

Evaluation of DPP4, TNF, and Lipid Profile Levels as Early Indicators in Infertility Among Iraqi Women

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Abstract

Infertility is one of the problems facing women and men. Obesity, lipid level disorders, and insulin resistance are among the risk factors that cause infertility, in addition to other relevant factors. The current study aimed to investigate the effect of obesity and lipids imbalance, together with studying the relationship for each dipeptidyl peptidase -4 (DPP-4) and tumor necrosis factor α (TNF- α) infertile women compared to healthy women, to determine the possibility that they are being diagnostic and indicative factors of the disease. The study included 50 participants, age range (15-34) years, who attended Kamal Al-Samarrai Hospital in Baghdad, Iraq. The participants were divided into two groups; patients group (30 infertile women) and control group (20 healthy women). Levels of fasting blood sugar (FBS), lipid profile, dipeptidyl peptidase -4 (DPP4), tumor necrosis factor α (TNF- α), follicle-stimulating hormone (FSH), luteinizing hormone (LH), insulin and testosterone were evaluated. FBS and lipid profile levels were determined on the basis of spectrophotometric methods. Levels of DPP4, TNF- α , FSH, LH, insulin and testosterone were estimated by their ELISA kits. BMI, HOMO-IR, Atherogenic index were calculated. The results showed high levels of FBS, lipid profile (except HDL), DPP-4, TNF- α , LH, Testosterone and decrease of FSH level in patients compared to the healthy women. In conclusion, increasing lipid profile levels, obesity, atherogenic index, insulin resistance values approve the association of infertility with obesity, lipid disorders, diabetes and atherosclerosis diseases. Furthermore, DPP-4 and TNF- α are useful as good diagnostic factors for women's infertility.

Keywords: Dipeptidyl peptidase -4, Follicle-stimulating hormone, Infertility, Luteinizing hormone, Obesity, Testosterone, Tumor necrosis factor α .

Introduction

Obesity is defined as abnormal weight or excessive fat accumulation, which can be identified by the body mass index, where a value of thirty or more indicates obesity and a value of twenty-five indicates overweight. A characteristic of this condition is the excessive accumulation of triglycerides in adipose tissues, which can be used as nutrients by other tissues via the lipolysis pathway in the event of an essential nutrient deficiency, as in cases of starvation, fasting, and strenuous activities¹.

The phenotype changes according to the transition from underweight to obesity. This transition is the degree of chronic accompanied by a low inflammation, which can be identified by elevating the levels of free fatty acids in the circulatory system with soluble factors of pro-inflammation such as TNF- α , in addition to the activation and permeation of immune cells to the sites of inflammation. Obesity is often associated with dyslipidemia, which can be detected by an increased level of low-density lipoproteins, a decreased level of high-density lipoproteins, and an

elevated triglyceride level. Chronic inflammation and lipid imbalance lead to an increased risk of heart disease and type II diabetes mellitus ².

The leading causes of infertility can be attributed to several reasons, including obstruction in the fallopian tube, instability of the menstrual cycle, hormonal disturbance in women, and defects that may occur during the ovulation process. In addition to advanced age in women and high obesity, that is expressed by the body mass index in couples. Also, unexplained infertility can be another cause ³. Recently, the use of herbs and alternative medicine has emerged as an alternative to traditional treatment to treat infertility in women, according to claims that indicate that herbal treatment regulates hormonal disorders and treats cases of oxidative stress that affect public health. At the same time, a consensus on this has not been achieved ⁴. The effectiveness of antioxidants that can be obtained as nutritional supplements was studied to investigate their effect on male fertility, which constitutes one of the cases of infertility in women. In general, it was found that the antioxidants positively impact stimulating fertility in men. It was also found that they help to reduce the state of infertility in women ⁵. Studies indicated the importance of lifestyle on fertility. For example, physical activity and limited sleep are inversely related to their effect on fertility. There is a lack of agreement between studies in this area, and the need remains for more studies to confirm such claims ⁶.

The relationship between total and local oxidative stress factors in the serum and follicular fluid of an Iraqi infertile woman was investigated. The study includes various cases of infertility, like infertility caused by men, infertility caused by polycystic ovarian syndrome, and unexplained infertility. The study indicated that the general oxidative stress may provide reliable data about local oxidative stress in both healthy women and patients with Polycystic ovary syndrome (PCOS), and these data may be suitable for interpretation of the role of oxidative stress in failure in vitro fertility in patients with various reasons for infertility⁷. In the same field, some relevant prediction factors of successful pregnancy by *in vitro* fertility for Iraqi infertile women, which include testosterone, insulin-like growth factors, and dehydroepiandrosterone, were estimated. The role of these parameters is associated with the quality of the ovum, embryo, and follicle growth. The study referred to the fact that both testosterone and dehydroepiandrosterone have a prognostic use as predictive factors for successful

pregnancy in both healthy women and infertile women with PCOS ⁸.

In another previous study, the association of growth differentiation factor 8 (GDF8) levels in follicular fluid to the results of in vitro fertilization in infertile women with PCOS and without PCOS (male infertility) was studied. It was found that the GDF8 level represents a sensitive marker for the quality of the embryo and a better acceptable predictor for the successful pregnancy rate with in vitro fertility ⁹. The levels of ceruloplasmin ferroxidase and iron in follicular fluid and their correlation with *in vitro* fertility success rate were investigated. The study referred to the augmentation of iron levels in the follicular fluid of women with PCOS may cause a decrease in the pregnancy success rate when applied with the *in vitro* fertility method ¹⁰. In a recent study ¹¹, oligoasthenozoospermia was studied regarding sperm methylation outcome on sperm factor. The study showed no significant alterations between infertile men and seminal assay, but it found a significant correlation between DNA methylation and semen level, but no relation with morphological index.

