Relationship of hepcidin with Iron and lipids in patients with polycystic ovary Syndrome

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Abstract
polycystic ovary Syndrome is a common disorders in the endocrine glands in woman reproductive age the ovary is characterized by the aggregation of small bags surrounded by theca interna layers bloated follicles .
The level of hepcidin and other biochemical parameters were examined in (60) patients with polycystic ovary syndrome attended Azadi and AL-Jumhori in Kirkuk city, in addition to (30) blood samples of healthy individual as control group. The results showed a significant decrease ($p < 0.01$) in the level of hepcidin in addition to the levels of TIBC ($p < 0.01$) with UIBC ($p < 0.01$) and Transferrin ($p < 0.01$) while there was a significant increase in the levels of Iron ($p < 0.01$) and TS% ($p < 0.01$) in patients Compared to healthy control group . The relation was studied between levels of hormone hepcidin and concentration Iron and lipid profile with body mass index the results showed hormone hepcidin decrease in obese women . The results showed Iron increase in the obese group this indicated the rate between, polycystic ovary syndrome and obesity in women for this parameter. The effect of age on patients was studied with hormone hepcidin and iron and lipid profile concentrations where the hormone hipcidin decrease in the duration (15-25) years and increase in duration (26-35) and (36-45) years , The results showed iron and TS% increase in the duration (15-25 years), and decrease in (26-35), (36-45) years, and UIBC showed an increase in (36 -45) years and decreases in the periods (15-25) and (26-35) years either cholesterol and LDL Showing increase in (26-35) years and a decrease in (15-25) and (36-45) .

Introduction
polycystic ovarian syndrome bags were first discovered in 1935 by Leventhal and Stein The syndrome was known in their name and other names were also known to include hyperandrogenia of the ovary , Hyperplasia ovarian follicles [1] In 1970, the syndrome was diagnosed as a disorder Hypothalamic-pituitary axis Which leads to malfunction due to the secretion of the hormone Lutein and hormone stimulating follicles [2] Hepcidin is a hepatic protein that has been detected as a mediator in natural immunity, increasing its level with inflammation and infection. This hormone plays an important role in regulating the absorption of iron from the gut , as well as in the storage process of iron.[3] It is a peptide made in the liver and discovered in 2000 [4]. The hepcidin is an antimicrobial peptide. It is the encoded gene of hepcidin. The prefix–Hep is the designation of hepcidin to the production of this peptide in the liver and which has a pesticide of the carbobacteria referred to by the cidin part [3]. The lipids is a natural substance that dissolves in organic compounds and no dissolves in water. It plays a major role in the body as a source of energy storage. It is also an essential component in cellular membranes and hormones such as steroids, yellow salts and prostaglandins. It also plays an active role in the formation of certain compounds such as vitamins [5]. They are also essential constituents of cellular membranes and hormones such as steroids [6] .

Materials and methods of work
The current study was conducted on (60) blood samples of patients with polycystic ovarian syndrome in a randomized manner and with ages between 17 to 45 years of patients who attended the Azadi and al-Jumhori teaching Hospitals in Kirkuk city, in addition to (30) blood samples of healthy individual as control group, who were previously diagnosed with the disease by specialist doctors. The blood sample were divided into two parts, which were placed in a 5 ml blood clotting tube to examine hepcidin, Iron, TIBC, UIBC, Transferrin, TS%, Cholesterol, Triglyceride, HDL, LDL and VLDL levels. The tests were performed using ELIZA to measure the level of hepcidin, using ready-made hepcidin preparation from China YH Biosearch,. The enzyme-linked immune sorbent assy (ELISA) based on biotin double antibody sandwich technology to assay Human hepcidin . Hepcidin samples were incubated in wells that are pre-coated with hepcidin monoclonal antibody then adding hepcidin antibodies labeled with biotin to combine with streptavidin-HRP, which forms the immune complex . The unbound enzymes were removed after incubation and washing, then adding the substrate A and B. The solution will turn blue and change to yellow with the effect of acid. The shades of solution and the concentration of Human hepcidin are positively correlated [7].
The tests were performed using spectrophotometer to measure the levels of parameters, using ready-made Iron preparation from Spainreact-Spanish, Iron concentration was calculated using a chromatic method that analyzes the ferric ions in a weak acidic medium. Iron ion is a complex binary iron with Ferrozine[8]. Using ready-made TIBC preparation from Biolab- French. The chromatic method was used to calculate total iron concentration associated with transferrin. The method depends on the amount of iron added to the serum for the purpose of saturation of protein as the iron is not associated with the deposition of magnesium carbonate basal and after the process of centrifugation is the determination of iron in the leachate. The TIBC value is derived directly from the following equation: Transferrin (µg\dL)= 0.7 x TIBC (µg\dL) .[9] The percentage of

