell cycle arrest, DNA repair, and antioxidant properties.

Objectives: To investigate the relationship of P53 serum level with hematological findings, remission, and survival status in de novo AML patients.

Methods: This is a cross-sectional study that enrolled 63 newly diagnosed de novo AML patients, and 15 sex- and age-matched healthy persons as a control group. Serum P53 levels were assessed using the enzyme-linked immunosorbent assay (ELISA) technique before initiating induction chemotherapy. The study was performed between November 2022 and May 2023 at the Hematology and Bone Marrow Transplant Center of the Medical City Complex in Baghdad.

Results: There were significantly lower P53 serum levels in AML patients before starting chemotherapy compared to the control group. However, no substantial difference in P53 levels was identified between AML patients achieving complete remission and those exhibiting no response, nor between alive and deceased individuals. Furthermore, there was a positive yet statistically non-significant correlation between serum P53 levels and age, and no significant relationship between P53 levels and sex or various hematological parameters.

Conclusion: P53 levels are low in AML patients. They are not associated with remission status or survival after six months and are not correlated with hematological values.

Keywords: AML; ELISA; Remission; Survival status; 53.

Introduction:

Acute myeloid leukemia (AML) is the predominant form of leukemia in adults. it is recognized by rapid proliferation of undifferentiated myeloid precursors (blasts) in both the bone marrow (BM) and peripheral blood (PB). This leads to BM failure and impaired erythropoiesis (1). The TP53 gene encodes the p53 tumor suppressor protein and is often called the "Guardian of the Genome." (2). The p53 protein functions as a tumor suppressor and transcription factor, governing cell division and inhibiting tumor development by preventing the proliferation of cells harboring altered or damaged deoxyribonucleic acid (DNA). It accomplishes this through orchestrating transcriptional control to induce apoptosis. In response to cellular stress or DNA damage, it elicits the activation of several transcriptional targets. The p53 protein orchestrates a diverse array of cellular reactions, metabolic changes, antioxidant properties, anti-angiogenic effects, autophagy, senescence, and apoptosis(3). As P53 plays a crucial role in hematopoietic stem cell activities, its abnormalities significantly impact the development, characteristics, and responsiveness to treatment of AML, often indicating a poor prognosis. Understanding the precise pathways responsible for p53 malfunction will provide valuable insights into the development of targeted treatments for AML (4).This study aimed to investigate the serum level of P53 in de novo AML patients to demonstrate its prognostic value and its relation to laboratory findings at diagnosis.

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Patients, Materials, and Methods:

This cross-sectional study was performed between November 2022 and May 2023 at the Hematology and Bone Marrow Transplant Center of the Medical City Complex in Baghdad on 63 patients who had just been diagnosed with de novo AML. They were selected using

a non-probability sampling method (sequential selection), and 15 healthy individuals, who were matched in terms of age and sex, as a control group. These individuals were conveniently selected from healthcare workers, friends, and relatives of the cases. They had normal complete blood counts and normal C-reactive protein, and did not complain from any illness. Patients under 18, those with secondary AML, AML-M3, other malignancies, pregnant or lactating women, and those with comorbid diseases were excluded from the study. A comprehensive assessment of the patient's medical records was conducted, focusing on the diagnosis, therapy, and any other relevant clinical data. Complete patient history and clinical examination were performed. The results of the patient's complete blood count (CBC), blood films, bone marrow aspirate (BMA), and flow cytometric immunophenotypic analysis were collected from patients' data records. Patients were assessed for response to remission induction therapy after one month of starting chemotherapy by assessment of CBC, PB, and BMA blast percentage. Patients < 60 years of age, received the 3+7 protocol, which consists of daunorubicin from days 1–3 and Cytarabine from days 1–7. Patients ≥ 60 years of age were treated with decitabine and oral venetoclax. Patients were split into two categories: The complete remission (CR) category, which included those who achieved CR [i.e., BM blast count <5%, no circulating blast cells, no extramedullary disease, absolute neutrophil count (ANC) ≥1.0 $\times 10^9$/L, platelet count (PLT) ≥100 $\times 10^9$/L], or CR with incomplete hematological recovery (CRi) (meeting all CR criteria except neutropenia <1.0 $\times 10^9$/L or PLT <100 $\times 10^9$/L), and the no response (NR) category (5).Patients were followed for up to six months to document their survival status and accordingly were divided into two groups based on whether they were alive or deceased. This study has obtained the approval of the Pathology Research Ethics Committee that of the College of Medicine, University of Baghdad (approval number: 144 on 4 October 2022). All participants involved in this study were informed, and their verbal consent was obtained before sample collection. Serum P53 level was determined by quantitative sandwich enzyme immunoassay ELISA technique using Human p53 tumor protein ELISA Kit, Catalog no. E1711Hu (BT LAB, China).

Statistical analysis

On version 26 of the Statistical Package for the Social Sciences (SPSS), a full explanation of each variable was made. The mean, standard deviation, and frequency (percent) were used to show the data, depending on the type of variable. The chi-square test was used to test the association between two variables. Both an independent sample t-test (for normally distributed data) and a Mann-Whitney U test (for non-normally distributed data) were used to find the difference between the two means of continuous variables. The Pearson correlation was used for data that was normally distributed and the Spearman correlation for data that was not normally distributed. A confidence level of 95% with a P-value equal to or less than 0.05 was considered significant.

Results

The mean age of the 63 AML patients was 50.0 ± 19.01 (Mean ± SD) years, ranging from 18 – 80 years. The male-to-female ratio was 1: 2.15 (20/43). The most prevalent clinical presentations were pallor (71%), and fever (68%) followed by bleeding (22%), splenomegaly (14%), hepatomegaly (11%), bone pain (9.5%), weight loss (8%), and lymphadenopathy (5%). There was a statistically significant lower mean P53 serum levels in AML patients when compared with the control group with a P-value of 0.001. The mean values for males and females were not statistically significant (Table 1).

Table 1: P53 levels in AML patients and controls and male and female patients

Characteristics	P53 (ng/L)(Mean ± SD)	P value ǂ
Control	1324.7 ± 1304.64	0.001
AML patients	586.0 ± 651.95	
Male (n=20)	450.7 ± 77.71	0.478
Female (n=43)	648.9 ± 782.25	

ǂ *Mann-Whitney U test*

The response to treatment after 28 days of starting chemotherapy was CR in 20 patients (31.7%), and NR in 32 patients (50.8%). Eight (12.7%) patients died during treatment and three patients (4.8%) died before treatment. The follow-up after six months to document the survival status revealed that 36 patients (57.1%) were still alive and 27 (42.9%) died. No statistically significant association was found between CR and NR categories with patients' sex (P= 0.213), and no significant difference in mean age (P= 0.609), Table 2. At the time when the cases were diagnosed with AML there was a statistically significant difference between the means of BM blast percentage in CR patients (38.5 ± 25.43) and NR patients (56.8 ± 28.62) with a P-value of 0.031. No statistically significant difference was observed between remission status and white blood cell count (WBC), hemoglobin (Hb), PLT, ANC, and PB blasts. No statistically significant difference was found in the mean serum level of P53 in AML patients between those who achieved CR and those with NR (P = 0.430).

Table 2: Distribution of CR and NR surviving AML patients by sex and difference between their mean hematological values

Variable		Remission				P value
		Complete remission		No response		
Sex	Male	9	45%	9	28.1%	0.213ǂ
	Female	11	55%	23	71.9%	
Age (years)		47.6 ± 21.60		50.6 ± 19.31		0.609*
WBC (×10^9 /L)		38.0 ± 48.31		39.7 ± 54.72		0.605ᵎ
Hb (g/dL)		8.5 ± 2.71		8.0 ± 1.93		0.371*
Platelets (×10^9/L)		58.0 ± 41.29		91.6 ± 82.71		0.058*
ANC (×10^9 /L)		7.0 ± 9.12		4.0 ± 6.14		0.585ᵎ
Peripheral blood blast (%)		39.8 ± 26.02		40.8 ± 30.40		0.992ᵎ
Bone marrow blast (%)		38.5 ± 25.43		56.8 ± 28.62		0.031ᵎ
P53 level (ng/L)		510.7 ± 176.95		688.7 ± 898.05		0.430ᵎ

ǂ *Chi-square test* **Independent t-test* ᵎ *Mann-Whitney U test*

Note: Eleven patients who passed away before or during treatment are not included because they did not receive or finish the induction chemotherapy.

After six months of follow-up, there was no statistically significant association between sex and or difference in mean age with survival status in AML patients (P-values = 0.815 and 0.200 respectively (Table 3). There were no statistically significant differences between the means of hematological parameters and P53 levels between alive and deceased patients (P-values > 0.05).

Table 3: The association of survival status with sex, and the differences in mean age, hematological parameters, and P53 levels between alive and deceased AML patients

Variables		Survival status after 6 months				P value
		Alive		Deceased		
		N=36	%	N=27	%	
Sex	Male	11	30.6	9	33.3	0.815ǂ
	Female	25	69.4	18	66.7	
Age (years)		47.4 ± 20.23		53.6 ± 16.96		0.200*
WBC (× 10^9/L)		33.3 ± 46.84		43.5 ± 54.82		0.917ᵎ
Hb (g/dL)		8.2 ± 2.47		7.9 ± 1.63		0.573*
PLT (×10^9/L)		68.3 ± 62.84		80.1 ± 72.23		0.739ᵎ
ANC (× 10^9/L)		5.2 ± 7.52		3.5 ± 6.22		0.151ᵎ
PB blast (%)		38.9 ± 25.73		46.3 ± 34.88		0.453ᵎ
BM blast (%)		49.1 ± 27.39		58.4 ± 31.26		0.199ᵎ
P53 level (ng/L)		579.3 ± 555.43		594.8 ± 773.37		0.117ᵎ

ǂ *Chi-square test* **Independent t-test* ᵎ *Mann-Whitney U test*

Table 4 shows positive but non-significant correlations between serum levels of P53 and age, Hb, PLT, and ANC in AML patients. There was a negative but non-significant correlation between serum levels of P53 and WBC, PB blast percentage, and BM blast percentage (P> 0.05).

Table 4: The correlations of P53 with age, and hematological parameters in 63 AML patients

Variable	P53 level	
	r	P
Age	0.116	0.366*
WBC	-0.007	0.959**
Hb	0.128	0.316*
PLT	0.146	0.255*
ANC	0.092	0.474**
PB blast %	-0.184	0.148*
BM blast %	-0.034	0.797*

**Pearson correlation* ***Spearman correlation*

Discussion

The mean age of AML patients was comparable to other Iraqi studies (6-8), and with British, Iranian, and Egyptian studies (9-11), respectively. The sex distribution of the patients in the current study revealed a female majority, which is consistent with another local study that reported a higher prevalence of AML in females (80%) compared to males (12). Other recent Iraqi studies have also revealed a modest female preponderance (13, 14), although AML is reported to be more frequent in males. The small sample size of the current study may have contributed to the differences in sex predominance. Regarding clinical characteristics at presentation, the current study found that the most common symptoms at the time of presentation were pallor, fever, and bleeding tendency, in addition to other complaints like bone pain and weight loss that were manifested to a lesser extent, which are in agreement with previous Iraqi studies (13, 15), and in studies from other countries (16, 17), which reported that pallor and fever were among the most common presenting symptoms in adult AML. The most frequent signs at presentation were splenomegaly, hepatomegaly, and lymphadenopathy, respectively, these results were consistent with an Iraqi (18), and an Indian (16) study. The CR in the current study is lower than that reported by Zayed, et al. in Egypt (19) (CR rate of 40%), Moulod, et al. in Iraq (20) (CR rate of 40%), Alwan, et al. in Iraq (CR rate of 69.5% in 115 patients diagnosed with de novo AML) (18), and Udupa, et al. in India (CR rate of 65.6%) (21). The discrepancies in the findings may be attributed to the different sample size, the risk stratification of these patients, and the efficiency of supportive care during the myeloablative period. In the present study, there was a significant decrease in the serum level of P53 in AML patients as compared to the control group, in agreement with an Egyptian study (22), which reported a significantly lower serum level of P53 in AML patients when compared with the control group, which was significantly increased after treatment when compared to its level before treatment. Unfortunately, we did not assess serum p53 after induction remission to compare the level before treatment in AML patients. In contrast to other Egyptian studies; El-Toukhy et al. in 2019 (11), and Abdel-Aziz in 2013 (23) reported a significant increase in the serum level of P53 at presentation by ELISA in AML patients. Suppression of the p53 level may occur as a result of other genes, such as P63, P73, among others, which may cause the inhibition, preventing an increase in P53 levels (24). There were no significant correlations between the serum level of P53 and the patients' age, or hematological parameters, and a non-significant association with sex. Similarly, El-Toukhy, et al. reported no statistically significant association between the age and sex of AML patients and P53 levels by ELISA. However, they found a strong relationship

between high P53 and Hb, BM blast percentage, and PB blast percentage (11).

Study limitation

The limitations of this study include a restricted sample size and a short duration of patients' follow-up.

Conclusions

P53 levels are low in AML patients. They are not associated with remission status or survival after six months or correlated with hematological values.

Authors' Declaration

We confirm that all the tables in the manuscript are ours. The project was approved by the Research Ethics Committee in the College of Medicine, University of Baghdad (issue number 144 dated 4 Oct 2022).

Conflict of interest: None

Funding: **None**

Authors' contributions

Study conception & design: (*Laith A. Jebur & Haithem A. Al-Rubaie*). Literature search: (*Laith A. Jebur*). Data acquisition: (*Laith A. Jebur*). Data analysis & interpretation:(*Laith A. Jebur*). Manuscript preparation: (*Laith A. Jebur & Haithem A. Al-Rubaie*). Manuscript editing & review: (*Haithem A. Al-Rubaie*).