Atherogenesis was developed due to the formation of plates in the inner layers of arteries. It occurs when an inflammation accompanies the lipid accumulation. This case varied significantly among individuals. The atherogenicity (AI) index is an excellent marker to assess the threat of atherogenic disorders and health subjects. The association between atherogenic index and cardiovascular disease in patients with type 2 diabetes was investigated. It was found that AI can be used as a reliable factor for detecting cardiovascular disease risk ¹². Moreover, it was found recently that high insulin resistance values are associated with atherogenic dyslipidemia. Insulin resistance value scales assist in categorizing the presence of atherogenic dyslipidemia ¹³. Lipid indices are considered essential factors for specific glycemic regulators. They possess a more reliable analytical value than classic indicators. Thus, cholesterol, LDL, TGs and atherogenic indices can be applied as essential predictors of glycemic control ¹⁴. Also, it was reported that the lipid profile could be used to identify patients with a higher risk of cardiovascular disease, notably when the lipid profile increased to higher levels or abnormal case ¹⁵. In a recent study ¹⁶, it was reported that the lipoprotein atherogenicity is more significant than that in LDL. Thus, the drugs

directed at these conjugated lipids are a substantial risk that can cause atherogenicity.

Lipid distribution and atherogenic factors have similar forms in infertile women as predictive factors, particularly TGs, which represent a based atherogenic index, consisting of a principal effect to develop atherosclerosis in this case¹⁷. Lipid disorders are considered the principal reason for developing female infertility. Subsequently, the levels of lipid profile and atherogenic indices are increasing in infertile women rather than in the healthy female group. Furthermore, an increase in atherogenicity idiocies among the patients group may lead to cardiovascular disease. So, periodic evaluation of lipid levels and atherogenicity indices is necessary for infertility¹⁸. The association between infertility and cardiovascular disease is observed through specific symptoms like obesity, early menopause, and irregularity of the menstrual cycle. The risk of CVD increased in the case of infertile women. It differed among the infertile females according to age and the type of infertility¹⁹.

The enzyme dipeptidyl peptidase-4 (DPP4) has a variety of functions and biological roles due to its different properties in lipid accumulation, stimulation of the immune system, and resistance to antitumor agents. Furthermore, the level of this enzyme was found to be increased in patients with hepatic disorders. In addition to the preceding, the DPP4 enzyme is in direct contact with hormones, as it is a mobile enzyme in plasma and presents in blood vessels in endothelial cells. It is expressed in cells of the immune system, such as helper T cells, as well as in endocrine glands and in specialized fibroblasts, as in mammary glands²⁰. Recently, it was found that the influence of fatness on Iraqi infertile females with the complications of coronavirus in terms of some relevant biochemical factors was investigated²¹; on the other hand, the association between obesity and diabetic complications was studied²².

The essential biological function of DPP4 is attributed to its role in splitting peptides like glucagon-like peptide-1 to regulate the metabolism²³. Dipeptidyl peptidase -4 is an abundant protein in cell membrane presence as a bound or soluble form. Its inhibitors include heterogeneous chemical drugs that possess various inhibition activities²⁴. Also, DPP-4 splits some immunoregulatory peptides in addition to liberating chemotactic elements and stimulating the activity with the migration of the immune cells. Correspondingly, these activities of DPP-4 assess to excretion of reactive oxygen species

and increase the action of phagocytic activity²⁵. The serum level of DPP-4 in patients with the grave disease was considerably increased. Furthermore, the level of DPP-4 was wholly related to the severity of hyperthyroidism in grave disease patients²⁶. In a recent study, it was recorded that the level of DPP-4 was significantly increased in the case of infertile patients with PCOS rather than healthy women²⁷. It was recorded that DPP-4 inhibitors are used as a savior in the case of glucose tolerance, and they possess an essential role in recovering liver activity. So, inhibitors of DPP4 could serve as drugs in the case of glucose intolerance associated with DPP-4, which occurs due to liver disorders, in addition to being applied in the therapy of liver diseases²⁸. In a more recent study²⁹, DPP-4 level was estimated in women with gestational diabetes mellitus and women with excessive gestational weight gain. The study reported that the level of DPP-4 in two patient groups was higher than in the healthy women group. It referred to that the DPP-4 level can be employed as a predictive factor for gestational diabetes mellitus.

Initially, Tumor necrosis factor alpha (TNF- α) was known as a factor that leads to necrosis tumor development. Then, it was found to possess other vital roles in the pathogenesis of immune diseases. Thus, the understanding of TNF- α role mechanisms will lead to facilitating the appropriate tools for the management of diseases³⁰. In a recent study³¹, it was found that the inhibitors of TNF have some excellent applications in infertile women with PCOS. Subsequently, the more significant general treatment effect can be applied in infertile PCOS patients receiving IVF-ET. On the other hand, one of the specialized studies in this field indicated that tumor necrosis factors could reverse the negative consequences of pregnancy during the first three months, but this is within the framework of research and investigation. So it required more studies to confirm its pharmacological effect in saving pregnancies at risk³². More recently, TNF- α level was evaluated in endometriosis of infertile women. The value of TNF- α was significantly increased with patients suffering from severe endometriosis disorders among other patients with infertility cases. The study suggested to further studies to understand the role and prognostic value of TNF- α in this type of disorders³³.

Women who are candidates for diabetes or are at the onset of type 2 diabetes have a more significant risk factor for infertility or miscarriage. This affects

childbearing compared to women who do not have diabetes³⁴. It was found that several medical conditions of infertility were linked to several disorders, such as type 2 diabetes, hypercholesterolemia, and high blood pressure. The study referred to the importance of health supervision specialists having a high level of knowledge to achieve a high level of care for pregnant women at risk of miscarriage and to understand the paths of metabolic complications that accompany the disease³⁵. Therapeutic measures for infertility remain limited, and most of them are

Materials and Methods

Blood sample collection

Every participant in the study carried out at the Kamal Al-Samarai Hospital in Baghdad, Iraq, provided verbally informed consent during the period from August 2022 to March 2023. A sample of fifty Iraqi women between the ages of fifteen and thirty years were chosen. Simultaneously, 30 obese infertile women and 20 healthy women were selected as the control group. Respondents were interviewed face-to-face to acquire information about their lifestyles and demographics through a pre-created questionnaire. The subject was asked to fast for ten to twelve hours before providing a blood sample. Every patient and the control group used a single-use disposable syringe that held five milliliters of blood. To separate the serum, 30 minutes of spinning at 3000 rpm was sufficient. An enzymatic immunoassay sandwich kit from (My BioSource, USA) was used to measure TNF α , DPP4, LH, FSH, Testosterone, and insulin in one milliliter of the patient's serum after it had been tested for blood

Results

Table 1 shows the BMI, FBS, lipid profile, and atherogenic index results in both infertile and healthy women groups. The results of BMI showed highly significant differences between healthy and patient groups. Also, it was found that FBS levels significantly increased in the patients group than in healthy subjects. Similarly, the results of the lipid profile (except HDL) revealed a significant increase in infertile women compared to the healthy group.

experiments on animals. Therefore, it becomes necessary to know the cellular mechanisms and the molecular characteristics of infertility caused by diabetes³⁶.

