Measurement of Ferritin and Transforming Growth Factor-β1 Levels in Iraqi Women with Polycystic Ovary Syndrome.

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Abstract

Background: Polycystic ovary syndrome (PCOS) is common heterogeneous disorder syndrome in females, characterized by chronic oligoovulation, polycystic ovary, and hyperandrogenism. This study aimed to the association of ferritin and transforming growth factor- β1 (TGF-β1) levels with insulin resistance, cardiovascular and type 2 diabetes risks. Patients and methods: (61) Iraqi women with PCOS patients diagnosed according to the Rotterdam criteria, were subdivided according to their Body Mass Index (BMI) to: (20) lean women with normal BMI: (18-24), (17) overweight women with BMI: (25-29) and (25) obese women with BMI >30. For the the purpose of comparison, (20) healthy Iraqi women were enrolled as controls matched for age. Fasting serum glucose (FSG), serum insulin, ferritin and TGF-β1 was quantitatively determine, Homeostatic model assessment (HOMA2) parameters were calculated. Results: Ferritin levels showed a high significant increase in the the obese and overweight group when compared with the lean group. TGF-β1 showed a significant increase in obese and overweight groups but not in the lean group. Pearson correlation analysis of patients groups revealed an a significant positive correlation between ferritin and (insulin, HOMA IR, and TGF- β1) and TGF- β1 with (FSG, insulin, and HOMA IR) while HOMA S (%) showed significant negative correlation with both ferritin and TGF- β1. Conclusion: Increase body iron stores, as reflected by serum ferritin concentrations, and TGF-β1 in PCOS patients and their correlation with insulin and insulin resistance could be association with the risk increased risk for cardiovascular and T2DM diseases.

Key words: ferritin, transforming growth factor –β1, PCOS, hyperinsulinemia.

Introduction

Polycystic ovary syndrome (PCOS) is a common heterogeneous disorder syndrome in females, with high prevalence. It is a multifactorial, heterogeneous, complex genetic, endocrine and metabolic disorder, diagnostically characterized by chronic anovulation, polycystic ovaries and biochemical and clinical manifestations of hyperandrogenism [1, 2]. PCOS is correlation with insulin resistance (IR) and increase the the risk for type II diabetes and possibly cardiovascular disease [3, 4, 5]. Obesity or overweight effect most of the patients with PCOS, suggesting adipose tissue dysfunction [6]. Transforming growth factor β (TGF-β) is a highly pleiotropic cytokine that in mammals exist in three isoforms (TGF-β1, TGF-β2, and TGF-β3) [7]. TGF-β is also normally found in the plasma (TGF-β1 isoform), and bound to ECM proteins throughout the body [8]. The etiology of PCOS is not well unclear; TGF-β1 is expressed in the ovary and has been implicated in the pathogenesis of abnormal follicle development and hyperandrogenism in PCOS [9]. Other factors, such as endocrine, environmental, and metabolic factors, might also play a role in dysregulated TGF-β signaling in PCOS patients [10].

Iron stores in the body exist fierstly in the form of ferritin. The ferritin molecule is an intracellular protein and considered as acute phase protein [11]. Oligoovulation may result in oligomenorrhea and/or amenorrhea reducing iron loss [12]. Insulin resistance and compensatory hyperinsulinemia may facilitate iron uptake by different tissues [13]. Androgen excess may enhance erythropoiesis and together with insulin resistance, may decrease hepcidin secretion thereby increasing iron absorption [14, 15]. Of these potential mechanisms, data in humans supported the corelation of reduced menstrual losses [16]. The aim of the present study is to explore the association between ferritin and TGF-β1 levels of PCOS patients with insulin resistance, cardiovascular and type 2 diabetes risks.

Experimental part

Subjects

This study was carried out in Baghdad city at Kamal AL-Samaree Hospital for Infertility and *In Vitro* Fertilization, for the period from November 2014 to April 2015. It included sixty one women newly diagnosed with PCOS were allocated into three groups as follows: (20) lean with BMI (18-24.9 Kg/cm^2), overweight women with BMI (25-29.9 Kg/cm^2), and (24) obese women with BMI over 30 Kg/cm^2. Additionally, twenty apparently healthy women were selected to serve as controls. The controls have a regular menstrual cycle with BMI range (18-25 Kg/cm^2), and with the comparable age of selected patients. The diagnosis of PCOS depende on the definition generated at to criteria of the Rotterdam, when at least two of the following features were present: oligo- or anovulation; clinical and/or biochemical signs of hyperandrogenism; and polycystic ovaries (presence of 12 or more follicles in each ovary measuring 2 –9 mm in diameter and/or increased ovarian volume (>10 cm^3) [17]. Exclusion criteria for women included in the study: Pregnancy, Current or previous (within six months) use of oral contraceptives, antiandrogens or other hormonal drugs, known cardiovascular, renal, liver, lung diseases and diabetes mellitus, and Cigarette smoking.