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تقييم مستوى بروتين (p53) في المصل لدى المرضى البالغين المصابين بابيضاض الدم النخاعي الحاد وارتباطه بالاستجابة للعلاج

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الخلاصة:
الخلفية: يبرز سرطان الدم النخاعي الحاد (AML) كشكل بارز من أشكال سرطان الدم لدى البالغين، والذي يتميز بالانتشار السريع لسلائف النخاع الشوكي غير المتمايزة، والذي يبلغ ذروته في فشل نخاع العظم (BM) وتكوين الكريات الحمر المعرضة للخطر. يعتبر البروتين الكاتب للورم p53 هو البروتين الأساسي في علم الأمراض، والذي يلعب دورًا محوريًا في التحكم في انقسام الخلايا وإحباط تكوين الورم عن طريق وقف تكاثر الخلايا ذات الحمض النووي التالف أو المتحور. فهو ينسق مجموعة من الاستجابات الخلوية، بما في ذلك إيقاف دورة الخلية، وإصلاح الحمض النووي، ووظائف مضادات الأكسدة.. فهو ينظم التفاعلات الخلوية المختلفة، بما في ذلك إيقاف دورة الخلية، وإصلاح الحمض النووي، وخصائص مضادة للأكسدة.
الهدف من الدراسة: استكشاف العلاقة بين مستويات مصل P53والنتائج المختبرية لدى المرضى الذين تم تشخيص إصابتهم بسرطان الدم النخاعي الحاد (AML).
المنهجية: سجلت هذه الدراسة المقطعية 63 مريضًا تم تشخيصهم حديثًا بمرض سرطان الدم النخاعي الحاد (AML) إلى جانب 15 فردًا يتمتعون بصحة جيدة من حيث العمر والجنس والذين يعملون كمجموعة مراقبة. تم تقييم مستويات P53 في الدم باستخدام تقنية مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA)، سواء في مرضى سرطان الدم النخاعي الحاد قبل بدء العلاج الكيميائي التعريفي أو في المجموعة الضابطة.
النتائج: لوحظ انخفاض ملحوظ في مستويات مصل P53 لدى المرضى مقارنة بالمجموعة الضابطة، مما يشير إلى وجود فرق كبير ومع ذلك، لم يتم تحديد أي تباين كبير في مستويات P53 بين مرضى سرطان الدم النخاعي الحاد الذين حققوا استجابة كاملة وأولئك الذين لم يظهروا أية استجابة، ولا بين الناجين والأفراد المتوفين. علاوة على ذلك، كان هناك ارتباط إيجابي ولكن غير مهم إحصائيًا. بين مستويات P53 في الدم والعمر، في حين لم يتم اكتشاف علاقة معنوية بين مستويات P53 والجنس أو البارامترات الدموية المختلفة.
الاستنتاج: مستويات P53 بين مرضى سرطان الدم النخاعي الحاد منخفضة. وهي لا ترتبط بحالة سكون المرض أو البقاء على قيد الحياة بعد ستة أشهر، ولا ترتبط بقيم الدم.
الكلمات الدالة: ابيضاض الدم القوي الحاد، مقايسة الامتصاص المناعي المرتبط بالإنزيم، بروتين P53، حالة الشفاء، البقاء على قيد الحياة.

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Research Article

The Association between Leptin and Asprosin Levels in Female Patients with Type II Diabetes Mellitus

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Abstract:

Background: Leptin and asprosin are adipokines secreted by white adipose tissue. The leptin and asprosin molecules have many functions in the central nervous system and other functions of the body: Appetite, glucose metabolism, insulin resistance, and cellular death.

Objectives: The study aims to determine the potential relationship between leptin and asprosin hormones in female patients with type II diabetes mellitus.

Methods: The present study was conducted in Al-Mahmodia Hospital / Baghdad and the laboratories of the College of Science for Girls / University of Baghdad / Iraq, for the period from 1/11/2023 to 1/2/ 2024. This study is a comparative analysis of several essential biomarkers found in the sera of individuals with diabetes via estimating leptin, asprosin, fasting blood glucose, glycated hemoglobin, body mass index, total cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in females with type II diabetes mellitus. The study consisted of 60 participants grouped into: Group I (30 females with diabetes), and group II (30 healthy females). The Biochemical parameters of every participant were ascertained. The quantification of leptin and asprosin in the serum was conducted using the enzyme-linked immunosorbent assay (ELISA).

Results: The levels of leptin and asprosin were markedly high in the diabetic group [(5.1 ± 0.69), and (10.3 ± 1.07)] compared with the control group [(2.0 ± 0.48), and (1.6 ± 0.16)] respectively, with a significant difference.

Conclusion: Despite the high levels of leptin and asprosin in female patients with Type II diabetes mellitus, the relationship between leptin and asprosin was a weak negative one.

Keywords: Asprosin; Fasting Blood Glucose; Glycated hemoglobin; Leptin; Type II diabetes mellitus.

Introduction:

The principal sources of energy for the body are glucose and fatty acids; glucose can be converted to cholesterol and fatty acids. Glucose and lipid metabolism issues give rise to diabetes, cardiovascular disease, and a fatty liver [1]. Diabetes mellitus is a subset of metabolic disorders known as hyperglycemia caused by the poor secretion of insulin from pancreatic [2,3,4]. Type II diabetes mellitus (DMII) is responsible for 90% of all clinical cases of diabetes [5]. Dyslipidemia, hyperglycemia, and insulin resistance (IR) are all conditions associated with type II diabetes mellitus (DMII) [6]. DM impacts lipid, energy, and glucose metabolisms as a result of inadequate glycemic control and resistance to insulin. Furthermore, DM is associated with elevated concentrations of low-density lipoprotein (LDL), triglycerides, and free fatty acids (FFA) in the plasma, while the level of high-density lipoprotein (HDL) decreases. As a result of macroangiopathy and microangiopathy, diabetes-induced long-term hyperglycemia impacts numerous systems, resulting in diabetic nephropathy, retinopathy, neuropathy, and atherosclerosis [7].Adipose tissue functions as a constituent of the endocrine glands, which generate a variety of signaling molecules that govern metabolism, energy expenditure, digestion, production, endocrine function, and immune system functionality [8]. Recent research has devoted a substantial amount of attention to examining the functions of adipokines, particularly asprosin and leptin, in the regulation of metabolism, given their emergence as noteworthy variables of interest [9]. Adipokines are involved in a multitude of physiological processes, encompassing fullness of stomach and appetite regulation, insulin sensitivity, adipogenesis, energy metabolism in insulin-sensitive tissues, endothelial function, maintenance of blood pressure, energy expenditure activity, hemostasis, and fat distribution within pancreatic cells [10]. Leptin is secreted primarily by adipose tissue [11], and it functions as a vital hormone in the regulation of energy balance. Leptin deficiency is associated with weight gain, increased food consumption, and adipose tissue accumulation [12,13]. Leptin's effects on improving glucose homeostasis are mediated either directly

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or indirectly by several target tissues, such as skeletal muscle, liver, central nervous system, and pancreas. Research indicated that subjects with diabetes have higher leptin levels than controls. Additionally, insulin levels, insulin sensitivity, age, sex, body mass index, central adiposity, and anti-diabetes therapy are all variables that influence leptin levels [14]. Asprosin, a new adipokine, is expressed and secreted primarily by white adipose tissue [15]. Asprosin is transported to the liver after being liberated from white adipose tissue. Hepatic gluconeogenesis is induced by asprosin, leading to elevated levels of circulating insulin and glucose. By traversing the blood-brain barrier, plasma asprosin directly stimulates orexigenic neurons via a cAMP-dependent signaling pathway, thereby inducing weight gain and stimulating appetite [16]. Numerous effects of asprosin have been documented on peripheral tissues and organs, as well as the central nervous system. Diabetes has been linked to elevated levels of serum asprosin, according to recent studies. Asprosin levels in the serum are significantly elevated in DMII patients compared to controls. It has also been shown that circulating asprosin is associated with fasting blood glucose and glycosylated hemoglobin [16]. This study aims to explore the relationship between leptin and asprosin in female patients with type II diabetes mellitus (DM) and in control subjects. Ultimately, we are trying to ascertain whether leptin and asprosin can serve as indicators for DMII. This finding may contribute to the development of innovative strategies to combat DMII and improve the lives of individuals affected by this prevalent metabolic disorder.

Participants and Methods

Thirty blood samples were collected from female patients with type II diabetes, and 30 blood samples were collected from female controls, in Al-Mahmodia Hospital / Baghdad and the laboratories of the College of Science for Girls / University of Baghdad / Iraq, for the period from 1/11/2023 to 1/2/ 2024. The ages of the patients ranged from 30 to 60 years. The controls were volunteers from the College of Science and Al-Mahmodia hospital workers. They were age-matched with the cases. Data was collected through face-to-face interviews with the patients and controls using a questionnaire designed for the study. Five ml of venous blood was drawn from the subjects after a period of 10 to 12 hours fasting using a 5 ml syringe. One milliliter of the blood was placed in a tube containing Ethylene diamine tetra-acetic acid (EDTA) to conduct the HbA1c test, and 4 ml were placed in a gel tube not containing an anticoagulant. The blood components were then separated in a centrifuge at a speed of 3000 rpm for 15 minutes for the purpose of conducting biochemical tests. A fasting blood glucose level and lipid profile assessment were conducted on 2 ml of the serum, while an evaluation of liver function (ALT, AST, and ALP) was conducted on 1ml of the serum. The remaining serum was transferred to Ependorf tubes and frozen at -20°C to quantify leptin and asprosin later by using (ELISA) technique.

Inclusion Criteria

1. Diabetes medical history: Each of the individuals was already diagnosed with type II diabetes mellitus, based on criteria of the Expert Committee on Diabetes Mellitus of the World Health Organization.
2. Patients between 30 and 60 years old.

Exclusion Criteria: Patients who are obese.

Results

Table (1) shows the mean ± SD values of leptin, asprosin, and body mass index (BMI) for the two study groups. A significant difference (p=0.0001) was found in leptin, and asprosin levels between patients and control groups [(5.1 ± 0.69), (2.0 ± 0.48), and (10.3 ± 1.07), (1.6 ± 0.16)] respectively. The mean BMI values did not differ significantly (p=0.811) between patients and controls.

Table 1: Mean ± SD levels of leptin and asprosin hormone in DMII patients and controls

Parameters	DMII Group (N=30)	Control Group (N=30)	P-value
Leptin (ng/ml)	5.1 ± 0.69	2.0 ± 0.48	0.001
Asprosin (ng/ml)	10.3 ± 1.07	1.6 ± 0.16	0.001
BMI (Kg/m^2)	28.1 ± 2.69	26.9 ± 2.79	0.811

Table (2) shows that females with DMII had significantly different FBG, HbA1c, TG, and HDL levels than the healthy females (p-value ≤0.05), while the mean levels of total cholesterol, and LDL showed no significant difference (P-value ≥0.05) between patients and controls.

Table 2: Mean ± SD of FBG, HbA1c, and lipid profile of the study groups

Parameters	(DMII) Group (N=30)	Control Group (N=30)	P-value
	189.4 ± 54.59		0.001
			0.001
FBG (mg/dl)	8.3 ± 1.65	93.1 ± 7.75	0.055
HbA1c %	208.6 ± 39.54	5.0 ± 0.51	0.002
Cholesterol (mg/dl)		178.5 ± 24.38	0.024
Triglycerides (mg/dl)	185.5 ± 50.06	112.7 ± 23.03	0.618
HDL (mg/dl)		44.6 ± 8.61	
LDL (mg/dl)	31.7 ± 6.43	109.5 ± 20.03	
	119.9 ± 16.77		

The mean ± SD values of Alkaline Phosphatase (ALP) showed a significant difference at (p<0.05) when comparing patients and controls. There was no significant differences between mean Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) levels between the study groups (P > 0.05), as shown in Table (3).

Table 3: Mean ± SD levels of liver enzymes in the DMII and control groups

Parameters	(DMII) Group (N=30)	Control Group (N=30)	P-value
AST (U/L)	19.7 ± 6.08	18.0 ± 5.67	0.688
ALT (U/L)	14.5 ± 4.78	12.7 ± 3.56	0.169

ALP (U/L)	77.4 ± 19.66	72.1 ± 9.95	0.004

As shown in Figures 1 and 2, a weak negative non-significant correlation was found between leptin and asprosin in both study groups (r = -0.095, p - value = 0.616) and (r = -0.023, p -value = 0.904) respectively.