The present study aims to assess the levels of DPP-4 and TNF- α in infertile women as diagnostic and predictive factors for early prediction of infertility, together with studying the effect of obesity on the development of infertility and its risk on the progress of atherogenicity.

glucose and lipid profiles (cholesterol, triglycerides, HDL, and LDL). The remaining serum was frozen at -20°C. BMI is calculated as follows: kg/m² or weight in kilograms divided by height in square meters.

Statistical analysis

These analyses were carried out using SPSS version 26. An independent sample t-test was used to determine the significance of differences between the mean and SD. A p-value of 0.05 or less was judged significant.

Including criteria

1. Female patients were selected with infertility and obesity
2. Female age range: 15-34 years old.

Exclusion criteria

1. Patients with DM, any apparently acute inflammation, and any immunity diseases.
2. Parathyroid disorder.

Furthermore, the calculated value of the atherogenic index in the patients group was higher than in the healthy group. Subsequently, these results confirmed the occurrence of dyslipidemia, which is the leading cause of infertility in women, besides disruption of glucose level in patients with infertility together with high disorders of lipid profile compared to the control group.

Table 1. Levels of FBS, lipid profile, BMI, IR and Atherogenic index in patients and healthy women

Parameters	Infertility women Group (No. 30)	Control women Group (No. 20)	p-value
BMI	32.68 \pm 2.22	25.7 \pm 3.12	**0.001
FBS (mmol/L)	7.51 \pm 0.42	2.76 \pm 0.22	**0.001
Cholesterol (mmol/L)	8.95 \pm 2.02	3.67 \pm 0.50	**0.001

Triglycerides (mmol/L)	6.28 ±1.47	1.35±0.17	**0.001
HDL (mmol/L)	0.42±0.22	1.87±0.49	**0.001
LDL (mmol/L)	4.85 ±1.75	1.35±1.19	**0.001
VLDL(mmol/L)	2.87±0.67	0.61±0.07	**0.001
Atherogenic Index	1.17±0.82	0.14 ±0.46	**0.001
Insulin resistance	7.44±2.49	0.98±0.57	**0.001

The results of Tumor necrosis factor alpha (TNF α), dipeptidyl peptidase-4 (DPP-4), and insulin are recorded in Table 2. The TNF- α level was higher (p=0.001) in the patients group than the control group. Similarly, the DPP-4 level was recorded to be a more substantial increase in the patients' group than in the control group. Also, the result of insulin level for the patients group was recorded as a considerable level in the case of the infertile women

group than healthy women group. Also, the level of both LH and testosterone in the patients group was found to be higher than the control group, whereas the level of FSH was higher in the healthy group than the patients'. Subsequently, the ratio of LH/FSH in the patients group is higher than the healthy group. Furthermore, the calculated value of insulin resistance was found to be 7.44±2.49, which represents the abnormal value.

Table 2. Levels of TNF α , DPP4, and hormones group in patients and healthy women

Parameters	Infertility women Group (No. 30)	Control women Group (No. 20)	p-value
TNF α (ng/ mL)	203.27±45.20	63.22 ± 19.24	**0.001
DPP4 (ng/ mL)	59.78±11.25	30.45±8.20	**0.001
Insulin (mIU/dL)	10.85±0.9	6.6±0.59	**0.001
FSH (mIU/ mL)	5.54±2.80	8.25±2.77	**0.001
LH (mIU/ MI)	8.78±4.63	4.83±1.92	**0.001
LH/FSH ratio	1.69±0.55	0.58±0.14	**0.001
Testosterone (ng/ mL)	0.70±0.25	0.30±0.10	**0.001

ROC Analysis

The receiver operator characteristics (ROC) curve data of DPP4 and TNF- α are recorded in Table 3 and illustrated in Figs. 1-2. The results showed that the area under the curve of DPP4 was 0.984, which confirms that the DPP4 factor represents an excellent factor for the diagnosis of the studied disease case. Furthermore, the value of area under the curve for TNF- α was 0.773, this result indicated the acceptable of this factor as diagnostic factor for the studied case.

Table 3. ROC curve data for DPP-4 and TNF- α

Parameters	Area Under the Curve	Std. Error	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
DPP4	0.984	0.009	0.967	1.000
TNF- α	0.733	0.055	0.626	0.840

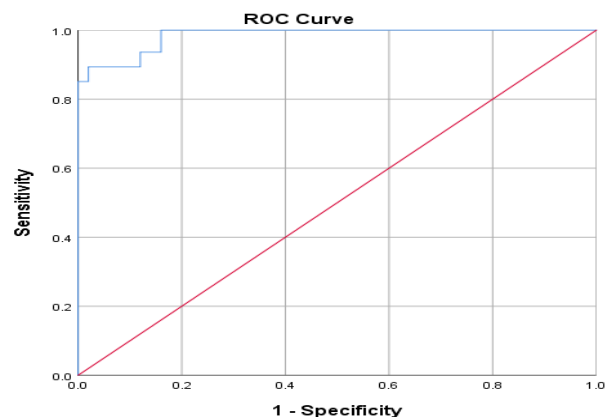


Figure 1. ROC curve for DPP-4

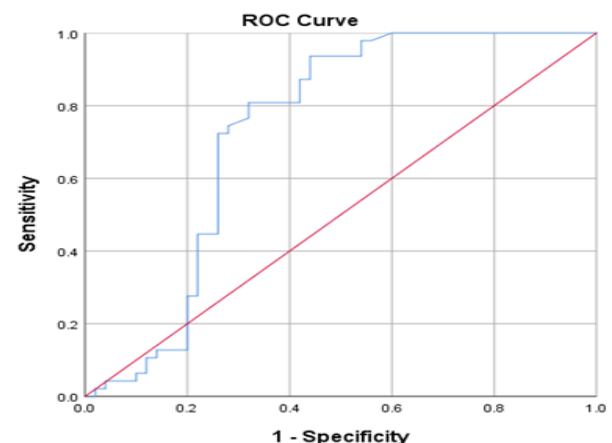


Figure 2. ROC curve for TNF- α

Discussion

The term infertility refers to the failure to conceive after a full year of regular unprotected intercourse; about 10% of couples worldwide suffer from infertility³⁷. In addition to obesity, there are some comorbidities, such as hyperlipidemia and inflammation, that are associated with infertility³⁸.