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saturation of transferrin with iron can be estimated and calculated by measuring the concentration of iron and total iron in the serum using the following equations Saturation %(T.S) = concentration Iron/ TIBC×100. Using ready-made cholesterol preparation from Biolab- French, The cholesterol was assessed in the serum by enzymatic method and using the diagnostic kits processed by the French company. This method is based on the enzymatic oxidation of the free cholesterol and cholesterol, the working reagent: consists of dissolving enzyme reagent R2 with the size of the regulated solution in R1[10]. Three triglycerides were estimated in serum using several kit analysis manufactured by the French company Biolab[11]. Determination of Serum LDL Cholesterol in serum according to the following equation [12]: LDL (Conc.) = Conc. of Cholesterol - Conc. HDL – VLDL. Determination of Serum VLDL Cholesterol in serum according to the following equation [13]: VLDL-C = Triglycerides/5. The tests were performed using Reflotron to measure the level of HDL , Determination of Serum HDL Cholesterol in serum method sample plasma EDTA for LDL and Chylomicrons vector reagents by magnesium ions and dextran sulfate [14] . User statistics by analysis of variance ANOVA

Results and discussion

The results in Table (1) and Figure (1) showed a significant decrease (P< 0.01) in the level of the hepcidin of the group of patients with polycystic ovarian syndrome compared with control group which was in agreement with the studie by Rashidi et al [15] and Luque-Ramírez [16] . This is due to the fact that high iron in patients with polycystic ovary syndrome contributes to a decrease in the level of hepcidin due to the accumulation of this iron in the body and increased absorption by the intestine and this disorder causes an increase in the androgen also responsible for the disorder in the levels of the hormone hepcidin.

Results showed an increase (p <0.01) in the level of Iron and this was in agreement with Al-Hakeim et al [17]. It is hypothesized that genetic factors, the absence of a regular menstrual blood loss, or even hyperinsulinemia resulting from insulin resistance, considering that insulin might stimulate intestinal iron absorption by upregulating the activity of hypoxia-inducible factor-1alpha and down regulating hepcidin expression [18][19].

Table (1) The concentrations hepcidin, Iron, TBIC, UIBC, Transferrin, TS% in the group of women with polycystic ovary syndrome and control group

parameters	Groups	N	Means ±SD	P-Value
HEP Pg\ml	control	30	1383±481	0.01
	patients	60	918±168	
I R O N µg\dL	control	30	84.4±36.5	0.01
	patients	60	113.0±34.2	
T I B C µg\dL	control	30	284.9±23.9	0.005
	patients	60	267.5±31.7	
U I B C µg\dL	control	30	200.6±54.7	0.01
	patients	60	159.2±46.7	
Transferrin µg\dL	control	30	199.5±16.7	0.011
	patients	60	188.5±22.2	
T . S%	control	30	30.7±16.9	0.004
	patients	60	41.4±13.7	

Figure (1) The level hepcidin on the group of patients and control group

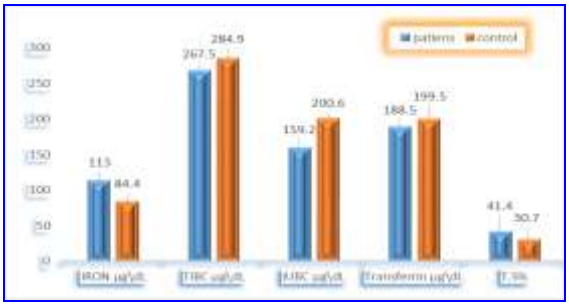

Figure (2) The effect of the concentrations Iron, TBIC, UIBC, Transferrin, TS% in the group of women with polycystic ovary syndrome and control group

The results in Table (2) and Figure (3) showed there is no significant difference between lipid levels and this study is not consistent with the Ahmed [20] and Luque-Ramírez [21]. This is due to the convergence of weights between the group of patients and the control group and the current study proves that polycystic ovary syndrome does not affect the fat levels of patients.