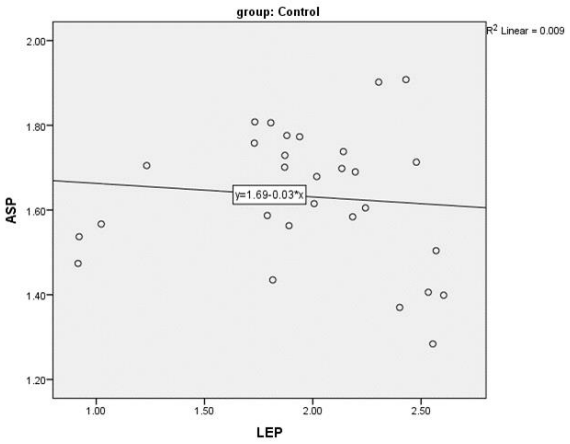

Figure 1: The correlation between leptin and asprosin in the control group

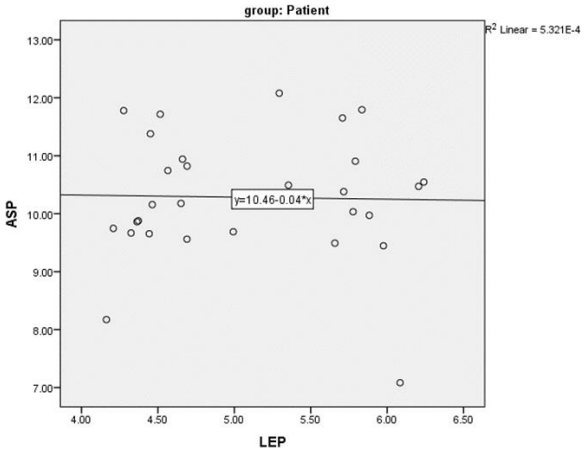

Figure 2: The correlation between leptin and asprosin in the DMII group

Discussion

The higher levels of leptin and asprosin in the DMII than the control group are in agreement with the findings of Peng et al, Katsiki et al, and Hameed et al [12,13,17], but are in disagreement with the findings of Onyemelukwe, et al [14]. The results of the current study and those of Peng, et al, suggest that the high levels of leptin may be related to its action of reducing glucose levels. Leptin can reduce blood glucose levels autonomously, specifically in hyperglycemic models of insulin deficiency [12]. The regulatory functions of leptin regarding energy expenditure and food intake via central signaling pathways are well-defined. Leptin transmits signals to the hypothalamus and hindbrain, which are central to inducing a reduction in food intake and an increase in energy expenditure [13,17,18]. Furthermore, central signaling pathways may be utilized by leptin to exert significant glucoregulatory and insulin-sensitizing effects [12,18]. The direct influence of leptin on neurons that express pro-opiomelanocortin (POMC) has been shown to contribute to the glucoregulatory effects of leptin signaling, thereby expanding upon these findings. Leptin receptor selective re-expression in POMC-expressing central neurons reduces circulating glucagon concentrations and ameliorates dyslipidemia, hepatic insulin resistance, and blood glucose. Moreover, by influencing lipid metabolism, leptin may potentially contribute to enhancements in insulin sensitivity [18]. The current study has also shown that the levels of asprosin hormone were also significantly higher in the DMII than in the control group. This agrees with the findings of Farrag et al and Naiemian et al [19,20]. Research has demonstrated that asprosin inhibits β-cell autophagy via the adenosine monophosphate-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signaling pathways, thereby inducing β-cell apoptosis [21]. Asprosin may cause β-cell dysfunction and impaired glucose tolerance in patients with DMII, according to a Wang et al [21]. Romere et al revealed that asprosin elevates the concentration of glucose in the circulation by promoting hepatic gluconeogenesis [22]. Since obese DMII cases were excluded from the study groups, the mean BMI was not significantly different between the DMII female patients and their controls. The current study showed that the serum levels of Cholesterol, TG, and, LDL were higher in the DMII group than the control group, while serum HDL level was lower in patients compared to controls. This is in agreement with another study that showed that patients with DMII had elevated levels of all lipid markers except HDL [23]. It had been suggested that the elevation in triglyceride levels could be attributed to an insufficiency of insulin, leading to impaired glucose utilization, hyperglycemia, and fatty acid mobilization from adipose tissues. Blood glucose is not utilized by tissues in individuals with diabetes, leading to hyperglycemia. The fatty acids are mobilized from the adipose tissue for energy production and surplus fatty acids are accumulated in the liver, and are converted to triglyceride [23]. Insulin stimulates the production of LDL receptors; therefore, chronic insulin deficiency may be linked to a reduction in LDL receptor levels. This results in the elevation of LDL particles and a subsequent rise in LDL-cholesterol levels in individuals with diabetes mellitus. Liver enzymes (ALT and AST) levels were not significantly different when comparing DMII patients and their controls. However, a significantly higher ALP level was found in fasting serum samples from diabetic patients when compared to the control group. 4. Few prior studies have documented that a considerable number of diabetics may also manifest elevated levels of ALP [24], which may be due to the direct hepatotoxic impact of fatty acid on the liver when it is excessively produced. Potential mechanisms underlying this phenomenon encompass high-concentration cell membrane disruption, mitochondrial dysfunction, toxin formation, as well as activation and inhibition of crucial

metabolic regulation steps [24]. Accordingly, individuals diagnosed with diabetes exhibit metabolic abnormalities that cause raising leptin and asprosin levels.

Conclusions

Serum levels of leptin and asprosin are higher in patients with DMII. A weak negative correlation exists between leptin and asprosin in those patients. Leptin and asprosin may influence glucose levels; thus, they may play a significant role in the diagnostic and therapeutic management of diabetes.

Authors' Declaration

We confirm that all the Figures and Tables in the manuscript belong to the current study. Authors sign an ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee in (The project was approved by the local ethical committee in College of Science for Women/University of Baghdad/Iraq) according to the code number (67621) on (8/ 11/2023).

Conflicts of Interest

The authors declare no conflict of interest.

Funding/Non.

Authors' Contributions

Duha Q.Bakr and Ahmed Y.Abed contributed to the design and implementation of the research, to the analysis of the results, and the writing of the manuscript.

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العلاقة بين مستويات اللبتين والاسبروسين لدى الإناث المصابات بداء السكري من النوع الثاني

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الخلاصة:
خلفية البحث: اللبتين والأسبروسين هي عبارة عن أديبوكينات تفرزها الأنسجة الدهنية. تلعب جزيئات اللبتين والأسبروسين العديد من الأدوار في الجهاز العصبي المركزي ووظائف الجسم الأخرى: الجوع وإستقلاب الجلوكوز ومقاومة الإنسولين (IR) وموت الخلايا.
الأهداف: معرفة العلاقة المحتملة بين هرمون اللبتين وهرمون الأسبروسين لدى الإناث المصابات بداء السكري من النوع الثاني DMII.
المنهجية: أجريت الدراسة الحالية في مستشفى المحمودية \ بغداد ومختبرات كلية العلوم للبنات \ جامعة بغداد \ العراق خلال الفترة الزمنية من 1\11\ 2023 إلى 1\2\2024. صممت هذه الدراسة لمقارنة بعض المؤشرات الحيوية في أمصال مرضى السكري من خلال تقدير هرمون اللبتين والأسبروسين ونسبة الجلوكوز في الدم الصائم والهيموجلوبين السكري ومؤشر كتلة الجسم والكوليسترول والدهون الثلاثية والبروتين الدهني عالي الكثافة والبروتين الدهني منخفض الكثافة وناقلة أمين الأسيارتات وناقلة أمين الألانين والفوسفاتيز القلوي لدى الإناث المصابات بداء السكري من النوع الثاني. تكونت مجموعة الدراسة من 60 عينة تم تقسيمها إلى مجموعتين: المجموعة الأولى (30 أنثى مصابة بداء السكري) والمجموعة الثانية (30 أنثى سليمة). تم تحديد المتغيرات الكيميائية الحيوية في جميع المشاركات. تم قياس تراكيز مصل اللبتين والأسبروسين باستخدام مقياس الإمتزازالمناعي المرتبط بالإنزيم.
النتائج: كانت مستويات هرموني اللبتين والأسبروسين مرتفعة بشكل ملحوظ لدى مجموعة المرضى (0.69 ± 5.1) و (1.07 ± 10.3) مقارنة بالمجموعة الضابطة (0.48 ± 2.0) و (0.16 ± 1.6)، على التوالي مع وجود فرق معنوي
الإستنتاجات: على الرغم من إرتفاع مستويات هرمون اللبتين والأسبروسين في الإناث المصابات بداء السكري من النوع الثاني إلا أن العلاقة بين اللبتين والأسبروسين كانت سلبية ضعيفة.
مفتاح الكلمات: داء السكري من النوع الثاني، اللبتين، الأسبروسين، جلوك وز الدم الصائم، الهيموجلوبين السكري.

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Research Article

Impact of DASH System on the Diet Pattern of Patients after Recovery from Myocardial Infarction

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Abstract:

Background: One of the top three causes of death and morbidity is cardiovascular disease Myocardial infarction is a clinical illness defined by quickly growing severe myocardial ischemia. It is the most frequent health problem in the world and the main cause of mortality.

Objectives: The study aims to assess the dietary pattern of food intake frequency for patients with post-myocardial infarction.

Methods: The quasi-experimental design has been carried out to determine the impact of the Dietary Approaches to Stop Hypertension system on patients with post-myocardial infarction, in the Al-Diwaniyah Teaching Hospital's cardiac outpatient clinic for the period from 17th April 2023 to 3rd May, 2024. Nonprobability (purposive) sample of (60) patients were selected who recovering from myocardial infarction at AL Diwaniyah Teaching Hospital. The study instrument comprised of (3) parts: **part I**: demographic details of the patient, which included seven items. **Part II:** Data on clinical characteristics, consisting of (3) items and **Part III:** Evaluation of diet frequency consisting of (8) items.

Results: The study results showed that most of the food groups were (less than the Recommended Daily Servings) in pre-test, then became (Equal to the Recommended Daily Servings) in post-test in the study group. While most of the food groups were (Less than the Recommended Daily Servings) in pre-test and post-test in the control group. Also, there were statistically significant differences between the study and control groups in the post-test measurements regarding the evaluation of the dietary pattern of food intake frequency.

Conclusions: The DASH system was effective in improving the dietary pattern of patients after recovery from myocardial infarction. And in assessing food intake frequency and the dietary pattern. There were statistically significant differences between the study and the control group on the post-test measures.

Keywords: Dietary Approaches; Dietary Assessment; Dietary Pattern; Healthy Diet; Hypertension; Myocardial Infarction.

Introduction:

In the world, one of the top three causes of death and morbidity is cardiovascular disease (CVD). The frequency of chronic illnesses has increased along with life expectancy, and the mortality rate from heart disease has exceeded the 25% mark in the late 20th century. By 2025, it is predicted to reach between 35-60%. Furthermore, non-communicable illnesses have emerged as the primary cause of death in addition to changes in lifestyle (**1**)**.** Myocardial Infarction (MI) is the most prevalent kind of CVDs. It results from coronary artery blockage and myocardial ischemia, and it is a leading cause of death for patients with CVD **(2).** In Iraq, Yemen, Egypt, Lebanon, and Jordan, there is a relatively high mortality rate from CVDs especially acute MI. The age standard of cardiovascular death rate is are due to cardiovascular diseases (3).

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more than twofold in comparison with the United States. According to mortality estimates, approximately 25%-40% of deaths in these countries There are several causes and risk factors attributed to the manifestations and progression of CVDs, these relate to both modifiable and non-modifiable risk factors. Non-modifiable risk factors relate to inherited syndromes, and genetic components, they cannot be controlled (4). Modifiable risk factors often have to do with lifestyle choices and actions, such as eating poorly, not exercising, smoking, and drinking alcohol. The evidence shows that approximately 80% of CVDs can be attributed to these modifiable behavioral risk factors. Consequently, to address the growing burden of CVDs, the most logical intervention is the development of preventive strategies to control CVDs with modifiable risk factors (5). The development and prevention of CVDs, the leading cause of death globally, are significantly influenced by diet. Although, the majority of conventional epidemiology research has

concentrated on specific foods or nutrients, the increasing number of dietary program research has made it possible to consider the intricacy and synergy of nutrients and foods consumption (6). Dietary Approaches to Stop Hypertension (DASH) diet originated in 1990. Several studies were funded in 1992 by the National Institute of Health for the United Kingdom to see whether particular dietary treatments may help reduce hypertension and cardiovascular diseases (7). DASH is regarded as a dietary pattern that emphasizes plant-based protein over animal protein and is high in fruits, vegetables, whole grains, and low-fat dairy products. Originally designed to help persons with hypertension control their blood pressure, DASH is now more commonly advised for populations at high risk of cardiovascular diseases. There are well-established advantages of the DASH diet for lowering blood pressure and body weight, but no long-term clinical trials have evaluated the diet's impact on mortality or the risk of subsequent cardiovascular events **(8).** DASH diet is an example of a very healthy eating pattern. It is low in saturated fat and higher in fiber, potassium, calcium, and magnesium, and it has a reduced consumption of refined carbohydrates. It is also said to be able to control and improve a number of CVD risk factors, including dyslipidemia, hypertension, and glucose intolerance **(9).**

The study aims to:-

1) Assess the dietary pattern of food intake frequency for patients with post myocardial infarction.
2) Determine the impact of the DASH system on patients' dietary patterns after recovery from myocardial infarction.

Methods and Material:

Study Design: The quasi-experimental design has been carried out to determine the impact of the DASH system on patients with post myocardial infarction, with the application of pre and post- test approaches for the study and control group in assessing their dietary habits by adherence and compliance to healthy diet. which has been achieved for the period from 17th January 2023, to 3rd May, 2024.

Study Setting: The study was conducted in the cardiac outpatient clinics of the Al-Diwaniyah teaching hospital with approval from Al-Diwaniyah Health Directorate.

Study Sample: Sixty patients were chosen as a non-probability (purposive) sample, at Al-Diwaniyah teaching hospital, patients who underwent cardiac outpatient clinics and had medical records were those who had recovered from myocardial infarction for at least four weeks. The patients were allocated into two groups, the study group and the control group, each with thirty patients.

Inclusion criteria:

- Patients who are in stable condition.
- Sex: Both males and females.
- Patients who agreed to participate in the study.
- Patients who are free from psychiatric illness.
- Age is greater than or equal to 30 years, less than or equal to 70 years.

Exclusion criteria:

- Patients who do not read and write
- Patients who refused to participate in the program.

Ethical considerations and approvals:

Protecting the values and dignity of participants is one of the most fundamental criteria to follow when collecting data. The researcher received this approval from the Medical Research Ethics Committee of AL-Diwaniyah health directorate According to the code number (15) on the date (28/5/2023).

Instrument Construction: For the present study, a questionnaire was designed and developed by the researcher, the questionnaire was constructed by reviewing previous literature and related studies for myocardial Infarction and the DASH diet. The study instrument comprised of (3) parts:-

Part I: (Socio-demographic characteristics data): It has seven items that are connected to sociodemographic traits, such as age, gender, residential area, marital status, socio- economic status, educational level, and occupational status.

Part II: (Clinical characteristics data): this part is concerned with data obtained from myocardial Infarction patients by observation and interview. The data consist of (3) items: Medical history, Family medical history of myocardial Infarction, and The recovery period from myocardial infarction.