Sample collection

A venous blood specimen was withdrawn on day 2-5 of the menstrual cycle about (5 ml) by disposable syringe from each woman between 08:00 and 12:00 A.M, after an overnight fasting to be placed in gel-containing tubes. After clotting, the specimens were centrifuged at 3000 rpm for 10 minutes to collect serum that was divide into aliquots (300 µl) in Eppendorff tube, to be useing in the estimation of FSG, on the same day; other aliquots were keep frozen at -20 C° until used.

Anthropometric indices measurements

Determination of Body Mass Index (BMI)

Body mass index was determine by dividing body weight in kilogram by the square of her height in meter. The equation used in medicine produce a unit of measure of kg/m^2 [18].

$$\mathbf{BMI} = \frac{\mathbf{Mass\ (kg)}}{\mathbf{Height(m^2)}}$$

Laboratory methods

Measurement of glycemic indices:

Serum glucose level was measure by the glucose oxidase method 0f Trinder 1969 (Linear, Spain) [19]. Serum insulin levels were determined using ELISA Kit [20] (DRG, diagnostic company, Germany) as described by the manufacture. The DRG Insulin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. HOMA2 parameters: HOMA2-IR, β-cell percentage (HOMA2- β %) and sensitivity percentage (HOMA2-S %) were calculated using an HOMA2 calculator [21].

Determination of serum ferritin and transforming growth factor beta-1(TGF-β1) levels

Serum ferritin level was quantitatively determine in patients and control groups using the indirect enzyme immune assay (ELISA) method, and serum TGF-β1 level was quantitatively determined using sandwich ELISA method, using (Demeditec Germany) kits [22, 23].

Statistical Analysis

Statistical Package for the Social Sciences, (SPSS) version 20.0 for Windows Software (SPSS Inc., Chicago, III, USA) was useing for statistical analysis. The data were normally distribute and were expresse as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare the parameters among groups (control, lean, overweight and obese) followed by post hoc test. Differences among groups were defined to be statistically significant if the corresponding *P*-value was equal and less than 0.05. Correlation between variables was determine by Pearson correlation coefficients (*r*- values).

Results

A significant increase in mean level of BMI was observed in overweight and obese groups in comparison with that of control group (26.84 and 35.04 *vs.* 22.7 Kg/cm^2) respectively, and no significant difference is found between lean group and control group (Table 1)

A significant increase in the mean level of FSG was observe in lean, overweight and obese groups in comparison with that of the control group (93.96, 98.3and 99.96 *vs.* 82.32mg/dl). And no significant difference was found between patients groups (Table 1).

A significant increase in the mean level of insulin was observe in groups (lean, overweight and obese) in comparison with that of the control group (14.65, 21.25 and 21.6 *vs.* 09.99 µlU/ml). Insulin showed a decrease in the mean of the lean group in comparison with an obese group (14.65 *vs.* 21.6µlU/ml) and the difference was significant (P<0.05). Also, between lean and overweight groups (14.65 *vs.* 21.25 µlU/ml) and the difference was significant, (Table 1).

A significant increase in mean level of HOMA2-B (%) was observe in overweight and obese groups in comparison with that of control group (165.29 and 157.71 *vs.* 136.4). Also, a significant increase was observed in overweight and obese groups in comparison with lean group (165.29 and

157.71 *vs.* 134.7). However, no significant difference was found in HOMA2 B cell (%) between control and lean groups, (Table 1).

A significant decrease in mean levels of HOMA2-S (%) was observe in lean, overweight and obese groups in comparison with that of the control group (59.95, 40.98 and 38.1 *vs.* 82.26). HOMA2-S (%) also showed a significant decrease in mean levels of the obese group in comparison with that of the lean group (38.1 *vs.* 59.95) and between overweight and lean groups (40.98 *vs.* 59.95).

A significant increase in mean levels of HOMA2-IR was observe in lean, overweight and obese groups in comparison with that of the control group (1.9, 2.73 and 2.8 *vs.* 1.27). Also, significant increase in the mean level of HOMA2-IR was observed in overweight and obese groups in comparison with that of the lean group (2.73 and 2.8 *vs.*1.9) as shown in the table (2).

The mean level of ferritin showed a significant increase in lean, overweight and obese groups in comparison with that of the control group (57.87, 60.7 and 67.3 *vs.* 45.14ng/ml). The mean level of TGF-β1 showed a significant increase in overweight and obese groups in comparison with that of the control group (21.12 and 22.1 *vs.* 16.76 pg/ml), and no significant difference was observe between control and lean groups (Table 2).

Person correlation analysis of ferritin and TGF-β1

As shown in the Table (2), ferritin level of the lean group showed significant positive correlation with insulin, HOMA2 IR and significant negative correlation with HOMA2 S % (Figure1). A ferritin level of the overweight group showed significant positive correlation with insulin, HOMA2 IR, and TGF-β1, and significant negative correlation with HOMA2 S (%, (Figure 2). A ferritin of the obese group showed significant positive correlation with insulin, HOMA2 IR and TGF-β1 and significant negative correlation with HOMA2 S % (Figure 3).