Heart disease (such as myocardial infarction and cardiovascular disease) is blood vessels disease has a strong and significant association with infertility³⁹. All lipid profiles (except HDL-C levels) show a substantial increase in the patients group compared with the healthy group at (P values ≤ 0.01). In contrast, HDL-C levels were significantly lower in patients than in control at (P values ≤ 0.01). The results of Dipanshu Sur et al.⁴⁰ and Eqbal A. Gatea et al.⁴¹ were similar to the results of the current research, which indicates a rise in fat levels such as TC, TGs, and LDL (8.95 ± 2.02 , 6.28 ± 1.47 and 4.85 ± 1.75 mmol./L, respectively) and on the contrary, a reduction in the level of HDL (0.42 ± 0.22 mmol./L) in patients group than healthy people (TC, TGs, LDL and HDL: 3.67 ± 0.50 , 1.35 ± 0.17 , 1.35 ± 1.19 and 1.87 ± 0.49 mmol/L, respectively), as shown in Table 1. The results of FSH, LH, Testosterone, and LH/FSH ratio in the current study were (5.54 ± 2.80 , 8.78 ± 4.63 mIU/mL, 0.70 ± 0.25 ng/mL and 1.69 ± 0.55 , respectively), which showed significant differences (P values ≤ 0.01) between patients and healthy groups (8.25 ± 2.77 , 4.83 ± 1.92 mIU/mL, 0.30 ± 0.10 ng/mL and 0.58 ± 0.14 respectively), as noticed in Table 2, the differences are attributed to the development of infertility.

Furthermore, in this study, it was found that TNF- α concentration in infertility women (203.27 ± 45.20 ng/ mL) is significantly higher (P values ≤ 0.01) than in healthy women (63.22 ± 19.24 ng/mL), as recorded in Table 2. Sayın et al.⁴² referred to similar outcomes. In contrast, levels of TNF α have no significant increase in groups of pregnant and non-pregnancy women^{43,44}; also, studies showed that tumor necrosis factor TNF inhibitors have specific applications in infertile women with POCS⁴⁵. The analysis performed by S. Aboeldalyl et al.⁴⁶ reported that inflammation markers level was higher in infertile women compared to control. In addition to such supporting evidence, in another recent study,⁴⁷ was supported by the above-mentioned studies, which referred to increasing in TNF α level in infertility condition, indicating to need for further studies with the

advanced strategies and selected patients to determine the TNF- α function in infertility.

It was found that the levels of factors indicating inflammation increased in the presence of infertility, including c reactive protein and the number of white blood cells. These factors are affected by body mass index and insulin resistance⁴⁸. In another study, it was reported that the level of TNF- α has a positive correlation with insulin and fasting blood sugar in infertile women. The study concluded that the level of the factor may be an indicator of the risk of diabetes and insulin resistance in infertile women⁴⁹. Subsequently, the results of TNF α in the current study agree with these studies.

Studies show that increased serum TNF- α levels in women with infertility are associated positively with BMI⁵⁰. Also, studies referred to the vital role of the enzyme DPP4 in the metabolism of glucose sugar, as well as its presence in many and varied cells such as heart muscle cells, smooth muscles, and blood vessels, which indicates that it may contribute to the occurrence and development of cardiovascular diseases⁵¹. Current results suggest that such elevations in TNF- α levels may be associated with insulin resistance in infertile patients. These findings also imply that, rather than the presence of obesity, the metabolic outcome of obesity or the progression towards clinically relevant disease states are the triggering factors for the elevation of TNF- α . In this study, the DPP4 level was significantly (P values ≤ 0.01) higher in infertile women (59.78 ± 11.25 ng/mL) compared with healthy women (30.45 ± 8.20 ng/mL), as recorded in Table 2; these results are in agreement with Anoop S et al.,⁵². It was found that a high level of the DPP4 enzyme is associated with obesity and insulin resistance, as it was also shown that the harmful levels of this enzyme are in people who suffer from excessive obesity and a high level of inflammation, which causes an escalation of the risk factor for heart disease. Furthermore, it was recently found that a high level of this enzyme is associated with polycystic ovary syndrome in comparison with healthy women⁵³. Also, current results agree with other studies,^{54,55} which reported the relationship between DPP4 with inflammation, metabolism, and fat homeostasis.

Many studies have been conducted to demonstrate the effect of obesity on infertility in women, including a very recent study that concluded that being overweight or obese affects the birth rate

or clinical pregnancy, and this is entirely consistent with the results of the current study, which shows the condition of obese infertile women compared to the control group⁵⁶. In another more recent study, it was reported that obesity causes many complications in women, including failure of the ovaries and uterus diseases, which lead to infertility. Therefore, this study recommends reducing weight before trying to get pregnant. The results of this study support the results of the current study, as obesity has an impact and relationship with the occurrence of infertility in women⁵⁷. Obesity in women has become a global epidemic because it is associated with metabolic and endocrine disorders. Thus, obese women are more vulnerable to reproductive problems, including infertility and fetal growth defects. Many factors work with obesity to cause infertility, including insulin resistance, hyperinsulinemia, androgen excess, infections, and hyperlipidemia. Subsequently, weight loss is one of the factors that reflects the effect of obesity on fertility, pregnancy, and offspring. So current findings agree with these outcomes of the mentioned parameters⁵⁸.