Part III: (Dietary assessment of food intake frequency): The instrument used for dietary assessment developed by (DASH Guideline National Heart, Lung, and Blood Institute, 2017), (Rastogi, et al, 2004). Food intake frequency was used to obtain a measure of dietary intake for each participant in the study and control group (pre and post program) by asking each patient to recall what they ate in the last 24 hours at the main servings and in between servings (snacks), This instrument consists of (8) food groups (Grains and grain products, 6 lists of foods, Vegetables, 12 lists of foods, Fruits, 10 lists of foods, Milk and milk products, 3 lists of foods, Meats and eggs, it consists of 4 list of food, Legumes, it consists of 5 list of food, Fats and oils, 4 lists of foods, and Sweets, 3 lists of foods).

Data Collection: The data collection was carried out through the interview and intervention technique for the study and control groups (pre and post program), the participants in the study group were exposed to the DASH system. Dietary assessment for each participant in the study and control groups to obtain a measure of dietary intake was carried out by a daily servings scale (1 servings, 2 servings, 3 servings, 4 servings, 5 and more, None), then computed total daily servings and compared with recommended daily servings (RDS) according to (DASH Guideline National Heart, Lung, and Blood Institute, 2017), comparison scale (Equal to RDS, Less than RDS, More than RDS). To obtain a measure of serving size, using household measures such as (cups, different sizes of dishes, tablespoon, and teaspoon). The data

were collected for the study sample in the period from 2nd June 2023, to 9th November 2023**.**

Statistical Data Analysis: The study data were analyzed using the statistical data analysis methodologies listed below using Microsoft Excel 2016 and the statistical package for social sciences (SPSS) version (26). Tables with frequencies and percentages are used in descriptive data analysis; inferential data analysis uses this method, which entails accepting or rejecting the statistical hypothesis, comprises: The Chi-Squared test was used to measure the degree of association between the study variables based on their type and to assess the independence distribution of the observed frequencies. Furthermore, the significant *P-value* for this study's comparison is ≤ 0.05.

Results:

Table (1) revealed that the majority of sample were in the same age group (45-54) years old (36.7%) in study group and (40%) in control group. Regarding gender, (63.3%) were males in both groups. In addition, (73.3%) of the study group and (66.7%) of the control group live in urban residential areas.

About marital status the results in table (1) indicated that (80% and 73.3%) of the two groups respectively were married. Regarding socio-economic status, the study group (satisfied to some extent; unsatisfied) had equal results (37.7%), were the control group equal to half (46,7) of them had unsatisfied response. Additionally, the results indicated that (36.7%) of the study group have primary school educational level, while for the control group the results indicated that (33.3%) of them are read and write. Regarding the occupational status, the results show that (40%) of the study groups are retired, while (36.7%) of the control group are housewives. The final result in this table was, as follows; 50% of study group were not smoking and the other 50% of them were smoker divided in cigarettes only (43.3%), and cigarettes and narghile smoking (6.7%) while in the control group 83.3% of them are non-smoker and 16.7% of them are smoking cigarettes only.

Table (1): Distribution for both Groups according to socio-demographic characteristics:

Socio-demographic Characteristic		Study Group n=30		Control Group n=30	
		F	%	F	%
Age (year)	35 – 44	5	16.7	5	16.7
	45 – 54	11	36.7	12	40
	55 – 64	6	20	6	20
	65 +	8	26.7	7	23.3
	Mean ±SD	55.1 ± 9.43		54.73 ± 8.94	
Gender	Male	19	63.3	19	63.3
	Female	11	36.7	11	36.7
Residential Area	Rural	8	26.7	10	33.3
	Urban	22	73.3	20	66.7
Marital Status	Single	0	0	3	10
	Married	24	80	22	73.3
	Divorced	0	0	1	3.3
	Widow	6	20	4	13.3
Socio-economic Status	Satisfied	8	26.7	4	13.3
	Satisfied to Some Extent	11	36.7	12	40
	Unsatisfied	11	36.7	14	46.7
Educational Level	Read and Write	6	20	10	33.3
	Primary School	11	36.7	10	33.3
	Intermediate School	5	16.7	2	6.7
	Secondary School	2	6.7	1	3.3
	Diploma	3	10	5	16.7
	Graduate	3	10	2	6.7
Occupational Status	Government Employed	9	30	8	26.7
	Self Employed	3	10	6	20
	Unemployed	0	0	2	6.7
	Retired	12	40	3	10
	House wife	6	20	11	36.7

F=frequency, %= percentage

Table (2) illustrates the clinical data of study sample. The study results of medical history (hypertension and diabetes), indicated that all the participants (study and control groups), in the study (100%) had hypertension; with diabetes in 50% of them. Regarding family medical history, those with such history represented (50% and 46.7%) of the study and control groups, respectively. Concerning recovery period from myocardial infarction, the results of study group showed that 50% had (8-9 weeks interval); in the control group (36.7%) had two period of recovery (6-7 weeks and 8-9 weeks) intervals respectively.

Table (2): Distribution for both groups according to clinical data:

Clinical Data				Study Group n=30		Control Group n=30	
				F	%	F	%
Medical History	Hypertension	Yes		30	100	30	100
		No		0	0	0	0
	Diabetes	Yes	Type 1	2	6.7	2	6.7
			Type 2	13	43.3	13	43.3
			Total	15	50	15	50
		No		15	50	15	50
Family Medical History		Yes	First Degree	15	50.0	14	46.7
			Second Degree	8	26.7	7	23.3
			Total	23	76.7	21	70
		No		7	23.3	9	30
Recovery Period from Myocardial Infarction			4-5w	0	0	2	6.7
			6-7w	8	26.7	11	36.7
			8-9w	15	50.0	11	36.7
			10-13w	7	23.3	6	20

F=frequency, %= percentage

Table (3): Shows the dietary assessment of the study sample responses at the pre and post-tests for the study group. This table indicates that most of the food groups were (Less than RDS**)** at the pre-test**,** then it became (Equal to RDS) at post-test regarding (grains & grain products, vegetables, fruits, milk & milk products and legumes). While the food groups were (More than RDS**)** at the pre-test**,** then it became (Equal to RDS) at the post-test regarding (Fats & Oils and Sweets).

Table (3): Assessment of the dietary pattern of food intake frequency at pre and post-test measurements (Study Group N=30):

No.	Food Group	Period	Dietary Assessment		
			Equal to RDS F (%)	Less than RDS F (%)	More than RDS F(%)
1	Grains & Grain Products	Pre-test	2(6.7)	28(93.3)	0(0)
		Post-test	27(90)	1(33.3)	2(6.7)
2	Vegetables	Pre-test	3(10)	26(86.6)	1(33.4)
		Post-test	25(83.3)	0(0)	5(16.7)
3	Fruits	Pre-test	5(16.6)	9(30)	16(53.4)
		Post-test	22(73.3)	2(6.7)	6(20)
4	Milk & Milk Products	Pre-test	1(33.3)	18(60)	11(36.7)
		Post-test	24(80)	3(10)	3(10)
5	Legumes	Pre-test	12(40)	7(23.3)	11(36.7)
		Post-test	19(63.3)	4(13.3)	7(23.7)
6	Fats & Oils	Pre-test	2(6.7)	4(13.3)	24(80)
		Post-test	29(96.7)	1(3.3)	0(0)
7	Sweets	Pre-test	12(40)	0(0)	18(60)
		Post-test	26(86.7)	0(0)	4(13.3)

F=frequency, %= percentage; RDS=Recommended Daily Servings

The dietary assessment in the pre-test and post-tests for the control group. This table indicates that most of the food groups were (Less than RDS) regarding (grains & grain products, vegetables, milk & milk products) and (More than RDS) regarding (fats, legumes and sweets) in the pre-test andpost-test as shown in Table (4)

Table (4): Assessment of the dietary pattern of food intake frequency at pre and post measurements (Control Group N=30):

No.	Food Group	Period	Dietary Assessment		
			Equal to RDS F (%)	Less than RDS F (%)	More than RDS F (%)
1	Grains & Grain Products	Pre-test	2(6.7)	23(66.7)	5(16.6)
		Post-test	1(3.3)	20(66.7)	9(30)
2	Vegetables	Pre-test	3(10)	21(70)	6(20)
		Post-test	3(10)	23(76.7)	4(13.3)
3	Fruits	Pre-test	4(13.3)	0(0)	26(86.7)
		Post-test	4(13.3)	0(0)	26(86.7)
4	Milk & Milk Products	Pre-test	2(6.7)	27(90)	1(3.3)
		Post-test	4(13.3)	23(76.7)	3(10)
5	Legumes	Pre-test	9(30)	4(13.3)	17(56.7)
		Post-test	11(36.6)	1(3.3)	18(60)
6	Fats & Oils	Pre-test	0(0)	3(10)	27(90)
		Post-test	0(0)	4(13.3)	26(86.7)
7	Sweets	Pre-test	2(6.7)	0(0)	28(93.3)
		Post-test	5(16.6)	0(0)	25(83.3)

F=frequency, %= percentage; RDS=Recommended Daily Servings

While there were statistically significant differences between the study and control group at post-test measurements regarding dietary pattern assessment of food intake frequency (Grains & Grain Products, Vegetables, Fruits, Milk & Milk Products, Legumes, Fats & Oils and Sweets) at *P. value <0.05*. as shown in Table (5)

Table (5): Comparison between study and control group according to dietary pattern assessment of food frequency intake at post measurements (N=60):

No.	Food Group	Group	Dietary Assessment			Statistical Measurements		
			Equal to RDS F (%)	Less than RDS F (%)	More than RDS F (%)	X^2 value	D.f	*P*
1	Grains & Grain Products	Study	27(90)	1(33.3)	2(6.7)	14.732	4	0.001
		Control	1(3.3)	20(66.7)	9(30)			
2	Vegetables	Study	25(83.3)	0(0)	5(16.7)	13.929	2	.0010
		Control	3(10)	23(76.7)	4(13.3)			
3	Fruits	Study	22(73.3)	2(6.7)	6(20)	18.462	2	.0010
		Control	4(13.3)	0(0)	26(86.7)			
4	Milk & Milk Products	Study	24(80)	3(10)	3(10)	31.186	4	.0010
		Control	4(13.3)	23(76.7)	3(10)			
5	Legumes	Study	19(63.3)	4(13.3)	7(23.7)	11.579	4	0.021
		Control	11(36.6)	1(3.3)	18(60)			
6	Fats & Oils	Study	29(96.7)	1(3.3)	0(0)	9.459	1	.0020
		Control	0(0)	4(13.3)	26(86.7)			
7	Sweets	Study	26(86.7)	0(0)	4(13.3)	9.231	1	.0020
		Control	5(16.6)	0(0)	25(83.3)			

F=frequency, %= percentage; RDS=Recommended Daily Servings; X^2= Chi square; D.f= Degree of freedom; P.= p. value.

Discussion:

Myocardial ischemia and coronary artery blockage result in MI, the most prevalent form of CVDs. Myocardial infarction is a leading cause of death for people with heart disease, despite better clinical treatment, greater awareness among the public, and widespread application of health advances (1). Therefore, the goal of current study was to assess the dietary pattern of food intake frequency for patients with post-myocardial infarction.

Patients' Socio-demographic Characteristics: Current results agreed with those of previous studies of Abdul-Ameer and Khuder (10), who found that 36.7% of patients with MI were within the age groups (40-49) and (50-59) years. in the study and control groups, respectively. Concerning gender, (63.3%) of study sample were males and (36.7%) were females, in both groups. This result agreed with the result of Hussein and Widad (9), who found that the majority of study sample were males. Concerning marital status, current results were similar to a previous study by Khasal and Atiyah (11), who found that the majority of study subjects are married. Regarding residential areas, current results agreed with the results of a previous study of Aldaggistany (12), and Kittan and Rajha (13) whose findings suggested that more participants are living in cities than in rural areas.

In socio-economic status, the study group (satisfied to some extent; unsatisfied) had equal results (37.7%), whereas the control group equal to (46.7%) of them had unsatisfied responses. The outcome of Herliani's (2) earlier investigation was in line with these findings, and Kittan and Rajha (13), who found that a high proportion of study groups has insufficient monthly income.

Concerning educational level, current results matched with the results of a previous study of Khasal and Atiyah (11), who found that the majority of study subjects can read & write and primary school graduates. In respect to the occupational status, current results showed that (40%) of the study group were retired, while (36.7%) of the control group were housewives. These results were consistent with the results of a previous study by Zaitsu (14), who found that a high proportion of the study groups were retired.

Clinical characteristics related to myocardial infarction patients from the study sample: Concerning medical history, current results were consistent with the results of a previous study of Abdul-hussain (15), who found that the majority of the study samples had an increase in blood pressure (the risky stage of hypertension). These results are also in agreement with the results of a previous study of Kittan and Rajha (13), who indicated that the majority of study participants' were suffering from diabetes. Regarding the family medical history, these results were in matching with the results of a previous study of Kadhim (16), also Sharif and Samir (17), who showed that more than half of the people in the study group and the control group have first-degree relatives who suffer from MI. Regarding the recovery period from myocardial infarction, the results of current study showed that in the study group, 50% had (8-9 weeks interval); in the control group (36.7%) had two period of recovery (6-7 weeks and 8-9 weeks) intervals, respectively. These results were consistent with the results of a previous study of Atrous (18), who indicated that the recovery period (2-3 months) is the best among MI patients who received the dietary program than patients who received dietary care in the hospital.

Dietary assessment of food intake frequency at pre- and post-test measurements: The outcomes of Novaković (19) support current findings, and Rastogi (20), who found that when compared to standard care at six months, adherence to dietary guidelines also improved the consumption of fruits, vegetables, grains, and legumes in a population of patients with MI at the pre-test and post-test, while the control group did not follow dietary recommendations regarding (Grains & Grain Products, Vegetables, Fruits, Milk & Milk
Products and Legumes).
Results presented in Table (5) were consistent with the results of a previous study of Jayawardena (21) who found that using the Chi-squared test, a significant difference was reported between the study and control groups at post-test measures of the appropriate daily intake of grains, vegetables, fruits, nuts, seeds, and legumes.
According to the researcher, applying a diet pattern rich in fruits, vegetables, fish, chicken, olive oil, legumes, and nuts to the study group has been shown to be a significant preventive factor against some disorders, such as AMI. The DASH diet plan has been recommended by several US health organizations as an effective nutritional strategy for the prevention and management of elevated blood pressure and CVDs. The protective effects of healthy dietary patterns on MI appear to be due to the sum of small dietary changes rather than the restriction of any single nutrient.
group members were done in small teams or face-to-face individual meetings.