As shown in Table (4), TGF-β1 of the lean group showed significant positive correlation with insulin and HOMA2 IR (Figure 4). TGF-β1 of the overweight group showed significant positive correlation with insulin, HOMA2 IR, and ferritin (Figure 2, d) and (Figure 5). TGF-β1of obese group showed significant positive correlation with (FSG, insulin, HOMA2 IR, and ferritin (Figure 3, d), while significant negative correlation with HOMA2 S (%) was found, (Figure 6).

Discussion

Ferritin levels in this study showed a higher significant increase of overweight and obese groups. Also lean group showed a significant increase in comparison with control group (Table 1).These results in agreement with Faranak (*et al*., 2011) [24] who found that serum ferritin levels increased in women with PCOS irrespective of their BMI, CRP, and IR, they due there results to oligomenorrhea and less blood loss. In another study, higher levels of ferritin only in overweight and obese women with PCOS and not in lean subjects and concluded that increase iron stores might contribute to IR and β-cell dysfunction in PCOS patients, however, the elevation in serum ferritin levels in PCOS may be a secondary, not a pathogenic [25]. Serum ferritin levels were elevated in amenorrhea patients in compared with regularly menstruating women, so the absence of regular menstrual blood loss in PCOS patients might contribute to iron overload [24]. Also similar result was found by Luque-Ramírez and his colleagues [26]. It was hypothesized that the absence of a regular menstrual blood loss, genetic factors or hyperinsulinemia resulting from IR. considering that insulin might induce intestinal iron absorption by up regulating the activity of hypoxia inducible factor 1alpha and down regulating hepcidin expression [27, 28]. That may have contributed to the elevation body iron stores and serum ferritin levels observed in PCOS patients, and not the decrease menstrual losses secondary to from oligo or amenorrhea.

In this study, we find a higher positive correlation between insulin, HOMA2 IR with ferritin; also a higher negative correlation between ferritin and HOMA2 S (%) of lean, overweight and obese groups Table (2) Figure (1), these results agree with [26]. Recent data support this hypothesis and

show that insulin sensitivity is inversely relate to ferritin concentrations in premenopausal women and that women with abnormal glucose tolerance have increased ferritin levels [16, 30]. Elevated ferritin levels were positively correlated with the prevalence of the metabolic syndrome and with insulin resistance and an increased risk of T2DM [31, 32, and 33]. The increased ferritin level, occurred independent of changes in serum inflammatory markers, indicates that body iron stores are increase in these women and do not result from the secondary role of ferritin as an acute phase marker [12]. Therefore, both androgen excess and insulin resistance might be the cause for increased serum ferritin levels. However, other study hypothesized that the reduced menstrual losses due to the oligomenorrhea present in most women with PCOS could contribute to their increased iron stores. Recent data suggest that insulin resistance is one of the major players explaining their increased serum ferritin levels, whereas serum ferritin levels did not change after restoring regular menses by using an oral contraceptive for six months, these levels decreased markedly after insulin sensitization with metformin [24].

In this study, high significant increase in TGF-β1 level was found only in obese group and significant increase in overweight group, while no significant difference in lean group in comparison with control group was noted (Table 1). This data are in agreement with the previous findings of two studies by [34,35] showing increased serum TGF-b1 levels in patients with PCOS. It is now clear that PCOS is a pro-inflammatory state, as stated by Gonzales (*et. al.*, 2006) [31]. A low chronic inflammatory state, characterized by elevation levels of proinflammatory molecules and acute phase proteins, can be present in obesity and insulin resistance [36, 37].

We found that TGF-β1 of overweight and obese groups showed a higher significant positive correlation with insulin resistance, insulin, and significant negative correlation with HOMA2 S % (Table 3) (Figure 2). In other studies, increased circulating TGF-β1 levels in various cardio metabolic complications, hypertension, obesity, insulin resistance, diabetes, and coronary artery disease were found [38, 39, 36 and 40]. These findings of increased TGF-β1 reveal the bioavailability in PCOS is consistent with an important role for TGF-β1 in the pathogenesis of the cardio-metabolic complications seen in Iraqi women with PCOS. Targeting TGF-β1 dysregulation may be particularly important in PCOS as women with PCOS are at increased risk of impaired glucose tolerance and type 2 diabetes due to underlying insulin resistance. Excessive TGF-β1 activity has been implicate in the pathogenesis of arterial disease in patients with altered glucose metabolism [41]. Cells in the vessel wall express the isoforms TGF-β1, TGF-β2, and TGF-β3, which regulate cell differentiation, cell proliferation, cell migration, production of the extracellular matrix, and immune cell functions. As a potent regulator of vascular cell responses, TGF-β1 plays an important role in atherosclerosis and vascular remodeling [42]. Dysregulated TGF-β1 activity may contribute to atherosclerosis by stimulating smooth muscle cells in the vasculature to proliferate and synthesize collagen. TGF-β1 also regulates endothelial cells, macrophages, and T cell responses in the vasculature. In endothelial cells, TGF-β1 regulates the expression of genes that promote inflammation, such as interleukin 6. Circulating and molecular markers of oxidative stress and inflammation are highly correlate with circulating androgens [43, 44, 45]. These findings raise the possibility that in PCOS, either hyperandrogenemia pre-activate mononuclear cells (MNC) to account for the hyperglycemia-induced inflammation, or conversely that glucose-stimulated inflammation promotes ovarian androgen production in PCOS. There is data to support that both mechanisms may occur [46, 47].