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Table (2)The levels of lipids on the group of patients and control group

parameters	Groups	N	Means±SD	P-Value
Cholesterol mg\dL	control	30	149.60±32.90	0.157
	patients	60	161.30±43.00	
Triglyceride mg\dL	control	30	119.1±47.50	0.737
	patients	60	115.60±47.50	
H D L mg\dL	control	30	23.63±7.12	0.197
	patients	60	25.79±8.04	
L D L mg\dL	control	30	101.50±28.00	0.135
	patients	60	112.20±37.70	
VL D L mg\dL	control	30	23.77±9.54	0.798
	patients	60	23.22±9.64	

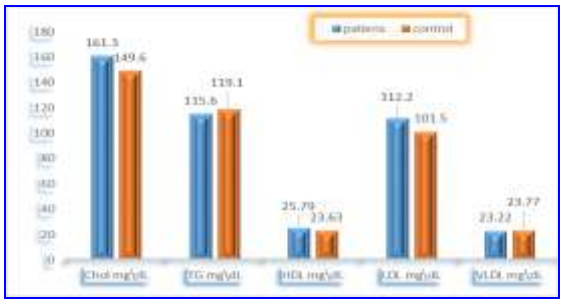

Figure (3) The levels lipids on the group of patients and control group

Body mass index (BMI) was considered and the patients with polycystic ovary syndrome were divided into three groups according to BMI G1 16-25 kg \ m2 (normal weight), G2 26-35 kg \ m2 (overweight) , G3 36-45 kg\m2 (obesity) these totals were compared to each other. The Table (3) showed increase in the first and second group and a decrease in the third groups .but iron showed increase in the third group and a decrease in the first and second groups .This finding suggested increased body iron stores in these women, raising the possibility that genes related to iron metabolism are altered in polycystic ovarian syndrome [22] .

Table (3) The concentration of hepcidin, Iron, TBIC, UIBC, Transferrin, TS% in BMI groups (G1, G2, G3)

parameters	Group (BMI)	N	Means±SD
HEP Pg\ml	G1(BMI)	22	931.80^{a}± 19.20
	G2(BMI)	26	919.20^{a}± 13.70
	G3(BMI)	12	866.70^{b}± 20.00
IRON µg\dL	G1(BMI)	21	109.76^{b}± 33.11
	G2(BMI)	26	102.88^{b}± 34.83
	G3(BMI)	12	127.00^{a}± 34.80
TIBC µg\ dL	G1(BMI)	21	269.76^{a}± 30.22
	G2(BMI)	26	265.65^{a}± 35.29
	G3(BMI)	12	275.83^{a}± 28.53
UIBC µg\ dL	G1(BMI)	21	160.00^{a}± 46.10
	G2(BMI)	26	162.77^{a}± 50.93
	G3(BMI)	12	148.80^{a}± 42.00
Transferrin µg\dL	G1(BMI)	21	188.74^{a}± 21.19
	G2(BMI)	26	185.96^{a}± 24.71
	G3(BMI)	12	193.08^{a}± 19.97
T.S%	G1(BMI)	21	41.32^{a}± 3.96
	G2(BMI)	26	39.45^{a}± 4.09
	G3(BMI)	12	46.26^{a}± 3.06

represents variables a, b that reflect the difference between a groups

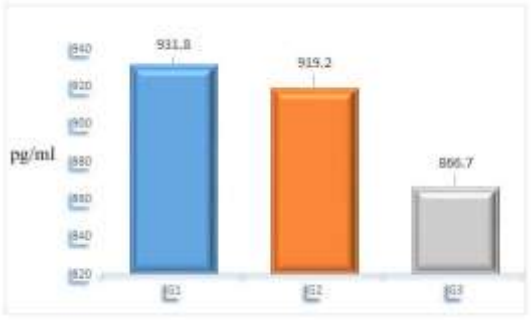

Figure (4) The level of hepcidin for body mass index (BMI) groups (G1, G2, G3)

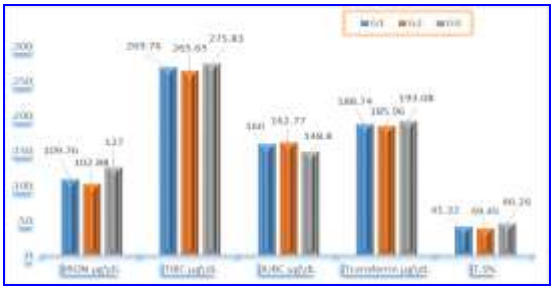

Figure (5) The concentration of Iron, TBIC, UIBC, Transferrin, TS% in body mass index (BMI) groups (G1, G2, G3)