Limitation of study:
Long duration for data collection. Also interviewing participants at the post-test was not easy, therefore, meetings for these group members were done in small teams or face-to-face individual meetings.

Conclusions:
Based on the findings of current investigation, the investigator can draw the following conclusions:
The DASH system is effective in improving patients' dietary pattern after recovery from myocardial infarction.
There were statistically significant differences between the study and control groups at post-test measurements regarding dietary pattern assessment of food intake frequency at *P* value <0.05.

Recommendations:
The research has recommended the following actions based on its findings, discussion, and conclusions:-
Including the DASH system in the treatment protocol for patients after recovery from MI.
Providing the patient with a booklet or guideline sheet that includes the instructions for the DASH system after recovery and being discharged from cardiac care unit.

Authors' Declaration:
We confirm that all tables and figures in the manuscript belong to the current study. Besides, the figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript; also we declare that the above research got the approval from the Medical Research Ethics Committee of AL-Diwaniyah health directorate According to the code number (15) on the date (28/5/2023).

Conflict of interest: none

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Authors' Contributions:
Sabri Shather Hadi: Study conception, study design, data collection, data analysis and interpretation. Khalida Mohammed Khudur: Drafting of the manuscript, Literature research and Critical revision.

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تأثير نظام داش على النمط الغذائي للمرضى بعد الشفاء من احتشاء عضلة القلب.

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خلفية البحث: أحد الأسباب الثلاثة الأولى للوفاة وانتشار المرض هو أمراض القلب والأوعية الدموية. احتشاء عضلة القلب هو مرض سريري يتم تعريفه بنقص تروية عضلة القلب الشديد. تعتبر المشكلة الصحية الأكثر شيوعًا في العالم والسبب الرئيسي للوفاة.
الهدف من هذه الدراسة: تهدف الدراسة إلى تقييم النمط الغذائي لتكرار تناول الطعام للمرضى بعد احتشاء عضلة القلب.
منهجية البحث: تم تنفيذ تصميم شبه التجريبي لتحديد تأثير نظام داش على المرضى بعد احتشاء عضلة القلب ، في العيادة الخارجية للقلب في مستشفى الديوانية التعليمي للفترة من 17 يناير 2023 إلى 3 مايو 2024. تم اختيار عينة احتمالية (غرضية) مكونة من (60) مريضا تعافوا من احتشاء عضلة القلب في مستشفى الديوانية التعليمي، وتم تقسيم العينة الى مجموعتين كل مجموعة تتكون من (30) كمجموعة دراسة و سيطرة . تكونت أداة الدراسة من (3) أجزاء: الجزء الأول: الخصائص الديموغرافية للمريض والمكون من (7) فقرات. الجزء الثاني: بيانات الخصائص السريرية ويتكون من (3) عناصر والجزء الثالث التقييم الغذائي لتكرار تناول الطعام ويتكون من (8) عناصر.
النتائج: أظهرت نتائج الدراسة أن معظم المجموعات الغذائية كانت (أقل من الحصص اليومية الموصى بها) في الاختبار القبلي، ثم أصبحت (تساوي الحصص اليومية الموصى بها) في الاختبار البعدي بالنسبة لمجموعة الدراسة. بينما كانت معظم المجموعات الغذائية (أقل من الحصص اليومية الموصى بها)) في الاختبار القبلي والبعدي بالنسبة لمجموعة السيطرة؛ كما كانت هناك فروق ذات دلالة إحصائية بين مجموعة الدراسة ومجموعة السيطرة في قياسات ما بعد الاختبار فيما يتعلق بتقييم النمط الغذائي لتكرار تناول الطعام.
الاستنتاجات: نظام داش كان فعالا في تحسين النمط الغذائي للمرضى بعد الشفاء من احتشاء عضلة القلب، وكانت هناك فروق ذات دلالة إحصائية بين مجموعة الدراسة ومجموعة السيطرة في القياسات البعدية فيما يتعلق بتقييم النمط الغذائي لتكرار تناول الطعام عند قيمة.
الكلمات المفتاحية: النظام الغذائي لوقف ارتفاع ضغط الدم ، التقييم الغذائي، النمط الغذائي، الغذاء الصحي، احتشاء عضلة القلب.

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Research Article

Assessment of the Correlation between Disease Activity and Serum Biomarker Anti-MCV and IL6 in Iraqi Patients with Rheumatoid Arthritis

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Abstract:

Background: Rheumatoid arthritis is an autoimmune disease characterized by autoantibodies against citrullinated antigens. The anti-cyclic citrullinated peptide test is commonly used to diagnose rheumatoid arthritis, whereas the anti-mutated citrullinated vimentin is another anti-citrullinated antibody that reacts with mutated citrullinated vimentin. Anti-mutated citrullinated vimentin antibodies have been suggested as a superior early arthritis diagnostic marker.

Objectives: This study aimed to evaluate the levels of IL6 and anti-mutated citrullinated vimentin biomarkers as well as to determine their potential correlation with disease activity in rheumatoid arthritis Iraqi patients.

Methods: The study included an overall sample of 120 individuals who were recruited from the Department of Rheumatology at Baghdad Teaching Hospital in Baghdad, Iraq, during the period from late August 2023 to early October 2023. They were subdivided into two primary groups. The first group consisted of 60 individuals diagnosed with RA who were further categorized based on disease activity. The second group consisted of 60 healthy individuals as controls. The age range of the participants was between 20 and 79 years. An enzyme-linked immunosorbent test was used to evaluate the blood level of anti-MCV and IL-6.

Results: There was no significant correlation between anti-mutated citrullinated vimentin and disease activity(p-value:0.374) also IL-6 and disease activity(p-value:0.792) but our findings showed there is a statistical accusation between Erythrocytes Sedimentation Rate, and C-reactive protein with disease activity(p-value:0.013 and 0.025) also a high positive correlation between anti-mutated citrullinated vimentin and duration of disease and anti-mutated citrullinated vimentin with Rheumatoid factor and no significant correlation between anti-mutated citrullinated vimentin and IL6.

Conclusion: anti-mutated citrullinated vimentin autoantibody shows a high correlation with the duration of disease and a positive correlation with Rheumatoid factor and has no significant correlation with disease activity.

Keywords: Anti-mutated citrullinated vimentin; Autoantibody; cytokines; IL6**;** Rheumatoid arthritis.

Introduction:

Rheumatoid arthritis (RA) is an autoimmune disease that is a polyarticular, inflammatory arthritis of the small joints of the body that are inflamed and symmetrically irrigated. This causes inflammation throughout the joints, which in turn causes morning stiffness, tenderness, pain, swelling, and reduced mobility (1). A patient's mental health and quality of life may be greatly impacted by long-term chronic pain that occurs repeatedly in each joint. This discomfort can also lead to joint deformities (2). Rheumatoid arthritis prevalence is steady at roughly 0.5-1.0% worldwide, while it is greater in particular communities, such as North American Indians. Rheumatoid arthritis may strike anyone at any age (3). Having a female-to-male ratio of 3:1, the medical issue is more common among women (4).

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Research has shown that the immune system plays a part in developing rheumatoid arthritis (RA) Inflammatory cytokines and enzymes that tear down cartilage and bone are released when immune cells infiltrate synovial joints, which is the first step in developing autoimmune disease (5). Immunoglobulin G's Fc region may be recognized by the Rheumatoid Factors (RF) antibody. In 1987, the ACR included RF in their criteria for classifying RA, and it was one of the first forms of autoantibodies found in the disease (6).Anti-citrullinated protein antibodies ACPA, a novel immunological marker for RA, are rapidly employed to give improved specificity and better prognostic signs for RA patients (7)**.** It has been suggested that ACPA activates immune cells and up-regulates the production of inflammatory cytokines, which might explain why inflammation is a key factor in the development of RA (8). A study by Mohammed et al. (2023) showed

that the diagnostic specificity of anti-CCP antibodies was greater in both early and established RA illness. Nevertheless, anti-MCV Exhibited enhanced sensitivity in detecting early rheumatoid arthritis compared to anti-CCP2. Utilizing anti-MCV antibodies to test for rheumatoid arthritis (RA) may assist in identifying the illness at its early stage. This can help choose the most appropriate first treatment, with more aggressive regimens being reserved for those with elevated levels of anti-MCV antibodies and predicted to have a severe and deforming course of the disease (9). One of the many members of the cytokine family that exhibits pleiotropic and redundant functional activity is the cytokine known as interleukin-6 (IL-6), which is considered to be the prototype kind (10). Interleukin-6 (IL-6) indicates that it has the potential to be a therapeutic target for the treatment of rheumatoid arthritis (RA). Both tocilizumab and sarilumab, which are both IL-6 inhibitors, have shown considerable effectiveness and safety in patients with rheumatoid arthritis who have not responded well to csDMARDs or tumor necrosis factor-alpha inhibitors. It is possible to take these drugs by themselves or in combination (11)**.** Thus the current study aimed to investigate the levels of IL6 and anti-mutated citrullinated vimentin biomarkers as well as to determine their potential correlation with disease activity in rheumatoid arthritis Iraqi patients.

Methods

Study population: A total of 120 individuals participated in this case-control study; 60 of them were diagnosed with rheumatoid arthritis (RA), while the other 60 served as healthy controls. The controls were selected to closely match the patients in terms of age and sex. The rheumatology outpatient clinic at Baghdad Teaching Hospital was the location where the patients were enrolled in the study between late August 2023 and early October 2023. A total of 60 Iraqi patients with RA were diagnosed by a rheumatologist using either the criteria established by the American College of Rheumatology (ACR) in 1987 or the criteria established by the European Alliance of Associations for Rheumatology (ACR-EULAR) in 2010. All patients were on treatment with different type of treatment. The inclusion criteria were adults over 18 years of age with either early or established rheumatoid arthritis, while exclusion criteria encompassed children and individuals with other autoimmune disorders such as systemic lupus erythematosus (SLE) and multiple sclerosis. A comprehensive medical history was collected for each participant, documenting basic demographic and clinical data, including age, gender, weight, height, family medical history and the duration of the disease. Body mass index (BMI) was calculated for all participants. The disease activity (SDAI score) was used to classify the patients into three different categories; mild, moderate, and severe, according to the disease activity of their condition. The SDAI is based on four variables: the patient's whole health condition on a visual analog scale from 0 to 10, the number of painful joints (0-28), the number of swollen joints (0-28), and C-reactive protein these four variable was used to elevate the disease activity. A control group of 60 persons who were in apparently good health and had no familial history of autoimmune illness were randomly selected.

Statistical analysis

For numerical variables that follow a normal distribution, the descriptive statistical analysis used the Mean±SD, whereas for variables that do not, the median (interquartile range) was employed. For the categorical data, percentages, and rates were computed. Using the Chi-squared test, we looked at the correlation between anti-MCV and several socio-demographic variables. To compare the means of the samples, we used the independent student *t*-test. Calculated the area under the curve (AUC) using the ROC curve to calculate the sensitivity and specificity of Ant-MCV. To find the factors that affect or are associated with IL6, multiple linear regression analysis using the enter approach. All statistical analyses were conducted using SPSS version 26. Using 0.05 threshold of significance to accept the findings.

Results

The demographic information of the cases and controls was broken out by age, gender, and age group, there were seven males and fifty-three females, with ages ranging from twenty to seventy-nine years old. The results demonstrated that the first two variables showed a good match between controls and patients (P = 0.732 and P = 0.676, respectively). There was a discernible disparity between the two sets of data. Table 1 showed the correlation between the disease activity and other parameters When the variables were compared with respect to disease activity using ANOVA, the mean Anti-MCV level were increased in the blood of RA patients with Low disease activity, i.e. 31.866 ± 54.141 (P= 0.424), compared to those with high disease activity (26.764 ± 56.739) (P=0.851) or moderate disease activity (23.008 ± 44.480) (P = 0.176). there was no statistical relation observed between Anti-MCV and disease activity P-value =0.374. The mean of IL6 values was increased in the blood of RA patients with moderate disease activity, i.e. 306.537 ± 59.450 (P=0.508), compared to those with low disease activity (301.505 ± 78.532) (P=0.552) or high disease activity (294.276 ± 86.155) (P=0.936) there was no significant relation was observed between IL6 and the disease activity P-value =0.792. While the mean of Anti-CCP values were increased in the blood of RA patients with moderate disease activity, i.e. 112.396 ± 179.584 (P=0.863), compared to those with low disease activity (83.902 ± 65.150) (P=0.888) or high disease activity (63.880 ± 72.146) (P=0.987) there was no significant relation was observed between Anti-CCP and the disease activity, P-value =0.981.

Table 1: Mean, and Std. deviation distributions of study parameters and disease activity of patients' group

Disease activity		Mean	Std. deviations	P-value (LSD)	
ESR mm/h	Low activity	27.550	10.709	A	0.015
	Moderate Activity	33.350	15.776	B	0.015
	High activity	45.730	28.894	C	0.507
	One-way ANOVA test (P-value): P-value=0.013				
CRP Mg/L	Low activity	25.910	26.300	A	0.047
	Moderate Activity	29.480	32.345	B	0.015
	High activity	31.150	31.847	C	0.842
	One-way ANOVA test (P-value): P-value=0.025				
Anti-CCP Ng/ml	Low activity	83.902	65.150	A	0.888
	Moderate Activity	112.396	179.584	B	0.863
	High activity	63.880	72.146	C	0.987
	One-way ANOVA test (P-value): P-value=0.981				
RF U/ml	Low activity	105.907	78.746	A	0.709
	Moderate Activity	102.856	82.334	B	0.610
	High activity	114.772	69.872	C	0.999
	One-way ANOVA test (P-value): P-value=0.858				
Anti-MCV U/ml	Low activity	31.866	54.141	A	0.424
	Moderate Activity	23.008	44.480	B	0.176
	High activity	26.764	56.739	C	0.851
	One-way ANOVA test (P-value): P-value=0.374				
IL6 Pg/ml	Low activity	301.505	78.532	A	0.552
	Moderate Activity	306.537	59.450	B	0.508
	High activity	294.276	86.155	C	0.936
	One-way ANOVA test (P-value): P-value=0.792				

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, Anti-CCP: Anti-cyclic citrullinated protein, RF: rheumatoid factor, Anti-MCV: anti-mutated citrullinated vimentin, LSD: Least significant difference, ANOVA: One-way analysis of variance.