Conclusions

1.Hyperinsulinemia and insulin resistance in Iraqi women with PCOS have an increased risk for T2DM disease.

2.Increased serum ferritin levels in PCOS patients and its correlation with insulin and IR support the hypothesis that insulin resistance is the cause of this increase.

3. Increased TGF-β1 level in overweight and obese Iraqi women with PCOS, but not in lean PCOS patients increase their risk for CVD diseases.
4. The TGF-β1 regulatory pathway appears to play a critical role in the development of PCOS and may be an important therapeutic target for PCOS.

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Table (1): Mean (±SD) level of BMI, FSG, insulin, HOMA2 parameters, ferritin and TGF-β1 of control and patients groups (lean, overweight and obese).

Parameters	Control Group N=20	Lean Group N= 20	Overweight Group N=17	Obese Group N=24	*P* value
BMI	22.7 ±1.68	22.84±1.65	26.84±1.34^{***a,b}	35.04±4.12 ^{***a,b,*c}	0.000
FSG (mg/dl)	82.32±5.93	93.96±9.85^{**a}	98.3±11.2^{***a}	99.96±11.3^{***a}	0.000
Insulin(µlU/ml)	09.99±1.87	14.65±5.28^{*a}	21.25±5.4^{***a,**b}	21.6±5.7^{***a,b}	0.000
HOMA2-β cell (%)	136.4±22.37	134.7±32.33	165.29±27.2^{**a,b}	157.71±23.74^{*a,b}	0.01
HOMA2-S cell (%)	82.26±18.22	59.95±20.85^{**a}	40.98±14.3^{***a,**b}	38.1±10.6^{***a,b}	0.000
HOMA2-IR	1.27±0.24	1.9±0.69^{*a}	2.73±0.71^{***a,**b}	2.8±0.75^{***a,b}	0.000
Ferritin(ng/ml)	45.14±14.2	57.87 ±7.31^{*a}	60.7±10.58^{**a}	67.3±18.3^{***a}	0.000
TGF-β1(pg/ml)	16.76±4.23	20.6±4.97	21.12±5.0^{*a}	22.1±5.2^{**a}	0.005

*P<0.05; **P<0.01; ***P<0.001. No asterisk: p≥0.05.

(a) Indicates significant difference between groups controls with lean, overweight and obese. (b) Indicates significant difference between group leans with overweight and obese. (c) Indicates significant difference between group overweight with obese.

Table (2): Pearson correlation analysis of Ferritin level in patients lean, overweight and obese groups.

Parameters	Ferritin		
	r Value lean	r Value overweight	r Value obese
Insulin (µIU/ml)	0.579**	0.682**	0.734**
HOMA2 S (%)	-0.462*	-0.553*	-0.627**
HOMA2 IR	0.589**	0.637*	0.711**
TGF-β1 (pg/ml)	0.366	0.691**	0.522*

*P< 0.05; **P<0.01; no asterisk: P≥0.05.

Table (3): Pearson correlation analysis of TGF-β1 in patients lean, overweight and obese groups.

parameters	TGF-β1		
	r Value lean	r Value overweight	r Value obese
FSG (mg/dl)	0.407	0.326	0.432*
Insulin (µIU/ml)	0.473*	0.594*	0.624**
HOMA2 S (%)	-0.348	-0.405	-0.587**
HOMA2 IR	0.496*	0.579*	0.648**

*P< 0.05; **P<0.01; no-asterisk: P≥0.05.