The effect of the age factor on the measured parameters was studied. Patients with polycystic syndrome were divided into three groups by age G1 (17-25 years), G2 (26-35) years, G3 (36-45) years, The table (5) shows an increase in the concentration of hepcidin in the second and third group and its decrease in the first group. Shows an increase in the concentration of Iron in the first group and its decrease in the second and third group. While UBIC was increased in the second and third groups and decreased in the first group. This indicates that the age factor affects this parameter. The TS% there increased in the first and second groups and a decrease in the third group.

Table (5) The age on hepcidin, Iron, TBIC, UIBC, Transferrin, TS% in groups (G1, G2, G3)

parameters	Group (Age)	N	Means±SD
HEP Pg\ml	G1(Age)	24	879.20^{b}± 84.10
	G2(Age)	21	947.60^{a}± 77.80
	G3(Age)	15	946.70^{a}± 45.70
IRON µg\ dL	G1(Age)	24	141.00^{a}± 28.05
	G2(Age)	21	107.14^{b}± 36.08
	G3(Age)	15	84.40^{c}± 24.69
TIBC µg\ dL	G1(Age)	24	263.04^{a}± 32.33
	G2(Age)	21	269.71^{a}± 31.69
	G3(Age)	15	279.70^{a}± 42.30
UIBC µg\ dL	G1(Age)	24	133.70^{c}± 7.20
	G2(Age)	21	162.60^{b}± 7.30
	G3(Age)	15	195.30^{a}± 5.40
Transferrin µg\dL	G1(Age)	24	187.19^{a}± 22.87
	G2(Age)	21	188.80^{a}± 22.18
	G3(Age)	15	195.77^{a}± 29.63
T.S%	G1(Age)	24	49.61^{a}± 6.20
	G2(Age)	21	40.16^{b}± 3.90
	G3(Age)	15	30.99^{c}± 8.21

Represents variables a, b, c that reflect the difference between a groups

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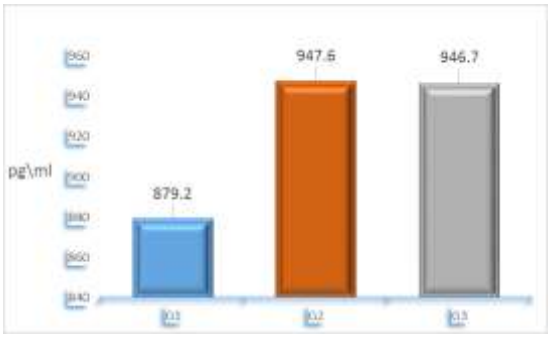

Figure (6) The level of hepcidin for the age groups (G1, G2, G3)

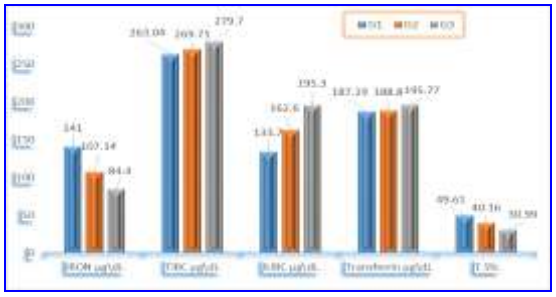

Figure (7) The concentrations Iron, TBIC, UIBC, Transferrin, TS% in the age groups (G1, G2, G3)

The table (2) showed an increased in the level of Cholesterol in the second and third group and decreased in the first group, While LDL was shown to increase in the second and third group and decreased in the first group, as for VLDL which increased in the second group and decreased in the first and third groups . This study is not consistent with the Al-Jabery et al [23] who indicated that the increase in the concentration of cholesterol and of lipoproteins in PCOS may be associated with the risk of coronary artery disease, this is what the study pointed out Bush [24]. The incidence of dyslipidemia disorder observed in 70% of women with PCOS may have been associated with high concentration Cholesterol and Triglyceride and LDL this is what a study found [25] .