The mean of ESR values were higher in RA patients with high disease activity (45.730 ± 28.894 mm/h) *P*= 0.507 compared to those with moderate activity (33.350 ± 15.776 mm/h) P=0.015 or low disease activity (27.550 ± 10.709 mm/h) *P* = 0.015. A statistical relationship was found between ESR and moderate and low disease activity The *P*-value is 0.013. Mean CRP values were increased in the blood of RA patients with high activity of the disease, i.e. 31.150± 31.847 (*P*=0.842), compared to those with moderate activity (29.480 ± 32.345) (*P*=0.015) or Low disease activity (25.910 ± 26.300) (*P*=0.047) there was statistical relation was observed between ESR and moderate and low disease activity *P*-value =0.025**.** Mean RF values were increased in the blood of RA patients with high activity of the disease, i.e. 114.772 ± 69.872 (*P*= 0.999), compared to those with moderate activity (102.856 ± 82.334) (*P*=0.610) or Low disease activity (105.907 ± 78.746) (*P* = 0.709). there was no statistical relation observed between RF and disease activity because autoantibody plays a weak role in disease activity. One-way ANOVA test (*P*-value): *P*-value =0.858**.** Table 2 showed the correlation between Anti-MCV and other parameters there were positive significant relation between Anti-MCV and RF (r:0.229, *P*= 0.039), highly positive correlation with Duration of disease and Anti-MCV (r:0.381, *P*=0.001), Inverse significant relation were identified between Anti-MCV and ESR (r:-0.228, P:0.040) and there was no significant correlation was found between Anti-MCV and Anti-CCP (r:-.0.099, *P*=0.226), CRP (r:-0.152, *P*=0.123, Smoking(r:0.047, *P*=0.361), BMI (r:-0.144, *P*=0.137), Treatment Type (r:0.154, *P*=0.121). Table 3 showed the correlation between IL6 and other parameters there were no significant identified between IL6 and other parameters like Age (r: -0.032, *P*=0.405), BMI (r:0.030, *P*: 0.409), Duration (r:-0.058, *P*: 0.330), ESR (r: 0.034, *P*= 0.397), CRP (r: -0.087, *P*: 0.254), Anti-CCP (r: 0.051, *P*: 0.350), RF (r: -0.117, *P*: 0.187), Anti-MCV (r: -0.150, *P*: 0.126), Smoking (r: -0.049, *P*: 0.354), Treatment type (r: -0.110, *P*= 0.201) .

Table 2. Correlation between Anti-MCV and RA parameters in studied patients

Patients with Rheumatoid arthritis (N=60)		
Pearson correlation		Anti-MCV
RF	r	0.229
	P-value	0.039
Anti-CCP	r	-0.099
	P-value	0.226
CRP	r	-0.152
	P-value	0.123
ESR	r	-0.228
	P-value	0.040
Smoking	r	0.047
	P-value	0.361
Duration	r	0.381
	P-value	0.001
BMI	r	-0.144
	P-value	0.137
Treatment Type	r	0.154
	P-value	0.121

ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein, Ani-CCP: Anti-cyclic citrullinated protein, RF: rheumatoid factor, and BMI: body mass index.

Table 3 Correlation between IL-6 and RA parameters in studied patients

Patients with Rheumatoid arthritis (N=60)		
Pearson correlation		IL6
Age	r	-0.032
	P-value	0.405
BMI	r	0.030
	P-value	0.409
Duration (yrs)	r	-0.058
	P-value	0.330
ESR	r	0.034
	P-value	0.397
CRP	r	-0.087
	P-value	0.254
Anti-CCP	r	0.051
	P-value	0.350
RF	r	-0.117
	P-value	0.187
Anti-MCV	r	-0.150
	P-value	0.126
Smoking	r	-0.049
	P-value	0.354
Treatment type	r	-0.110
	P-value	0.201

ESR: Erythrocyte sedimentation rate, CRP:C-reactive protein, Ani-CCP: Anti-cyclic citrullinated protein, RF: rheumatoid factor, Anti-MCV: mutated citrullinated vimentin and BMI: body mass index

Discussion

This study found the mean± standard deviation of the age of patients diagnosed with rheumatoid arthritis (RA) was 50,02±12.907 years. This finding was consistent with previous research conducted on Iraqi RA patients by Mohammed AM et al(2023) and Rashid MK et al(2023) (12, 13). and other international studies (14-16). These studies mentioned that the onset of RA typically occurs during the middle years of life and that the disease is most commonly affects individuals who are over the age of 40 years**.** The present study indicated that RA is more common in females than in men, according to the gender difference in susceptibility at a ratio of 4:1 autoimmune disease is more common in females due to hormonal factors. This finding is roughly to the findings of a local study that was conducted by Albarzinji et al. (2023)(17, 18). This study proposes that even in treating RA patients with different types of treatments, the biomarker Anti-MCV is still detected in sera of these patients. This Anti-MCV autoantibody showed high sensitivity and less specificity (82.7%,72.1%) respectively. Similarly, a study conducted by Lee et al. (year) demonstrates that anti-MCV is more sensitive, but it is less specific, and it has worse diagnostic accuracy than anti-CCP in RA patients (19).Moreover, there is no significant association between anti-MCV and IL6, as well as between anti-MCV and anti-CCP, CRP, disease activity, smoking, body mass index, and the type of therapy. Their findings contradict the findings of previous studies, which demonstrate a high correlation between anti-MCV and anti-CCP (20, 21) The findings of this investigation were comparable to those of Nigm et al. (Year) who found no association between anti-MCV and disease activity (20, 22) and disagree with other study show correlation Anti-MCV with disease activity(23) Because Anti-MCV shows high positive correlation with duration of disease. In addition, a study by Nigm et al. (2022) showed that the Anti-MCV used to diagnose RA is independent of other marker and shows higher level in newly diagnosed RA. In this study most patient have duration of disease lasting more than 5 years also all patients were on treatment. No association was found between anti-MCV and CRP, according to research conducted by Nigm et al. (22).According to this research, there was a substantial correlation between Anti-MCV, ESR and RF, as well as a very high correlation with the duration of disease has been found. In agreement with the findings of the research carried out by Al-Shukaili et al (year) which demonstrated a substantial positive connection between anti-MCV and RF (24). Also another study similar to our study showed a significant association between Anti-MCV, ESR, and duration of disease (25).This study showed no significant correlation between IL6 and Anti-CCP, RF, ESR, CRP, and Anti-MCV similar to the study conducted by Abeer et al. (year) showed no significant correlation between IL6 and ESR, CRP (12). Also it was similar to a study conducted by Matsumoto et al. (year) who showed no correlation between IL6 and Anti-CCP(26). All patients in this study were on treatment with many types of anti-inflammatory drug. IL6 shows high sensitivity and specificity (98.7% - 91.3%) respectively. This study was similar to a study conducted by Abeer et al. (year) showed high sensitivity (92.50%) but this study disagreed with our study by showing very low specificity (42.50%)(12). The patients in this study suffered from the disease for a long period and type of treatment may influence. Thus, the current study aimed to investigate the levels of IL6 and anti-mutated citrullinated vimentin biomarkers as well as to determine their potential correlation with disease activity in rheumatoid arthritis Iraqi patients.

Limitations and recommendations

Sample size is always a challenge in such studies, but it is recommended that, where possible, future studies elucidate the anti-MCV antibodies how contributions to immunopathogenic mechanisms, as these may enhance disease diagnosis, management, and therapeutic options to confirm the present findings.

Conclusion

Anti-MCV autoantibody shows a high correlation with the duration of disease and a positive correlation with RF, but has no significant correlation with disease activity.

Authors' declaration

Adnan Al-Rubaee is an editorial board member but did not participate in the peer review process other than as an author.

We here by confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.

Ethical Clearance: As part of an MSC degree in microbiology, the study received approval from the Ethical Committee of the Research and Ethics Committee of the Immunology, Department of Microbiology College of Medicine Baghdad University, Baghdad, Iraq (October 2023).

Conflicts of interest: None

Funding: None.

Authors' contributions

Study conception & design: (Mohammed .M.Al-Ani, Nizar. A.L.Al-Ani). Literature search: (Dania.A.K.Ali). Data acquisition: (Dania.A.K.Ali). Data analysis & interpretation: (Dania.A.K.Ali). Manuscript preparation: (Dania.A.K.Ali). Manuscript editing & review: (Adnan Al-Rubaee, Mohammed .M.Al-Ani& Nizar. A.L.Al-Ani).

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تقييم العلاقة بين نشاط المرض والمؤشرات الحيوية العراقيين المصابين بالالتهاب المفاصل الرثوي في مصل المرضى IL6 ,Anti-MCV

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الخلاصة
خلفية البحث: التهاب المفاصل الرثياني أو الداء الرثياني أو التهاب المفاصل الروماتويدي هو أحد أمراض المناعة الذاتية التي تتميز بوجود الأجسام المضادة الذاتية ضد المستضدات السيترولينية. يستخدم اختبار الأجسام المضادة المقاومة للببتيد السيترولييني الحلقي (AntiCCP)بشكل شائع لتشخيص التهاب المفاصل الروماتويدي، في حين أن الفيمتين السيترولييني المضاد للتحور (Anti-MCV)هو جسم مضاد آخر مضاد للسيترولينات يتفاعل مع الفيمتين السيترولييني المتحور. تم اقتراح الأجسام المضادة (MCV)كعلامة تشخيصية مبكرة لالتهاب المفاصل.
الاهداف: يهدف هذا البحث إلى تقييم مستويات المؤشرات الحيوية لـ IL6 وAnti MCV مع نشاط المرض، وتحديد العلاقة المحتملة بين Anti-MCV وIL6 في المرضى الذين يعانون من التهاب المفاصل الرثوي.
المواد وطرق العمل: مئة وعشرون شخصا تم تقسيمهم الى مجموعتين، المجموعة الأولى 60 شخصا مصاب بالتهاب المفاصل الرثوي تم تقسيمهم بالاعتماد على نشاط المرض , و المجموعة الثانية 60 شخص سليم تتراوح أعمارهم (20-79) تم استخدام مقايسة الممتز المناعي المرتبط بالأنزيم (ELISA) لتحليل مستويات Anti-MCV و IL6في الدم
النتائج: لم يكن هناك ارتباط كبير بين مضادات MCV ونشاط المرض وكذلك IL6 ونشاط المرض ولكن النتائج التي توصلنا إليها تظهر أن هناك ارتباط إحصائي بين ESR وCRP مع نشاط المرض، كما توجد علاقة إيجابية قوية بين مضادات MCV ومدة المرض ومضادات MCV معRF,النتائج التي توصلنا إليها لا تظهر أي علاقة ذات دلالة إحصائية بين Anti-MCV و IL6.
الاستنتاجات: تظهر الأجسام المضادة لـ MCV ارتباطا عاليا مع مدة المرض وارتباطا إيجابيا مع RF، وقد تؤدي هذه النتيجة إلى جعلها أداة تشخيصية جديدة لتشخيص RA بالإضافة الى استخدام RF و.Anti-CCP.
الكلمات المفتاحية: التهاب المفاصل الرثوي، الأجسام المضادة الذاتية، مضادات MCV، الحركيات الخلوية ، IL6.

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Research Article

Imbalance between Pro and Anti-Inflammatory Cytokines in Rheumatoid Arthritis in Iraqi Patients

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Abstract:

Background: Rheumatoid arthritis is an autoimmune chronic inflammatory illness that affects the whole body and is characterized by non-articular involvement and inflammatory arthritis. It often develops by an interaction between genes and environmental factors.

Objectives: This research was designed to investigate the effect of the imbalance between pro- and anti-inflammatory cytokines on patients with rheumatoid arthritis and in comparison, with healthy controls.

Methods: Two groups (One hundred) of patients with Rheumatoid arthritis (66female, 34 male) and 50 healthy control group (28female, 22 male)) were chosen in this study to investigate the effect of some laboratory parameters such as cytokines IL1, IL4, IL6, and IL20 measured by Enzyme-linked Immunosorbent Assay (ELISA)on patients visiting health institutions in the Al-Anbar Governorate / Iraq. The results of the laboratory tests of patients who had symptoms of rheumatoid arthritis were compared with the laboratory test results of healthy people for comparison.

Results: The results indicated that the level of the cytokine IL1 increased significantly in the rheumatoid arthritis patient group (23.24 pg/mL) when compared with the group of healthy people (3.48 pg/mL). The results showed that the level of cytokine IL6 significantly increased in the rheumatoid arthritis patient group (50.66 pg/mL) compared to the healthy group (3.36 pg/mL). The results of cytokine IL20 significantly increased in the rheumatoid arthritis patient group (46.03 pg/mL) compared to the healthy group (15.02 pg/mL). The cytokine IL4 level showed a significant increase within the rheumatoid arthritis patient group (51.95 pg/mL) compared with the healthy people group (9.09 pg/mL).

Conclusion: The levels of cytokines IL1, IL4, IL6, and IL20 increased significantly in the rheumatoid arthritis patient group compared to the group of healthy people.

Keywords: Anti-Inflammatory Agents; Arthritis; Cytokines; Interleukin; Rheumatoid.