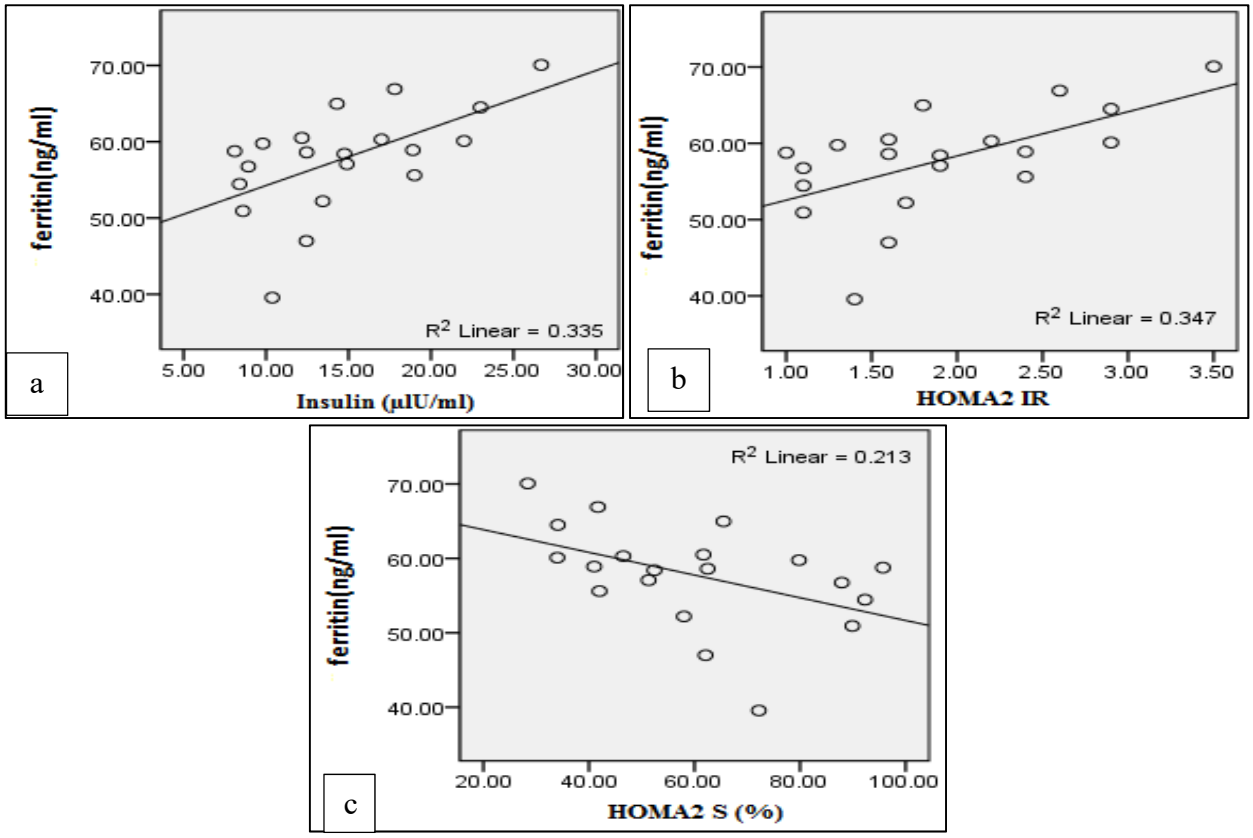

Figure (1): The significant correlation between ferritin in patients lean group with (a) insulin, (b) HOMA2 IR, (c) HOMA2 S %.