Table (6) The levels lipids for the age groups (G1, G2, G3)

parameters	Group (Age)	N	Means±SD
Cholesterol mg\dL	G1(Age)	24	146.34 [c]± 9.21
	G2(Age)	21	177.60 [a]± 8.10
	G3(Age)	15	162.35 [b]± 4.53
Triglyceride mg\dL	G1(Age)	24	116.19 [a]± 4.25
	G2(Age)	21	124.20 [a]± 5.30
	G3(Age)	15	117.90 [a]± 7.30
H D L mg\dL	G1(Age)	24	25.90 [a]± 4.78
	G2(Age)	21	24.41 [a]± 6.82
	G3(Age)	15	28.84 [a]± 7.19
L D L mg\dL	G1(Age)	24	96.92 [c]± 4.32
	G2(Age)	21	125.75 [a]± 8.55
	G3(Age)	15	114.31 [b]± 4.53
V L D L mg\dL	G1(Age)	24	23.50 [b]± 2.64
	G2(Age)	21	31.58 [a]± 3.99
	G3(Age)	15	20.35 [b]± 3.49

Represents variables a, b, c that reflect the difference between a groups

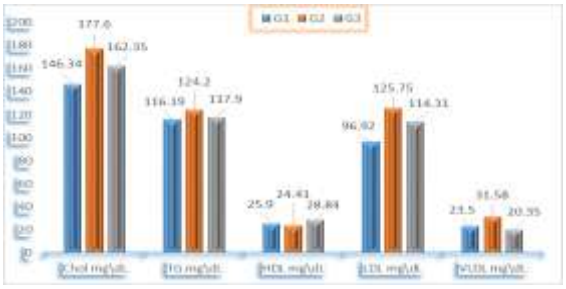

Figure (8) The levels lipids for the age groups (G1, G2, G3)

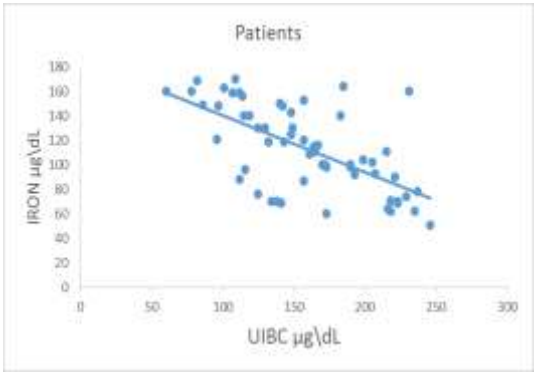

Figure (9) Correlation of UBIC and Iron Of patients (r= -0.632)

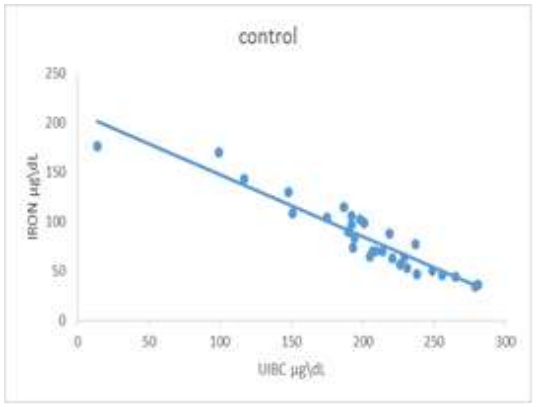

Figure (10) Correlation of UBIC and Iron Of control (r= -0.94004)

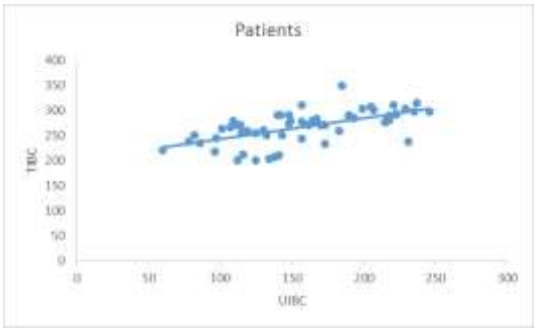

Figure (11) Correlation of UBIC and TIBC Of patients (r= 0.617)

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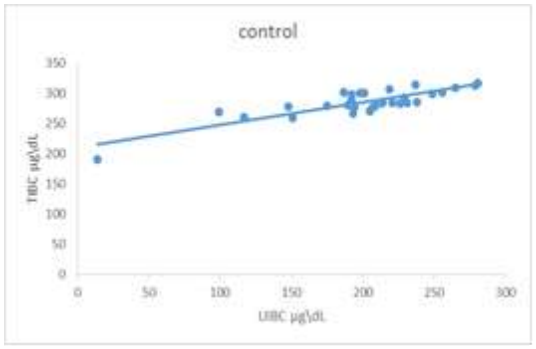