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Introduction

Rheumatoid arthritis (RA) is a kind of autoimmune disease that affects the whole body and is characterized by non-articular involvement and inflammatory arthritis. In many instances, it is a chronic inflammatory disease that develops by an interaction between genes and environmental factors. The synovial joints are the primary target of this condition. The condition normally begins in the smaller joints of the periphery. It is typically symmetric, and if it is not treated, it eventually spreads to the joints of the proximal extremities (1). Over time, joint degradation, including bone erosion and cartilage loss, may be caused by an infection. For RA to be diagnosed in the early stages, the symptoms should have persisted for less than six months; and for RA to be considered as established, the symptoms should have persisted for more than six months. Chronic inflammatory disorder is a disease that worsens with time and increases the risk of death if not treated (2). The diagnosis of this condition might be difficult in the early stages of RA, as no laboratory test can identify the presence of the disease. A complete clinical approach is essential for diagnosing and preventing joint injury that might be disabling. To be effectively treated, patients suffering from RA need treatment that includes both pharmacological and non-pharmacological therapy. The early treatment of rheumatic illness with disease-modifying, anti-rheumatic medications is now considered the standard of therapy. Many patients, despite receiving this therapy, eventually become disabled and have severe morbidity during their illness (3).

This study aimed to explore the impact of the imbalance between pro- and anti-inflammatory cytokines among patients with RA in the Al-Anbar Governorate, Iraq, in comparison with normal healthy controls.

Patients and Methods

This is a case-control study on 150 individuals, two groups were chosen to study the effect of some laboratory parameters, such as cytokines that cause rheumatoid inflammation and cytokines that are anti-rheumatic, for patients visiting health institutions in Al-Anbar Governorate /Iraq for the period from 2nd of Jan. 2023 to the 30th of Aug. 2023, who had symptoms of rheumatoid arthritis, while comparing

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the results of their laboratory tests with the results of healthy people. The first group (control group) consisted of 50 healthy people without rheumatoid arthritis. The second group (patient group) consisted of 100 patients with rheumatoid arthritis. The 100 Iraqi RA cases were diagnosed by a rheumatologist according to the American College of Rheumatology (ACR)1987 criteria or ACR-EULAR (European Alliance of Associations for Rheumatology)2010 criteria. (4) The ages of the patients ranged from 28 to 70 years for both study groups and for both sexes.
Five milliliters of blood were drawn from each participant using sterile procedures. Blood samples were centrifuged to separate the serum from the supernatant, and then the supernatant was carefully discarded. The separated serum was moved to Eppendorf tubes, then aliquoted and stored at -20°C until the IL Enzyme-linked Immunosorbent Assay (ELISA) test was performed to measure the serum IL concentration as per the manufacturer's instructions (Shanghai/ China). Information was taken from the patient, which included the patient's age, gender, weight, height, and medical history.

Statistics Analysis: The Mean ± SD was used for normally distributed numerical variables and the median (interquartile range) was used for non-normally distributed numerical variables in the descriptive statistical analysis. Rates and proportions were calculated for categorical data. The association between socio-demographic characteristics and IL was examined by the Chi-square test and the Fisher exact test. Data of patients were analyzed using SPSS version 25.0 software. Significant results were accepted at the 0.05 level

Results

Demographic characteristics of participants: The two research groups in this study had ages ranging from 28 to 70 years. Details are shown in Table 1. The results of Table 1 indicated that the average age of patients with rheumatoid arthritis was 58.33 years, their ages ranged from 32 to 70 years, while the average age of healthy people was 47.60 years, and their ages ranged from 28 to 65 years.

Table 1 showed that women are more susceptible to rheumatoid arthritis, as 66 women out of a total of 100 cases were recorded, while the number of infected males was 34 males.

Table 1 indicated that the two study groups differed in average BMI values, as the patient group had higher BMI values, recording 38.60 kg/m^2 compared to 27.25 kg/m^2 for the control group.

The values of the patient group indicated that patients with rheumatoid arthritis suffer from obesity, while the values of the control group were within normal ranges.

Laboratory tests: Table 1 shows that the two tested groups differed significantly in the ESR values. The group of patients with rheumatoid arthritis achieved a significant increase in the ESR value (P value ≤ 0.001), reaching 24.24 mm/hr, while the control group recorded 15.34 mm/hr. The elevation of ESR value in patients with rheumatoid arthritis may indicate the presence of an inflammatory process in the body.

Table 1 indicated that the level of the cytokine IL1 increased significantly in the group of patients with rheumatoid arthritis to 23.24 pg/mL. While the control group (healthy people) recorded 3.48 pg/mL (P value ≤ 0.001).

Table 1 showed that the average level of the cytokine IL-6 for the two groups studied differed significantly in its values. The group of patients with rheumatoid arthritis achieved a significant increase of 50.66 pg/mL, while the group of healthy persons recorded 3.36 pg/mL, (P value ≤ 0.001).

The results of measuring the levels of the cytokine IL20 in the two study groups showed that the level of the cytokine had increased significantly in the group of rheumatoid arthritis patients compared to the group of healthy people (46.03 vs. 15.02) pg/mL, (P value ≤ 0.001).

Table 1 showed that the level of the cytokine IL4 showed significant superiority within the rheumatoid arthritis patient population, where it was recorded at 51.95 pg/mL, while the cytokine level was 9.09 pg/mL in the group of people without rheumatoid arthritis, (P value ≤ 0.001).

Table 1: Demographic characteristics of the included participant

Variable	Group	Mean	No.	SD.	P values
Age (year)	Control	47.60	50	9.71	0.001
	Rheumatoid arthritis	58.33	100	9.48	
BMI (Kg/m^2)	Control	27.25	50	3.28	0.001
	Rheumatoid arthritis	38.60	100	3.79	
ESR (mm/hr)	Control	15.34	50	2.57	0.001
	Rheumatoid arthritis	24.24	100	7.19	
IL-1 (pg/mL)	Control	3.48	50	0.95	0.001
	Rheumatoid arthritis	23.24	100	6.68	
IL-6 (pg/mL)	Control	3.36	50	0.92	0.001
	Rheumatoid arthritis	50.66	100	12.04	
IL-20 (pg/mL)	Control	15.02	50	2.38	0.001
	Rheumatoid arthritis	46.03	100	10.65	
IL-4 (pg/mL)	Control	9.09	50	4.56	0.001
	Rheumatoid arthritis	51.95	100	11.99	

Discussion

Demographic characteristics of participants: The results of this table were consistent with the results of the study conducted by researcher Kato et al. (2017) which showed that the average age of patients with rheumatoid arthritis was 55.8 years during the year 2002-2003, 57.0 years during the year 2007-2008, and 59.9 years during the year 2012-2013(5) .

In a study conducted in 2023 by Yu and others, it was shown that the risk of developing rheumatoid arthritis is greatly affected by patient's age (6). The study showed that early detection of the disease and treatment in the early stages impact controlling the disease and reducing its complications. It also showed that health education and health programs that aim to control the disease in middle-aged patients have a major role in treating the disease. According to Radu *et al.* (2021) risk factors for developing rheumatoid arthritis are age, gender, heredity, and environmental stressors such as smoking and air pollutants(7).

Also, Maranini *et al.* (2022) indicated that Rheumatoid arthritis is a long-term inflammatory condition that impacts females with a ratio of 3:1 (female/male) (8). The role of gender in the susceptibility of females to rheumatoid arthritis may be due to hormonal factors and the effect of sexual dysmorphism (9). Alpizar-Rodriguez (2019) indicated that pregnancy and breastfeeding have been linked with a decreased risk of rheumatoid arthritis (10).

In a study conducted by Abuhelwa *et al.* (2020) showed that out of a total of 5428 people, 32.8% were above normal weight (overweight), 30.4% were obese, and 33.9% had a normal BMI (11). Feng X *et al*. (2019) showed that the elevation in BMI was associated with an increasing risk of rheumatoid arthritis development (12).

Laboratory findings

In a study by Jassim *et al.* (2021) it was concluded that there is a significant increase in ESR values for the rheumatoid arthritis patient group in comparison to healthy people (13).

IL1 contributes to many inflammatory diseases by initiating and enhancing immune and inflammatory responses (14). It plays a role in several systemic autoinflammatory syndromes and juvenile rheumatoid arthritis.

It also contributes a pathogenic role in inflammation and tissue destruction. It is a cytokine made up of eleven structurally similar proteins, involved in or controlling inflammation, which acts primarily by binding to specific receptors on the plasma membrane of target cells (15)**.**

It has been observed that the cytokine IL6 mainly in the neurological and cardiovascular systems is accountable for the systemic symptoms of rheumatoid arthritis. In general, patients with rheumatoid arthritis have been seen to have elevated serum levels of IL6 and IL6R in the serum and synovial fluid of the affected joints (16). IL6 is produced by infections and other types of inflammation directly, mostly by macrophages in response to infections or molecular patterns associated with inflammation. IL6 removes infectious organisms and promotes the acute phase and immunostaining reactions to mend injured tissue, acting as a protective measure. For both innate and adaptive immunity, IL6 is essential (17).

The role of the cytokine IL20 is to participate in the process of synovial angiogenesis in rheumatoid arthritis because it stimulates the expression of many angiogenic mediators such as fibroblast growth factor 2, vascular endothelial growth factor, and MMP in endothelial cells, thus promoting the infiltration of synovial tissue with Immunological cells (18)**.** IL20 has important inflammatory effects through leukocytes chemotaxis to the synovial membrane and sites of bone erosion in particular. IL20 also stimulates the production of several cytokines involved in the inflammatory process, including IL6, IL8, matrix metalloproteinase (MMP), and monocyte chemoattractant protein (MCP)-1, leading to joint inflammation and destruction (19)**.** Hussein *et al.* (2022) found that the level of IL20 significantly increased in patients with RA which recorded 58.5 ng/L as compared with 15.1 ng/L for healthy controls and they concluded that the cytokines IL20 was correlated with disease activity of rheumatoid arthritis (20).

The results of Table 1 were consistent with the results reached by Giri *et al*. (2021) in which they confirmed that IL4 levels were high in patients with rheumatoid arthritis compared to people who do not have the disease (21). Rheumatoid arthritis is now suspected to be driven by pathogenic Th17 cells that secrete interleukin 17 (IL17) and may be regulated by IL4 (22).

Limitation: Insufficient sample size for statistical measurements.

Conclusions:

In this study, the results indicated that the level of the cytokine IL1, cytokine IL6, cytokine IL20, and cytokine IL4, increased significantly in the rheumatoid arthritis patient group, compared to the group of healthy people. Ther is imbalance between pro- and anti-inflammatory cytokines among patients with RA in comparison with normal healthy controls.

Authors' declaration:

We confirm that the table presented in the manuscript is our original work.

The project was approved by the local ethical committee in the College of Science, University of Çankiri according to the code number (47-03.12.11.2021).

Conflict of Interest: None

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Authors' contributions

Study conception & design: (Shakir M. Salih, Sevki Adam). Literature search: (Shakir M. Salih). Data acquisition: (Shakir M. Salih). Data analysis & interpretation: (Shakir M. Salih). Manuscript preparation: (Shakir M. Salih). Manuscript editing & review: (Shakir M. Salih, Sevki Adam).

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اختلال السيتوكينات المؤيدة والمضادة للالتهابات في التهاب المفاصل الروماتويدي لدى المرضى العراقيين

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الخلاصة:
خلفية البحث: التهاب المفاصل الروماتويدي (RA) هو مرض التهابي مزمن مناعي ذاتي يصيب الجسم كله ويتميز بمشاكل غير مفصلي وغير مفصلي. في كثير من الحالات، يحدث المرض من خلال التفاعل بين الجينات والعوامل البيئية.
الاهداف: تم تصميم هذا البحث للتحقيق في تأثير عدم التوازن بين السيتوكينات المؤيدة والمضادة للالتهابات في المرضى الذين يعانون من التهاب المفاصل الروماتويدي ومقارنة نتائجهم مع الأشخاص الأصحاء الذين لا يعانون من المرض.
طرق العمل: تم اختيار مجموعتين (مائة) مريض يعانون من التهاب المفاصل الروماتويدي (66 أنثى ، 34 ذكر) و 50 مجموعة مراقبة صحية (28 أنثى ، 22 ذكر) في هذه الدراسة للتحقيق في تأثير بعض المعلمات المختبرية - مثل السيتوكينات IL1 و IL4 و IL6 و IL20 التي تم قياسها بواسطة مقايسة الممتز المناعي المرتبط بالإنزيم (ELISA) - على المرضى الذين يزورون المؤسسات الصحية في محافظة الأنبار / العراق. تمت مقارنة نتائج الاختبارات المعملية للمرضى الذين يعانون من أعراض التهاب المفاصل الروماتويدي مع نتائج الاختبارات المعملية للأشخاص الأصحاء - للمقارنة.
النتائج: أشارت النتائج إلى أن مستوى السيتوكين IL1 زاد معنويا في مجموعة مرضى التهاب المفاصل الروماتويدي (pg/mL23.24) بالمقارنة مع مجموعة الأشخاص الأصحاء (pg/mL3.48) ،. أظهرت النتائج أن مستوى السيتوكين IL6 زاد معنويا في مجموعة مرضى التهاب المفاصل الروماتويدي (pg/mL50.66) مقارنة بالمجموعة السليمة (3.36 pg/mL). زادت نتائج السيتوكين IL20 بشكل معنوي في مجموعة مرضى التهاب المفاصل الروماتويدي (pg/mL46.03) مقارنة بالمجموعة السليمة (15.02 pg/mL). أظهر مستوى السيتوكين IL4 زيادة معنوية داخل مجموعة مرضى التهاب المفاصل الروماتويدي (pg/mL51.95) مقارنة بمجموعة الأشخاص الأصحاء (pg/mL9.09).
الاستنتاجات: ارتفع مستوى السيتوكين IL1 و IL4 و IL6 و IL20 بشكل ملحوظ في مجموعة مرضى التهاب المفاصل الروماتويدي مقارنة بمجموعة الأشخاص الأصحاء.
الكلمات المفتاحية: العوامل المضادة للالتهابات، التهاب المفاصل، السيتوكينات، الإنترلوكين، الروماتويد.