A more recent study⁵⁹, focuses on studying the level of DPP-4 enzyme in women with gestational diabetes during the pregnancy period, and the results of the study indicated that the level of this enzyme increases in women with diabetes even after childbirth. The enzyme plays a vital role in glucose metabolism, and inhibiting its activity positively

Conclusion

Depending on the obtained results, it was found that both of dipeptidyl peptidase-4 (DPP-4) and tumor necrosis factor- α (TNF- α) represent good markers for monitoring and diagnosing women's infertility disease. Furthermore, the importance of these results was enhanced by ROC analysis data, which indicated that DPP-4 could be an excellent marker and TNF- α is an acceptable marker for this

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Author's Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been

affects the regulation of glucose levels. Furthermore, the results of the DPP-4 in the current study revealed a significant increase in the patients group than healthy women. These results were supported by ROC result, which found that the DPP-4 parameter represents an excellent factor for diagnosis of this case of women's disease based on AUC value (0.984); this result agrees with numerous previous studies⁶⁰⁻⁶².

Also, the current result of DPP-4 was consistent with a recent study, which found that the DPP-4 level was increased in the case of polycystic ovarian syndrome⁶³. The results of the current study are also consistent with the results of a more recent study, which indicated that infertility can share common pathways with other diseases, such as cardiovascular diseases, which can be expressed in terms of a high atherogenic index. It also indicated that infertility can occur through ovulation abnormalities and uterine fibroids as well as with low levels of HDL and high levels of cholesterol, TGs, and LDL⁶⁴. The results of TNF in the current study showed consistency with the results of a more recent study regarding a higher level of this factor in patient women compared to non-patient ones. This demonstrates a distinctive immunological profile in patients who underwent infertility treatments. Additionally, the result of ROC showed the acceptable value of TNF- α as a diagnostic factor in the case of women with infertility⁶⁵.

purpose. Also, the results of obesity, lipid profile, atherogenic index and insulin resistance are associated with infertility in women and atherosclerosis disease as a significant risk factor. Accordingly, this study suggests adopting a suitable diet and exercising regularly to maintain an average weight and avoid diseases caused by obesity, such as infertility and atherosclerosis.

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- included with the necessary permission for re-publication, which is attached to the manuscript.
- No animal studies are present in the manuscript.
- Authors sign on ethical consideration's approval.

- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Author's Contribution statement

KK, and FM: participated in designing the research idea, determining the study's factors, interpreting the results, preparing research, discussing the results,

and determining the study's conclusions. FE. took part in the diagnosis of infertile women.

References

- Cheong LY, Xu A. Intercellular and inter-organ crosstalk in browning of white adipose tissue: molecular mechanism and therapeutic complications. *J Mol Cell Biol.* 2021; 13 (7): 466-479. <https://doi.org/10.1093/jmcb/mjab038>
- Endalifer ML, Diress G, Epidemiology, predisposing factors, biomarkers, and prevention mechanism of obesity: a systematic review. *J obesity.*2020; 2020: 1-8. <https://doi.org/10.1155/2020/6134362>
- Mishra A, Sharma MD, Tandon A, Ahsan F, Rayal R, Gaurav N, et al. Impacts and Causes of Female Infertility: An Observational Study. *Sci Temper.* 2022; 13 (2): 19-24. <https://doi.org/10.58414/SCIENTIFICTEMPER.2022.13.2.03>
- Feng J, Wang J, Zhang Y, Jia L, Zhang D, Zhang J, et al. The efficacy of complementary and alternative medicine in the treatment of female infertility. *Evid Based Complement Alternat Med.* 2021; 2021: 1-21. <https://doi.org/10.1155/2021/6634309>
- Majzoub A, Agarwal A. Systematic Review of Antioxidant Types and doses in male infertility: Benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. *Arab J Urol.* 2018; 16 (1): 113-124. <https://doi.org/10.1016/j.aju.2017.11.013>
- Zhao F, Hong X, Wang W, Wu J, Wang B. Effects of physical activity and sleep duration on fertility: A systematic review and meta-analysis based on prospective cohort studies. *Front Public Health.* 2022; 10: 1-14. <https://doi.org/10.3389/fpubh.2022.1029469>
- Mahdi A, Abdul Wadood S, Hashem R. Association Between Systemic and Local Oxidative Stress of Infertile Women Undergoing IVF/ICSI. *Iraqi J of Sci.* 2019; 60 (9): 1888-1897. <https://doi.org/10.24996/ajs.2019.60.9.1>
- Abdul Wadood S, Mahdi QA, Hashem R. Predictive Value of DHEAS, TT AND IGF1 in Successful Pregnancy Outcomes of Patients Undergoing IVF/ICSI-ET. *Biochem Cell Arch.* 2019; 19 (2): 3979-3787. <https://doi.org/10.35124/bca.2019.19.2.3979>
- Hashem R, Abdul Wadood S, Mahdi S. The Impact of Follicular Fluid Growth Differentiation Factors 8 Levels on IVF/ ICSI Outcomes. *Biochem Cell Arch.* 2019; 19 (1): 215-221.
- Kadhun BS, Al-Shammaree S. Association of Iron Status in Follicular Fluid with Pregnancy Outcomes in Infertile Women Undergoing IVF/ICSI. *Iraqi J Sci.* 2021; 62 (6): 1779-1786. <https://doi.org/10.24996/ajs.2021.62.6.3>
- Mahmmud HG, Ali AM. Effect of Sperm Global Methylation Level on Sperm Parameter. *His Med.* 2023; 9(1): 2472-2477. <https://doi.org/10.107720/2409-5849.v9.1.2023.29>
- Kim SH, Cho YK, Kim YJ, Jung CH, Lee WJ, Park JY, et al. Association of the atherogenic index of plasma with cardiovascular risk beyond the traditional risk factors: a nationwide population-based cohort study. *Cardiovasc Diabetol.* 2022; 21 (1): 81-91. <https://doi.org/10.1186/s12933-022-01522-8>
- Paublini H, González AA, Cortés CB, Gil PT, Sbert PR, Manent JI. Relationship between Atherogenic Dyslipidaemia and Lipid Triad and Scales That Assess Insulin Resistance. *Nutrients.* 2023; 15 (9): 2105-2119. <https://doi.org/10.3390/nu15092105>
- Mohammed MS, Ahmed HS. Atherogenic Indices in Type 2 Diabetic Iraqi Patients and Its Association with Cardiovascular Disease Risk. *J Fac Med Baghdad.* 2023; 65(9): 179-186. <https://doi.org/10.32007/jfacmedbagdad.2075>
- Nkechinyere I, Charles OC, Kingsley OK, Godwin OO, Samuel FA, Patrick OO, et al. Investigation On Lipid Profile and Atherogenic Indices as Markers of Cardiovascular Disease Among Students. *Am J Innov. Res Appl Sci.* 2020; 11(5): 129-135.
- Björnson E, Adiels M, Taskinen M, Burgess TS, Chapman MJ, Packard CJ, et al. Lipoprotein(a) Is Markedly More Atherogenic Than LDL: An Apolipoprotein B-Based Genetic Analysis. *J Am Coll Cardiol.* 2024; 83 (3): 385-395. <https://doi.org/10.1016/j.jacc.2023.10.039>
- Desmond CO, Chinedu MI, Modestus EU, Joseph AC, Emeka EK. Assessment of Lipid and its Atherogenic Index in Female Infertility in Owerri, South East Nigeria. *Eur J Res Med Sci.* 2016; 4(2): 13-20.
- Emokpae MA, Eromosele CO, Ajibade HO. Alterations in Lipid Profile and Indices of Atherogenicity among Infertile Women Seeking Conception by Assisted Reproductive Technology. *J Infertil Reprod Biol.* 2020; 8 (3): 61-65.