Figure (12) Correlation of UBIC and TIBC Of control (r= 0.854)

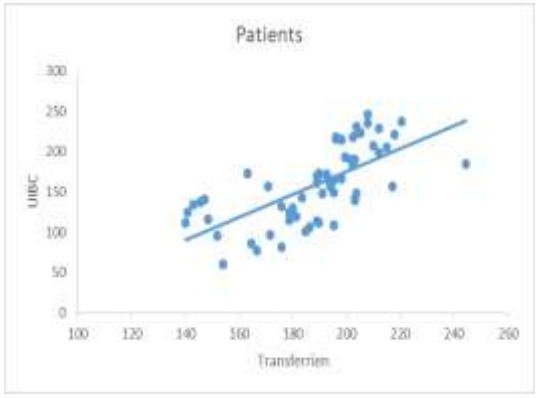

Figure (15) Correlation of Transferrin and UIBC Of patients (r= 0.673)

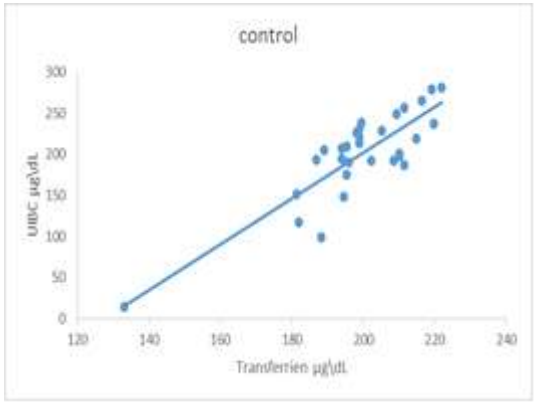

Figure (16) Correlation of Transferrin and UIBC Of control (r= 0.854)

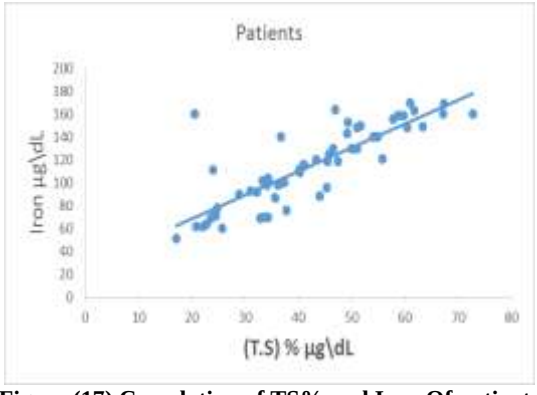

Figure (17) Correlation of TS% and Iron Of patients (r= 0.833)

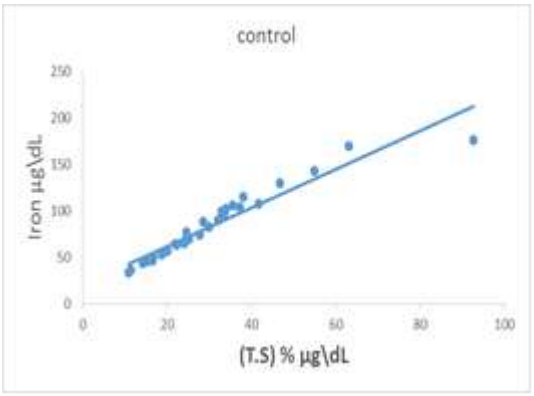

Figure (18) Correlation of TS% and Iron Of control (r=0.958)

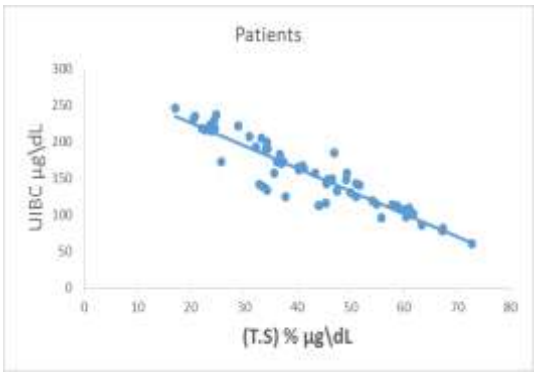

Figure (19) Correlation of TS% and UIBC Of patients (r= -0.921)