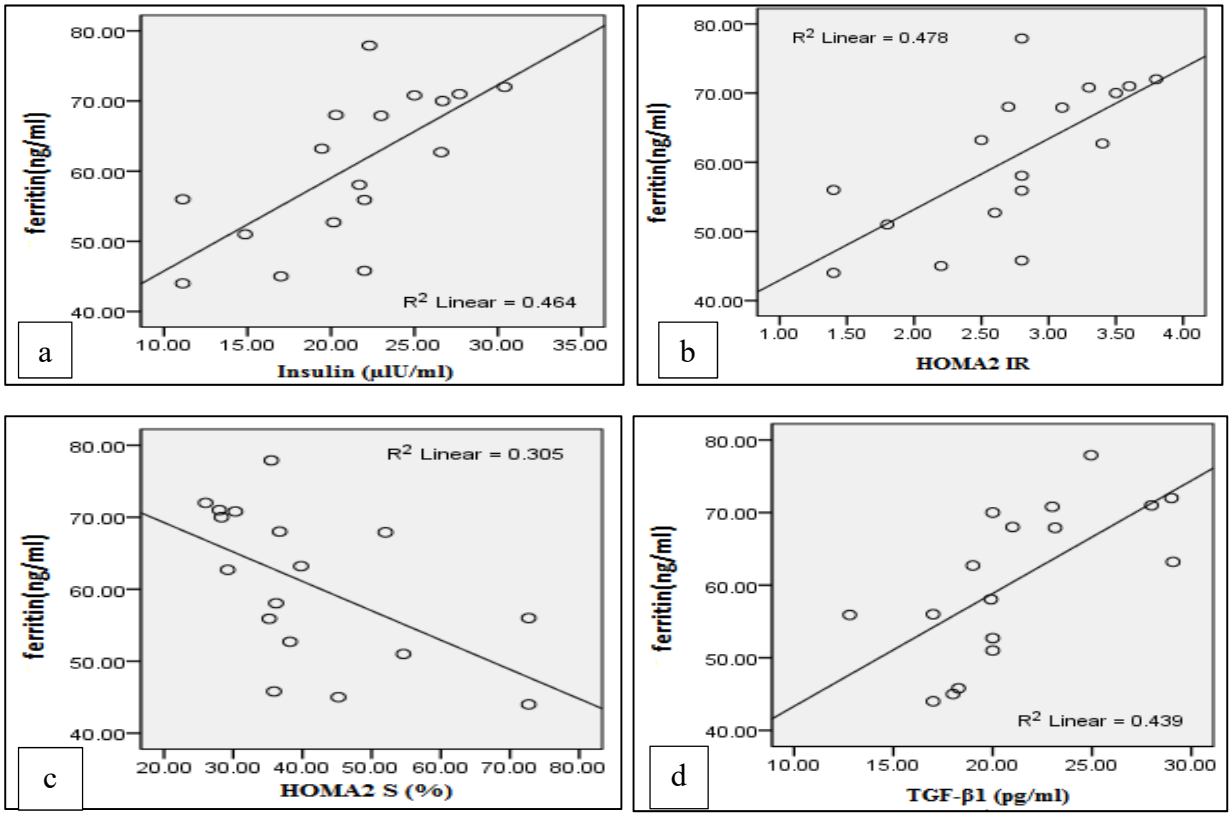

Figure (2): The significant correlation between ferritin in patients overweight group with (a) insulin, (b) HOMA2 IR, (c) HOMA2 S %, and (d) TGF-β1.

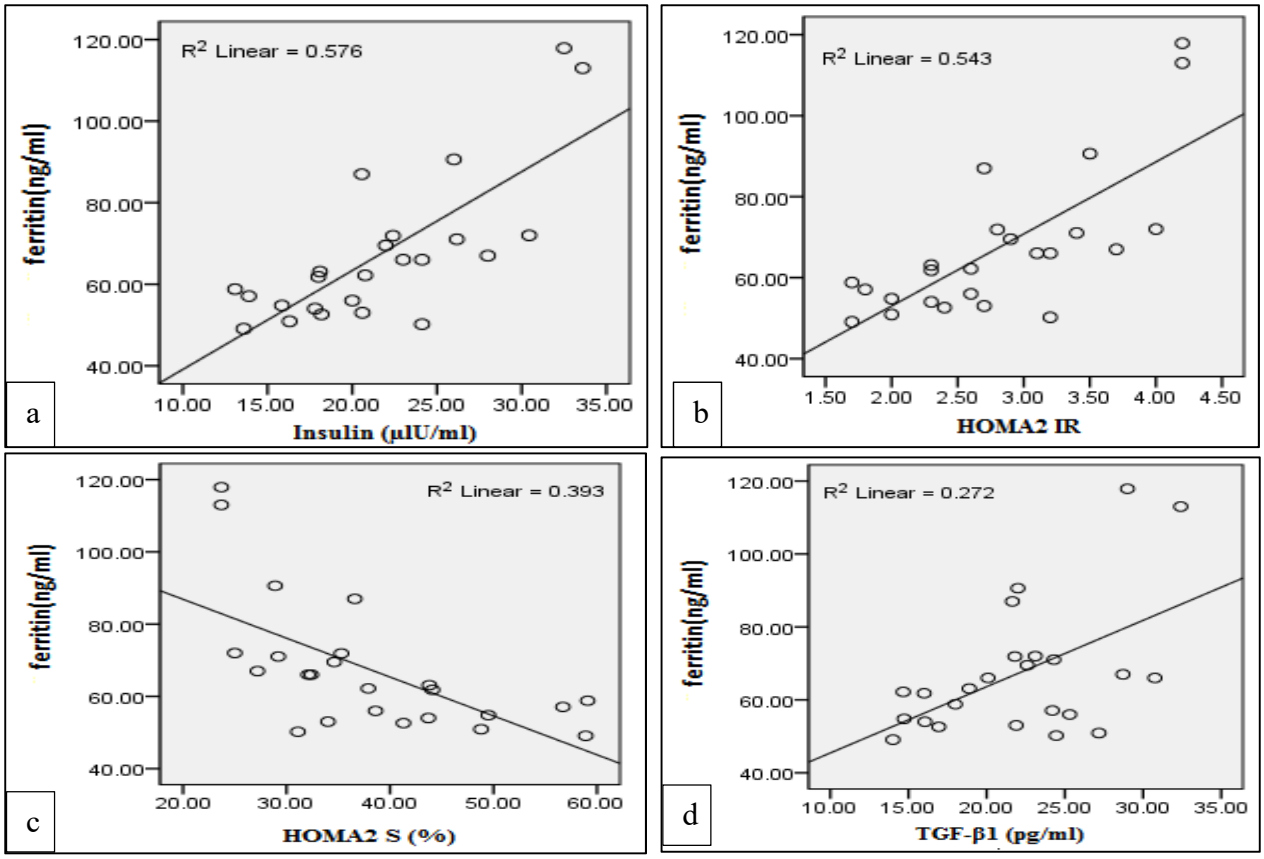

Figure (3): The significant correlation between ferritin in patients obese group with (a) insulin, (b) HOMA2 IR, (c) HOMA2 S % and (d) TGF-β1.