19. Farland LV, Wang YX, Wang Gaskins AJ, Edwards JW, Wang S, Magnus MC. Infertility and Risk of Cardiovascular Disease: A Prospective Cohort Study. *J Am Heart Assoc.* 2023; 12(5): 1-8. <https://doi.org/10.1161/JAHA.122.027755>
20. Sharma A, Virmani T, Sharma A, Chhabra V, Kumar G, Pathak K, et al. Potential Effect of DPP-4 Inhibitors Towards Hepatic Diseases and Associated Glucose Intolerance. *Diabetes Metab Syndr Obes.* 2022; 15: 1845–1864. <https://doi.org/10.2147/dmso.s369712>
21. Kaleel FM, Ghudhaib KK, Ali FE, The Influence of Obesity and IL-6 on Infertile Iraqi Women with COVID-19 Complications. *Baghdad Sci J.* 2023; 20 (4) (special issue): 1532-15139. <https://doi.org/10.21123/bsj.2023.9081>
22. Hamed GS. Allawi AA, Ghudhaib KK, Correlation of Pentosidine with Kidney Diseases in Iraqi Patients with Diabetic Nephropathy. *Iraqi J Sci.* 2021; 62 (10): 3436–3442. <https://doi.org/10.24996/ijis.2021.62.10.2>
23. Rohmann N, Schlicht K, Geisler C, Hollstein T, Knappe C, Krause L, et al. Circulating sDPP-4 Is Increased in Obesity and Insulin Resistance but Is Not Related to Systemic Metabolic Inflammation. *J Clin Endocrinol. Metab.* 2021; 106 (2): e592–e601. <https://doi.org/10.1210/clinem/dgaa758>
24. Kanasaki K. The role of renal dipeptidyl peptidase-4 in kidney disease: renal effects of dipeptidyl peptidase-4 inhibitors with a focus on linagliptin. *Clin Sci.* 2019; 133 (1): 151. <https://doi.org/10.1042%2FCSC20180031>
25. Yazbeck R, Jaenisch SE, Abbott CA. Dipeptidyl peptidase 4 inhibitors: Applications in innate immunity. *Biochem Pharmacol.* 2021;188: 1-11. <https://doi.org/10.1016%2Fj.bcp.2021.114517>
26. Chang X, Ding X, Wang J, Cai Q, Wang G, Liu J. The serum concentration and activity of DPP4 is positively related with the severity of hyperthyroidism in patients with Graves' disease. *Ann Med.* 2023; 55(1): 1-7. <https://doi.org/10.1080%2F07853890.2023.2226910>
27. Abolghasemi M, Mahjoub S, Esmaeilzadeh S. Serum dipeptidyl peptidase-4 activity and progranulin level in polycystic ovary syndrome patients. *Caspian J Intern Med.* 2022; 13(1): 70–75. <https://doi.org/10.22088%2Ffcjim.13.1.70>
28. Sharma A, Virmani T, Sharma A, Chhabra V, Kumar G, Pathak K, et al. Potential Effect of DPP-4 Inhibitors Towards Hepatic Diseases and Associated Glucose Intolerance. *Diabetes Metab Syndr Obes.* 2022; 15: 1845–1864. <https://doi.org/10.2147/DMSO.S369712>
29. Tatus MN, Pelech A, Bień K, Mekler J, Santiago M, Trojnar ZK, et al. Association of DPP-4 Concentrations with the Occurrence of Gestational Diabetes Mellitus and Excessive Gestational Weight Gain. *Int J Mol Sci.* 2024; 25(3): 1829-1841. <https://doi.org/10.3390/ijms25031829>
30. Jang D, Lee H, Shin Y, Song HR, Park JH, Kang TB, et al. The Role of Tumor Necrosis Factor Alpha (TNF- α) in Autoimmune Disease and Current TNF- α Inhibitors in Therapeutics. *Int J Mol Sci.* 2021; 22 (5): 2719-2794. <https://doi.org/10.3390/ijms22052719>
31. Liang J, Zhang Y, Xiao C, Cao S, Tian Y, Wang N, et al. Application value of tumor necrosis factor inhibitors in in vitro fertilization-embryo transfer in infertile women with polycystic ovary syndrome. *BMC Pregnancy Childbirth.* 2023; 23: 247-254. <https://doi.org/10.1186/s12884-023-05546-0>
32. Zhong Z, Wang Y, Wang G, Zhou F. Case Report: TNF-Alpha Inhibitors to Rescue Pregnancy in Women with Potential Pregnancy Loss: A Report of Ten Cases. *Front Immunol.* 2022; 13: 1-6. <https://doi.org/10.3389/fimmu.2022.900537>
33. Rahmawati AW, Ahsan F, Widyanugraha MY. Role of TNF Superfamily Members Lymphotoxin- α , sCD40L, and TNF- α in Endometriosis-Related Infertility. *J Endometr Pelvic Pain Diso.* 2024; Online First. <https://doi.org/10.1177/22840265241231722>
34. Mattsson K, Condori EN, Elmerstig E, Vassard D, Schmidt L, Ziebe S, et al. Fertility Outcomes in Women with Pre-existing Type 2 Diabetes a Prospective Cohort Study. *Fertil Steril.* 2021; 116 (2): 505-513. <https://doi.org/10.1016/j.fertnstert.2021.02.009>
35. Szaboova R, Devendra S. Infertility in a young woman with Type 2 diabetes. *London J Prim Care.* 2015; 7(3): 55–57. <https://doi.org/10.1080%2F17571472.2015.11494378>
36. Huang R, Chen J, Guo B, Jiang C, Sun W. Diabetes-induced male infertility: potential mechanisms and treatment options. *Mol Med.* 2024; 30: 11-25. <https://doi.org/10.1186/s10020-023-00771-x>
37. Damone AL, Joham AE, Loxton D, Earnest A, Teede HJ, Moran LJ. Depression, anxiety and perceived stress in women with and without PCOS: a community-based study. *Psychol Med.* 2019; 49 (9): 1510-1520. <https://doi.org/10.1017/S0033291718002076>
38. Zegers-Hochschild F, Adamson GD, Dyer S, Racowsky C, Mouzon J, Sokol R, et al. Poell, The international glossary on infertility and fertility care. *Fertil Steril.* 2017; 108 (3): 393-405. <https://doi.org/10.1016/j.fertnstert.2017.06.005>
39. Nayaker BS, Thomas S, Ramachandran S, Loganathan S, Sundari Mala K. Polycystic ovarian syndrome-associated cardiovascular complications: An overview of the association between the biochemical markers and potential strategies for their prevention and elimination. *Diabetes Metab Syndr.* 2017; 11(2): S841-S851. <https://doi.org/10.1016/j.dsx.2017.07.004>
40. Sur D, Chakravorty R. Association of Coronary Heart Disease Risk and Lipid Profile in Indian Women with polycystic ovarian syndrome. *J Clin*