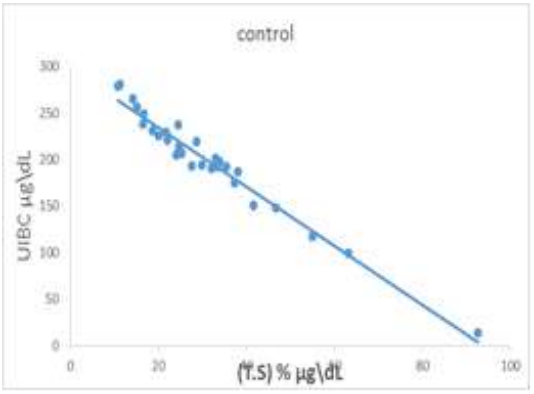

Figure (20) Correlation of TS% and UIBC Of control (r= -0.982)

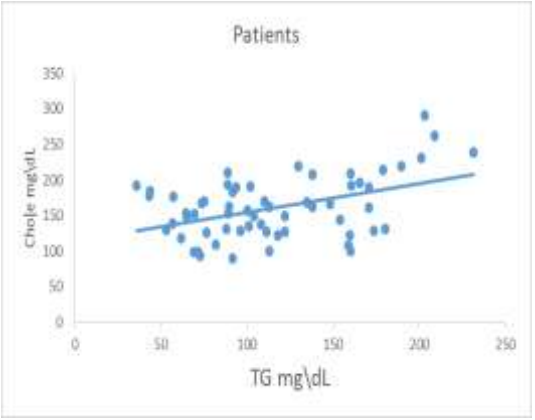

Figure (21) Correlation of TG and Cholesterol Of patients (r= 0.444)

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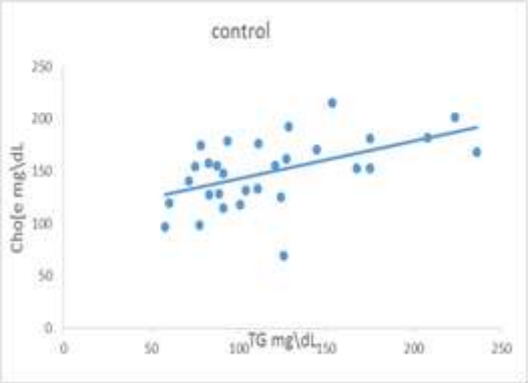

Figure (22) Correlation of TG and cholesterol Of control (r= 0.522)

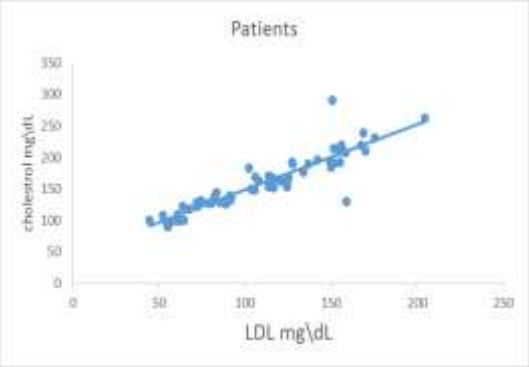

Figure (23) Correlation of LDL and cholesterol Of patients (r= 0.903)

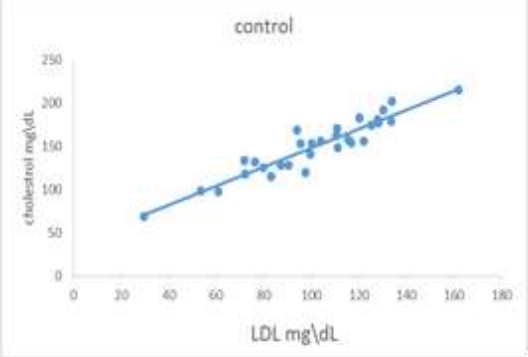

Figure (24) Correlation of LDL and cholesterol Of control (r= 0.939)