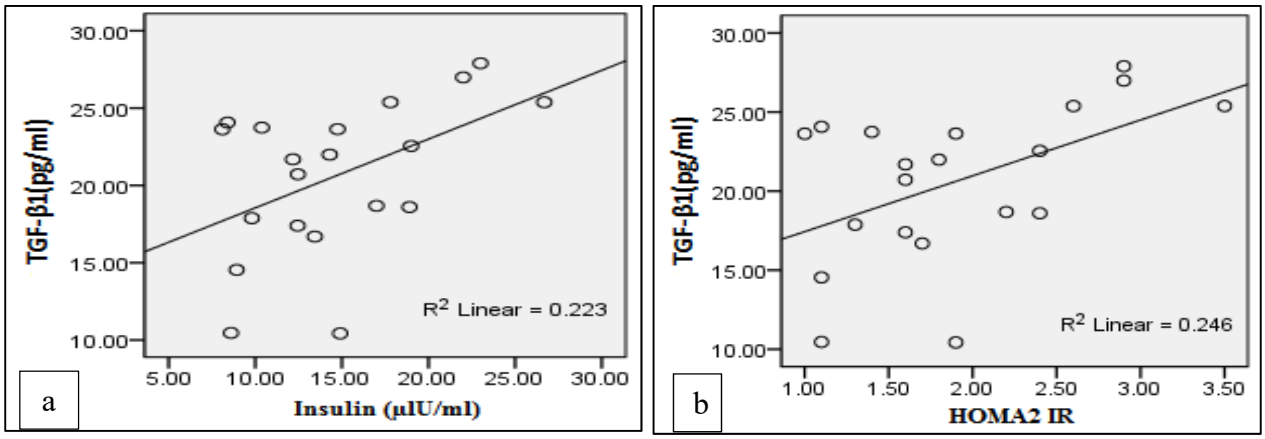

Figure (4): The significant correlation between TGF-β1 in patients lean group with (a) insulin, (b) HOMA2- IR.

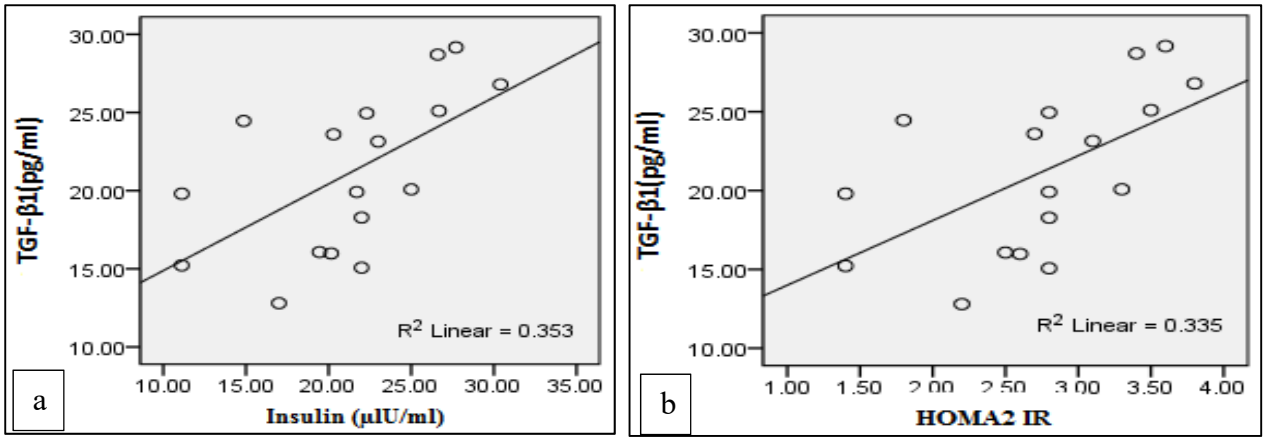

Figure (5): The significant correlation between the TGF-β1in patients overweight group with (a) insulin, (b) HOMA2- IR.