- Gynecol Obstet. 2016; 5(1): 23-26.
<http://dx.doi.org/10.14740/jcgo375w>
41. Gatea EA, Hamdan IA, Eabaid FA. Effect of Polycystic Ovarian Syndrome on the Lipid Profile and sexual Hormones. *J Pharm Sci Res.* 2019; 11 (5): 2048-2050.
 42. Sayin NC, Gücer F, Kaplan PB, Yüce MA, Sentürk C, Yardim T, et al. Elevated serum TNF- α levels in normal-weight women with polycystic ovaries or the polycystic ovary syndrome. *J Reprod Med.* 2003; 48(3): 165-170.
 43. Khadem N, Mansoori M, Attaran M, Attaranzadeh A, Zohdi E. Association of IL-1 and TNF- α Levels in Endometrial Secretion and Success of Embryo Transfer in IVF/ICSI Cycles. *Int J Fertil Steril.* 2019; 13(3): 236-239.
<https://doi.org/10.22074/ijfs.2019.5668>
 44. Moin AS, Sathyapalan T, Atkin SL, Butler AE. Inflammatory Markers in Non-Obese Women with Polycystic Ovary Syndrome Are Not Elevated and Show No Correlation with Vitamin D Metabolites. *Nutrients* 2022; 14(17): 3540-3554.
<https://doi.org/10.3390/nu14173540>
 45. Liang JX, Zhang Y, Xiao C, Cao S, Tian Y, Wang N, et al. Application Value of Tumor Necrosis Factor Inhibitors in *in vitro* Fertilization-embryo Transfer in Infertile Women with Polycystic Ovary Syndrome. *BMC Pregnancy Childbirth.* 2023; 23 (247): 1-8.
<https://doi.org/10.1186/s12884-023-05546-0>
 46. Aboeldalyl S, James SC, Seyam E, Ibrahim EM, Shawki HE, Amer S. The Role of Chronic Inflammation in Polycystic Ovarian Syndrome—A Systematic Review and Meta-Analysis. *Int J Mol Sci.* 2021; 22(5):2734.
<https://doi.org/10.3390/ijms22052734>
 47. Rudnicka E, Kunicki M, Suchta K, Machura P, Grymowicz M, Smolarczyk R. Inflammatory Markers in Women with Polycystic Ovary Syndrome. *Biomed Res Int.* 2020; 2020: 1-10.
<https://doi.org/10.1155/2020/4092470>
 48. Gurbuz T, Gökmen O, Madenli AA, Dilbaz B. R-Spondin1 and tumor necrosis factor-alpha in infertile women with polycystic ovary syndrome: relationships with insulin resistance and other parameters. *J Health Sci Med.* 2023; 6 (2): 449 – 455.
<https://doi.org/10.32322/jhsm.1210721>
 49. Osibogun O, gunmoroti O, Michos ED. Polycystic ovary syndrome and cardiometabolic risk: Opportunities for cardiovascular disease prevention. *Trends Cardiovasc Med.* 2020; 30 (7): 399-404.
<https://doi.org/10.1016/j.tcm.2019.08.010>
 50. Araya AV, Aguirre A, Romero C, Miranda C, Molina MC, Ferreira A. Evaluation of tumor necrosis factor alpha production in ex vivo short term cultured whole blood from women with polycystic ovary syndrome. *Eur Cytokine Netw.* 2002; 13 (4): 419-424.
 51. Chen SY, Kong XQ, Zhang KF, Luo S, Wang F, Zhang JJ. DPP4 as a Potential Candidate in Cardiovascular Disease. *J Inflamm Res.* 2022; 15: 5457-5469.
 52. Anoop S, Misra A, Bhatt SP, Gulati S, Pandey RM, Mahajan H. High circulating plasma dipeptidyl peptidase-4 levels in non-obese Asian Indians with type 2 diabetes correlate with fasting insulin and LDL-C levels, triceps skinfolds, total intra-abdominal adipose tissue volume and presence of diabetes: a case-control study”, *BMJ Open Diab Res Care.* 2017; 5: 1-8. <https://doi.org/10.1136/bmjdr-2017-0003931>
 53. Maryam A, Soleiman M, Sedighe E. Serum dipeptidyl peptidase-4 activity and progranulin level in polycystic ovary syndrome patients. *Caspian J Int Med.* 2022 13 (1): 70–75.
<https://doi.org/10.22088/cjim.13.1.70>
 54. Trzaskalski NA, Fadzeyeva E, Mulvihill E. Dipeptidyl Peptidase-4 at the Interface Between Inflammation and Metabolism. *Clin Med Insights Endocrinol.* 2020; 13: 1-10.
<https://doi.org/10.1177/1179551420912972>
 55. Barchetta I, Cimini FA, Dule S, Cavallo MG. Dipeptidyl Peptidase 4 (DPP4) as A Novel Adipokine: Role in Metabolism and Fat Homeostasis. *Biomedicines.* 2022; 10 (9): 2306-2326.
<https://doi.org/10.3390/biomedicines10092306>
 56. Jeong HG, Cho S, Ryu KJ, Kim T, Park H. Effect of weight loss before in vitro fertilization in women with obesity or overweight and infertility: a systematic review and meta-analysis. *Sci Rep.* 2024; 14: 6153-6161. <https://doi.org/10.1038/s41598-024-56818-4>
 57. Chuisaca FG, Peláez GB. Infertility and Obesity in Young Adult Women. *J Popul Therap Clin Pharmacol.* 2024; 31 (2): 2564-2574. <https://doi.org/10.53555/jptcp.v31i2.4239>
 58. Wei W, Zhang X, Zhou B, Ge B, Tian J, Chen J. Effects of Female obesity on Conception, Pregnancy and the Health of Offspring. *Front Endocrinol.* 2022; 13: 1-5. <https://doi.org/10.3389/fendo.2022.949228>
 59. Tatus MG, Pelech A, Bie K, Mekler J, Santiago M, Trojnar ZK, et al. Association of DPP-4 Concentrations with the Occurrence of Gestational Diabetes Mellitus and Excessive Gestational. *Int J Mol Sci.* 2024; 25(3): 1829-1841.
<https://doi.org/10.3390/ijms25031829>
 60. Salman Z A, Ghudhaib K K. Association of Osteopontin and Alkaline Phosphatase in Male Patients with Diabetes Mellitus Type 2 and Periodontitis. *Iranian J War Public Health.* 2022; 14(1): 105-109.
<https://doi.org/10.29252/ijwph.14.1.105>
 61. Salman Z A, Ghudhaib K K, Fadhil R. Evaluating Osteocalcin and Osteonectin in serum male patients with type 2 Diabetes mellitus and periodontitis. *Eurasian Chem Commun.* 2022; 4: 295-302.
<https://doi.org/10.22034/ecc.2022.325049.1307>
 62. Ibrahim RK, Ghudhaib KK, Allawi AAD. Determining ACE-2 Level and Some Relevant