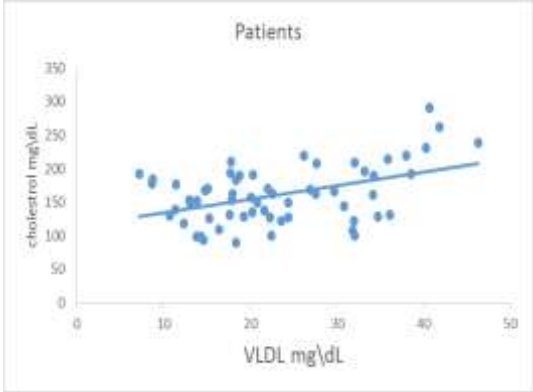

Figure (25) Correlation of VLDL and cholesterol Of patients (r= 0.447)

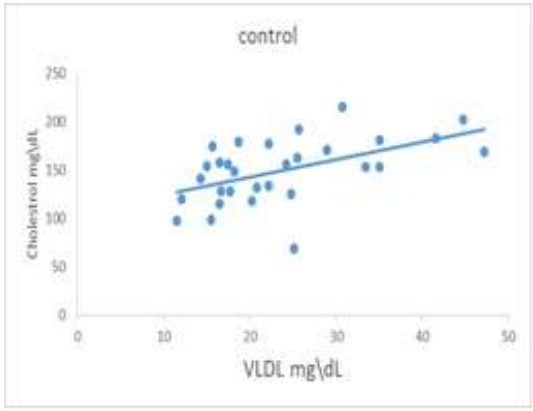

Figure (26) Correlation of VLDL and cholesterol Of control (r= 0.526)

Conclusion

It was concluded that hepcidin level in patients with polycystic ovary syndrome was lower than in control group.

There was no correlation between hepcidin and all other parameters but there was some correlations among parameters used .

There was a positive correlations between UIBC with TIBC and Transferrin and Transferrin . Iron with TS% and between cholesterol and TG and LDL with VLDL. There was a negative correlations between UIBC with Iron and TS% .

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علاقة الهيبسيدين مع الحديد ومستوى الدهون في المريضات المصابات بمتلازمة المبيض متعدد الاكياس

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الملخص

متلازمة المبيض متعدد الاكياس هي من الاضطرابات الشائعة في النساء بعمر الانجاب ومن اهم نتائجها عدم الانجاب ويتميز المبيض بتجمع أكياس صغيرة محاطة بطبقات الجريب الداخلي المتضخمة للجريبات المكيسة.

وتضمنت الدراسة تقدير مستوى الهيبسيدين وعدد من المتغيرات الكيموحيوية في مصل دم المريضات المصابات بمتلازمة المبيض متعدد الاكياس، فقد شملت الدراسة الحالية (60) مريضة من النساء اللواتي يعانين من تكيس المبايض بالإضافة الى مجموعة السيطرة والتي تضمنت (30) عينة دم.

واظهرت نتائج الدراسة الحالية انخفاض معنوي ($p <0.01$) في مستوى هرمون الهيبسيدين وكذلك في المتغيرات (($p <0.01$) TIBC ، ($p <0.01$) UIBC ، ($p <0.01$) Transferrin)، بينما أظهرت متغيرات أخرى ارتفاع في مستوياتها وهي (($p <0.01$)Iron، ($p <0.01$) T.S%) في المرضى مقارنة مع مجموعة السيطرة. تم دراسة علاقة مؤشر كتلة الجسم BMI مع هرمون الهيبسيدين وتراكيز الحديد والدهون واظهر هرمون الهيبسيدين انخفاض في النساء البدينات مما يؤكد عدم وجود علاقة بين السمنة ومتلازمة تكيس المبايض في النساء. اظهر الحديد ارتفاع في مجموعة البدينات مما يؤكد وجود علاقة بين السمنة ومتلازمة تكيس المبايض في النساء لهذا المتغير. تم دراسة تأثير العمر بين المرضى مع هرمون الهيبسيدين وتراكيز الحديد والدهون حيث اظهر هرمون الهيبسدين انخفاض في الفترة الزمنية من (15–25) سنة وارتفاع في الفترة الزمنية من (26–35) و (36–45) سنة ، اظهرا الحديد والنسبة المئوية للتشبع TS% ارتفاع في الفترة الزمنية (15–25) سنة وانخفاض في (26–35) و(36–45) سنة واظهر UIBC ارتفاع في (36–45) سنة وانخفاض في الفترتين (15–25) و (26–35) سنة اما الكولسترول و LDL فقد اظهرا ارتفاع في (26–35) سنة وانخفاض في (15–25) و (36–45) سنة .