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Review Article

A Review Article: Impact of Growth Hormone Treatment on Height in Children with X-Linked Hypophosphatemic Rickets

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Abstract

Background: X-linked hypophosphatemic rickets (XLHR) is the most frequent form of inherited rickets. In children, stunted growth and disproportionately short stature are frequently the early signs of XLHR.

Objective: To review different opinions in the review of literature about the use of growth hormone in the treatment of XLHR.

Methods: This review article followed a systematic literature review approach, there are no exclusion criteria.

Main Findings: Growth retardation may continue even after receiving appropriate conventional treatment (phosphate supplements and active analogs of vitamin D) in XLHR, even if it is initiated early in childhood. Recently, regardless of a well-controlled disease, treatment with recombinant human growth hormone (rhGH) was suggested as an effective way of supporting growth in children with XLHR exhibiting a lack of growth. It is necessary to follow until reaching adult height to assess the long-term effects of rhGH treatment on the ultimate height.

Conclusion: The addition of rhGH to optimal medical treatment might represent a promising option in the significant portion of affected patients with XLHR and growth failure. Follow-up is needed until the final height is reached to evaluate the long-term benefit of rhGH treatment on final height. Further studies will be necessary to determine the most efficient treatment protocol concerning doses, duration, and age of initiation or rhGH in short children with XLHR. However, further studies would be needed to study the addition of rhGH to optimal medical treatment in short children with XLHR.

Keywords: Growth hormone; Rickets; Short stature; X-linked dominant; X-linked hypophosphatemic.

Introduction

Definition: X-linked hypophosphatemic rickets (XLHR), is the most frequent form of inherited rickets affecting 3.9 - 5 / 100,000 live births (1). Genetics of XLHR: X-linked hypophosphatemic rickets is a hereditary disease caused by the loss of function mutations in the Phosphate phosphate-regulating endopeptidase X-Linked (*PHEX*) gene which is localized on Xp22.1. *PHEX* genes encode a particular endopeptidase which is highly displayed in the cells of the teeth (odontoblasts) and bones (osteocytes, osteoblasts) (2). Studies show that when PHEX function is lost, fibroblast growth factor 23 (FGF23) is secreted more readily; which can cause hypophosphatemia, urinary phosphate loss, and insufficient production of calcitriol (1, 25(OH) 2 Vitamin D) (3). Both heterozygous females and hemizygous males are affected by XLHR, which is inherited in an X-linked dominant pattern. Hemizygous males with a *PHEX* pathogenic variant pass it on to all of their daughters (who will be heterozygote affected) but not to any of their sons (non-affected). Male and Female progeny who inherit the pathogenic variant will be affected.

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Affected females have a 50% chance of passing on the pathogenic mutation to each child. When a family member inherits a *PHEX* pathogenic variation,the degree of symptoms can vary; however, intrafamilial clinical variability is not related to the affected family member's sex. It is possible to do prenatal and preimplantation genetic testing for XLHR if a family member with the *PHEX* pathogenic variation has been discovered (1).

Clinical and biochemical features of XLHR: Hypophosphatemia can present in the neonatal period, alkaline phosphatase can be elevated at the first month of life, and an early treatment with high doses of vitamin D may not prevent growth failure. Patients with the X-linked disorder do not show muscle weakness, tetany, or hypocalcemia. Adults, especially males, with XLHR may develop progressive ankylosis of the spine and major joints, simulating ankylosing spondylitis (4). Clinically, children with XLHR are characterized by short stature, and progressive leg bowing that develops as toddlers begin to stand and walk associated with radiological features of rickets, in addition to dental abscesses (5).

Biochemically, XLHR can be identified by raised serum alkaline phosphatase levels, low 1.25 dihydroxy vitamin D3 levels (calcitriol), normal

serum calcium and 25-OH vitamin D3 levels, and most importantly is hypophosphatemia and phosphaturia due to inadequate renal tubular reabsorption of phosphate) (5,6).

Growth status in patients with XLHR: In children, stunted growth and disproportionately short stature are frequently the early signs of XLHR. The length at birth is stated to be normal in children with XLHR (1). However, during the first few years of life, growth retardation becomes noticeable (7), leading to a mean height that is of 2 standard deviation scores (SDS) or more below the reference population's mean height (2). Unfortunately, growth retardation may continue even after receiving appropriate traditional therapy (phosphate supplements and active analogs of vitamin D), even if it is initiated early in childhood. In fact, the majority of studies have shown that approximately 50% of treated XLHR children remain short (< − 2 SDS) when growth has finished, which leads to a poor final adult height (8). There is yet, no fully effective treatment for XLHR. Growth retardation and skeletal abnormalities in XLHR patients have been proven to improve with using calcitriol in addition to oral phosphate (9). However, compliance with this regimen over the long term is challenging, and for some patients, the treatment outcomes are unsatisfactory. Many patients never show signs of catch-up growth or attain normal stature, even with the most effective medical therapy possible (10). Cohorts of treated patients have mean adult heights ranging from -2.8 to -1.7 SDS (11). Moreover, the medical therapy has been linked to substantial adverse consequences such as nephrocalcinosis, hypercalciuria, hypercalcemia, and secondary and tertiary hyperparathyroidism (12). Improper adherence to treatment with frequent phosphate dose and secondary hyperparathyroidism are two important factors that lead to inadequate metabolic control and stunted growth in children with XLHR (9). In fact, progressive disproportional stunting was revealed by a new study on the linear growth of a large cohort of XLHR patients receiving consistent calcitriol and phosphate medication (13), while the degree or the extent of leg bowing seemed to be just a weak link. This was primarily caused by consistently reduced leg growth over the prepubertal growth stage and was significantly correlated with the degree of hypophosphatemia. Trunk growth, however, was less negatively impacted in XLHR. Together, these discordant growth patterns produce a considerably raised sitting height index, and as a general rule, the shortest patients appear with the highest degree of bodily disproportion (13).

Growth Hormone treatment in children and adolescents with XLHR: Recently, regardless of a well-controlled disease, treatment with rhGH was suggested as a means of supporting growth in children with XLHR exhibiting poor growth. It is well established that growth hormone (GH) affects growth by acting through insulin-like growth factor-1 (IGF-1), which is crucial for the maturation and differentiation of growth plate chondrocytes (2), promotes the mineralization of the bone matrix, collagen secretion, and osteoblastogenesis (14). Furthermore, it is hypothesized that GH may increase serum phosphate levels by increasing renal phosphate reabsorption, both directly and indirectly via IGF-1 (15). In fact, the proximal renal tubule had been proved to have GH and IGF-I receptors. Consistent with this, research suggests that improved renal phosphorus reabsorption in association with enhanced 1α-hydroxylase enzyme activity are likely responsible for the favorable effects of rhGH treatment on phosphate metabolism (9). It is important to note that during rhGH treatment, patients with XLHR may experience an unexpected rise in the serum phosphate level due to a temporary decline in urine phosphate excretion (16). Numerous studies have demonstrated that rhGH treatment can accelerate growth in patients with short XLHR patients, particularly when initiated in the prepubertal period (5, 9, 15, 16). However, the limited patient numbers, lack of controls, absence of randomization, and very short observation periods make it difficult to judge these types of studies. Furthermore, GH may also exacerbate pre-existing body disproportion in XLHR patients, according to certain theories, as during rhGH treatment, the standardized sitting height increased by 1.6 SD compared with baseline values (17). In a retrospective longitudinal analysis study done by André et al (2) in 2022, there were two groups of children with XLHR: One that received rhGH treatment and the other that did not; the mean duration of GH therapy was 4.4 ± 2.9 years. This study is the first to clarify and corroborate the idea that GH promotes height in short kids with XLHR. It also shows that GH raises final adult height in children who continue to exhibit short stature even after receiving the best possible medical treatment. The greatest increase in height was seen throughout the first two years of treatment, and despite rhGH discontinuation, the height increase persisted until the final height was reached. Most notably, rhGH treatment enabled these children to attain a satisfactory final adult height (155.5 ± 6.3 cm in girls and 165.5 ± 6.4 cm in boys). A study conducted by Baroncelli et al. (18) in 2001, on a small number of children and teens with XLHR who received rhGH also suggests that GH treatment shows a favorable impact on final adult height. Zivicnjak et al. conducted a study in 2011 on 16 pre-pubertal children with XLHR who were short (height: −3.3 SDS), eight of whom received rhGH treatment for three years. The results revealed a considerable improvement in linear growth (+1.1 SDS) without a discernible alteration in the body proportions (13). An insightful study done by Rothenbuhler et al (5) in 2017, on 19 patients with XLHR, after two years of treatment with rhGH revealed that height SDS improved with rhGH therapy from −2.35 ± 0.8 SDS at the starting point to −1.62 ± 0.8 SDS (p=0.01) after a year and −1.2 ± 1.0 SDS (p=0.04) after two years. A substantial correlation was observed between the age at which

rhGH was initiated and the number of centimeters acquired throughout the duration of the study. Pre-pubertal children responded more favorably to rhGH. Burosumab, a human monoclonal IgG antibody that blocks FGF23's effects, has been a treatment option for XLHR since 2018 (19). Research on children demonstrated that this medication increases serum phosphate level and plasma calcitriol while restoring renal reabsorption of phosphate. In children with XLHR, it significantly improved bone malformations and rickets. But still, it seems that FGF23 blockage has little influence on growth velocity (19, 20).

In a recent appealing study, Ertl et al. (21) in 2022 conducted the first research on the growth of XLHR patients receiving concurrent burosumab and rhGH therapies. The study included 36 XLHR patients who were treated with Burosumab for a minimum of one year following conversion from traditional medical treatment. Twenty-three of them were given Burosumab exclusively, and the remaining patients maintained rhGH therapy after switching to Burosumab. After a year, children treated with Burosumab alone had only slight changes in their height SDS. In contrast, throughout the year of combination of Burosumab and GH therapy, children's height was definitely improved. This study suggests that continuing treatment with rhGH after switching from conventional therapy to Burosumab might be beneficial for the final height (21).

On the other hand, Smith and Remmington in 2021 included two studies (20 participants) in their review. The results showed that rhGH treatment could improve the height-standard deviation (SDS) score (z-score), but they were uncertain whether growth hormone or other treatments caused the transient increase in serum phosphate and maximal tubular phosphate reabsorption. They also concluded that they do not have strong enough evidence to recommend rhGH therapy for children. X-linked hypophosphatemia (22).

Conclusion:

The addition of rhGH to optimal medical treatment might represent a promising option in the significant portion of affected patients with XLHR and growth failure. Follow-up is needed until the final height is reached to evaluate the long-term benefit of rhGH treatment on final height. Further studies will be necessary to determine the most efficient treatment protocol concerning doses, duration and age of initiation or rhGH in short children with XLHR. However, further studies would be needed to study the addition of rhGH to optimal medical treatment in short children with XLHR.

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Authors' contributions:

Study conception & design: (Munib A. Al-Zubaidi and Wasnaa H.Abdullah). Literature search: (Wasnaa H.Abdullah). Data acquisition: (Munib A. Al-Zubaidi and Wasnaa H.Abdullah). Data analysis & interpretation: (Munib A. Al-Zubaidi and Wasnaa H.Abdullah). Manuscript preparation: (Wasnaa H.Abdullah). Manuscript editing & review: (Munib A. Al-Zubaidi and Wasnaa H.Abdullah).

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تأثير علاج هرمون النمو على الطول لدى الأطفال المصابين بالكساح الناقص الفوسفات المرتبط بالكروموسوم X

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الخلاصة
يعتبر الكساح الناقص الفوسفات المرتبط بالكروموسوم X هو الشكل الأكثر شيوعًا للكساح الوراثي في الأطفال، غالبًا ما يكون النمو المتقزم وقصر القامة غير المتناسب من العلامات المبكرة لـهذا النوع من الكساح. قد يستمر تأخر النمو حتى بعد تلقي العلاج التقليدي المناسب (مكملات الفوسفات ونظائر فيتامين د النشطة) حتى لو تم البدء في العلاج في وقت مبكر من الطفولة. أظهرت غالبية الدراسات أن حوالي 50٪ من الأطفال الذين عولجوا من هذا الكساح يظلون قصار القامة عند انتهاء النمو، مما يؤدي إلى طول نهائي ضعيف عند اكمال البلوغ. في الآونة الأخيرة وبغض النظر عن المرض الذي يتم التحكم فيه جيدًا، تم اقتراح العلاج بهرمون النمو البشري المؤتلف كطريقة فعالة لدعم النمو لدى الأطفال الذين يعانون من هذا النوع من الكساح والذين يعانون من نقص النمو. يُفترض أن هرمون النمو قد يزيد من مستويات الفوسفات في المصل عن طريق زيادة إعادة امتصاص الفوسفات الكلوي، سواء بشكل مباشر أو غير مباشر عبر عامل نمو الأنسولين 1. في الواقع، ثبت أن الأنبوب الكلوي القريب يحتوي على مستقبلات هرمون النمو وعامل نمو الانسولين 1.كما أثبتت العديد من الدراسات أن علاج هرمون النمو الطبيعي يمكن أن يسرع النمو لدى المرضى الذين يعانون من قصر القامة ، وخاصة عند البدء في العلاج في فترة ما قبل البلوغ. قد يمثل إضافة هرمون النمو الطبيعي إلى العلاج الطبي الأمثل خيارًا واعدًا في نسبة كبيرة من المرضى المصابين بقصر القامة في مرض الكساح الناقص الفوسفات وفشل النمو. هناك حاجة إلى المتابعة لتقييم الفائدة طويلة المدى لعلاج هرمون النمو الطبيعي على الطول النهائي. ستكون هناك حاجة إلى مزيد من الدراسات لتحديد بروتوكول العلاج الأكثر كفاءة فيما يتعلق بالجرعات ومدة وعمر بدء العلاج أو هرمون النمو الطبيعي في الأطفال قصار القامة الذين يعانون من الكساح الناقص الفوسفات المرتبط بالكروموسوم X.

الكلمات المفتاحية: الكساح الناقص الفوسفات المرتبط بالX، قصر القامة، هرمون النمو.

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