- Biochemical Parameters and studying the effect of Gender in Iraqi Diabetic Patients with Glomeruli and Renal Tubules Fibrosis as Early Prediction Marker. Baghdad Sci J. 2023; 20 (6): 2256-2264. <https://dx.doi.org/10.21123/bsj.2023.ID>
63. Abolghasemi M, Mahjoub S, Esmailzadeh S. Serum Dipeptidyl Peptidase-4 Activity and Progranulin Level in Polycystic Ovary Syndrome Patients. Caspian J Intern Med. 2022; 13(1): 70-75. <https://doi.org/10.22088/cjim.13.1.70>
64. Ganjare R, Mangalaram A. Association between infertility and risk of cardiovascular diseases in young women. Fi000 research. 2024; 13: 510-5018. <https://doi.org/10.12688/fi000research.146939.1>
65. Albayati HM, Abdulhameed WA. TNF-alpha and IL-10 Levels in Iraqi PCOS and Non-PCOS Patients Undergoing ICSI: An Immunological Perspective. Al-Rafidain J Med Sci. 2024; 6(1): 121-126. <https://doi.org/10.54133/ajms.v6i1.558>

تقييم مستويات ثنائي الببتيديل ببتايديز-4 ، عامل التنخر الورمي ونمط الدهون كمؤشرات مبكرة للعقم بين النساء العراقيات

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الخلاصة

العقم هو أحد المشاكل التي تواجه النساء والرجال. تعد السمنة واضطرابات مستوى الدهون، وكذلك مقاومة الأنسولين، من عوامل الخطر التي تسبب العقم، بالإضافة إلى العوامل الأخرى ذات الصلة. تهدف هذه الدراسة إلى معرفة تأثير السمنة واختلال توازن الدهون مع دراسة علاقة كل من ثنائي الببتيديل ببتايديز-4 وعامل نخر الورم-الفا لدى النساء المصابات بالعقم مقارنة بالنساء غير المصابات به، لتحديد مدى إمكانية اعتمادها كعوامل تشخيصية ودالة على المرض. شملت الدراسة 50 مشاركاً، الفئة العمرية (15-34) سنة، الذين راجعوا مستشفى كمال السامرائي في بغداد، العراق. تم تقسيم المشاركين إلى مجموعتين؛ مجموعة المرضى (30 امرأة تعاني من العقم) ومجموعة السيطرة (20 امرأة سليمة). تم تقييم مستويات FBS، وانماط الدهون، و DPP4، و TNF- α ، و FSH، و LH، والأنسولين والتستوستيرون. وتم تحديد مستويات FBS والدهون على أساس الطرق الطيفية. و تقدير مستويات DPP4 و TNF- α و FSH و LH والأنسولين والتستوستيرون بواسطة كتات ELISA الخاصة بهم. تم حساب مؤشر كتلة الجسم، HOMO-IR، ومؤشر تصلب الشرايين. أظهرت النتائج ارتفاع مستويات FBS، الدهون (باستثناء HDL)، DPP-4، TNF- α ، LH، والتستوستيرون وانخفاض مستوى هرمون FSH لدى المرضى مقارنة بالنساء الأصحاء. تؤكد زيادة قيم مستويات الدهون ومؤشر تصلب الشرايين ومقاومة الأنسولين ارتباط العقم بالسمنة واضطرابات الدهون والسكري وتصلب الشرايين. علاوة على ذلك، فإن DPP-4 و TNF- α مفيدان كعوامل تشخيصية جيدة للعقم عند النساء.

الكلمات المفتاحية: ثنائي الببتيديل ببتايديز-4، هرمون محفز الجريب، العقم، الهرمون اللوتيني، السمنة، تستوستيرون، عامل التنخر الورمي الفا