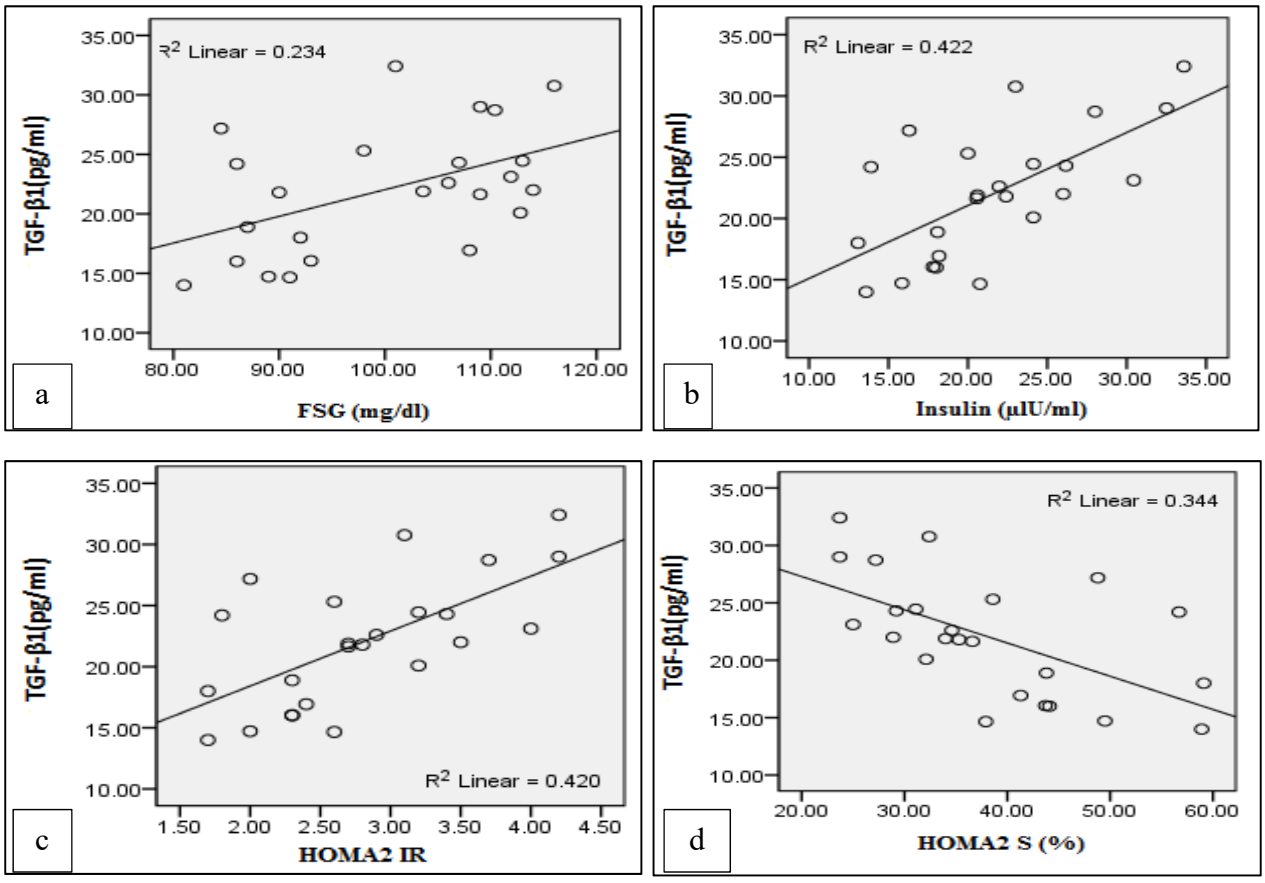

Figure (6): The significant correlation between TGF-β1in patients obese group with (a) FSG, (b) insulin, (c) HOMA2 IR, and (d) HOMA2 S %.

قياس مستويات ferritin و transforming growth factor-β1 في النساء العراقيات المصابات بمتلازمة تكيس المبيض

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الخلاصة

متلازمة تكيس المبايض هي اضطراب الغدد الصماء الاكثر شيوعا في الإناث, تشخص بعدم الإباضة المزمنة, تكيس المبيض و فرط الاندروجين. الهدف من هذه الدراسة توضيح العلاقة مابين مستويات ferritin و transforming growth factor-β1(TGF-β1)مع مقاومة الانسولين وخطر الاصابة بامراض الاوعية القلبية و السكري من النوع الثاني. طراق العمل: (61) نساء عراقيات مصابات بمتلازمة تكيس المبايض شخصت طبقا لمعايير Rotterdam, تم تقسيمها على مجاميع طبقا لدالة كتلة الجسم BMI: (20) نساء نحيفات مع دالة كتله جسم طبيعية (BMI: 18-24) , (17) نساء فوق الوزن مع (BMI: 24.9-29.9) و (24) نساء بدينات مع (BMI > 30) وتمت المقارنة مع (20) نساء عراقيات اصحاء متماثلين من حيث العمر. تم تمثيلهم من حيث; سكر مصل الدم (FSG),تم تقدير كميات مستويات مصل الانسولين, ferritin و TGF-β1,تم حساب عوامل Homeostatic model assessment (HOMA2).
النتائج: لوحظ زيادة معنوية عالية لل ferritin في مجموعة المرضى البدينات و فوق الوزن عندما قورنت مع مجموعة النحيفات. كما لوحظ زيادة معنوية لل TGF-β1 في مجاميع المرضى البدينات وفوق الوزن . بينما لم يظهر في مجموعة النحيفات. ارتباط Person لمجاميع المرضى اظهر وجود ارتباط معنوي موجب مابين مستوسات ferritin و (الانسولين , HOMA2-IR, و TGF-β1) وTGF-β1 مع (FSG , الانسولين, HOMA2-1R). بينما HOMA2 S % اظهر ارتباطا معنويا سالبا مع كلا من ferritin و TGF-β1.
الاستنتاج: زيادة مؤشر خزن الحديد بالجسم كانعكاس لزيادة تركيز ferritin و زيادة تركيز TGF-β1 في مرضى متلازمة تكيس المبيض وعلاقتهم بتركيز الانسولين ومقاومة الانسولين يمكن ان يرتبطا مع زيادة الخطر بالاصابة بامراض الاوعية القلبية والسكري من النوع الثاني.

الكلمات المفتاحية: متلازمة تكيس المبايض،ferritin,TGF-B1 ،فرط الانسولين