Estimation of the hepcidin level and some Biochemical parameters in patients of polycystic ovary Syndrome in Kirkuk city

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ABSTRACT

Polycystic ovary Syndrome is a common disorder in the endocrine glands, which is characterized by the aggregation of small bags surrounded by theca internal layers bloated follicles .

The level of hepcidin and some other biochemical parameters were examined in (60) patients In addition to (30) blood samples of healthy control .

The results showed a significant decrease in hepcidin level as well as E2 and progesterone while there was a significant increase in prolactin and testosterone in polycystic ovarian syndrome patients Compared to healthy control. There was no correlation between hepcidin and all other parameters but there was some correlations among parameters used, there was positive correlations between FSH with LH and Testosterone as well as between E2 and Testosterone and between T4 and T3 .

Introduction

The ovary is considered as an endocrine gland with external and internal secretion, Their tissue consists of two regions: - An internal area called Medulla , included a central nucleus rich in blood vessels that are located between the muscular connective tissue , It is the major part of the ovary, Surrounded by Tunica albuginea [1], The outer area is called the Cortex which is a thin surface-white, color containing thousands of primary follicles [2] The cortex is made up of a more cohesive cellular tissue more than the medulla and containing ovarian follicles, the primary follicle surrounded by a single layer of follicular flat cells. When it grows , small irregular spaces appear within the mass and are filled with follicular fluid , the follicle is called the secondary follicle , Then they combine with the secondary follicle together to form a single cavity called laurel. The egg cell, surrounded by a group of follicular cells, is moved to the side and is clearly visible in the laurel . After the maturity of graffian follicle they explode and release the egg, shrinks the wall of follicle and becomes a temporary glandular structure called the corpus luteum It then decomposes and dissolves shortly after, replacing its cells with a connective tissue and becomes a white scar called [3] corpus albicans . The polycystic ovary syndrome is orginally genetic in origin because it is known that the syndrome has a strong in hereditary effect and that one or more genes may contribute to the heterogeneous phenotype, clinical and biochemical pattern of the syndrome [4]. 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 [5] 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 [6] 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. [7] It is a peptide made in the liver which was discovered in 2000 [8]. The hepcidin is an antimicrobial peptide . It is the encoded gene of hepcidin. The prefix – Hep is the designation of hepcidin which has a pesticide of the carbobacteria referred to by the cidin part [7] .

Materials and methods

The current study was conducted on (60) samples of patients with polycystic ovarian syndrome in a randomized manner and with ages ranging from 17 to 45 years of patients who attended the Azadi and al-

Jumhori teaching Hospitals in Kirkuk city, who were previously diagnosed with the disease by specialist doctors. The blood sample were divided into two parts, which were placed in a 5ml blood clotting tube to examine hepcidin LH, FSH, PRL, Progesterone, E2, Testosterone, TSH, T3 and T4 levels . The tests were performed using mini VIDAS and 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 .

The levels of other hormones (LH, FSH, PRL, Progesterone, E2, Testosterone, TSH, T3, and T4) have been measured in the test kit using mini VIDAS.

Results and discussion

The results in Table (1) showed a significant decrease ($P < 0.01$) In the level of the hepcidin of the group of patients with polycystic ovarian syndrome compared to the control group which was in agreement with the studies by[9,10] They attributed the decrease in levels of serum hepcidin due to an increase in the concentrations of the follicle, especially in patients with menopause, because of ferritin elevation in irregular menstrual cycle. Results showed an increase in the level of prolactin which was in agreement with [11] The reason for this increase was due to changing in the axis of the pituitary gland, which increases its effect on the secretion of the rest of other hormones (E2 ، LH ، FSH) whereas (3-7)% of patients with polycystic ovarian syndrome had an elevation in serum prolactin levels, (measured on the third day of the menstrual cycle). The level of E2, showed a significant decrease ($P < 0.05$) in the group of patients compared with the control group. These results were in agreement with [12] due to feeding disturbance and menopause. The results of other research confirmed that the reason was due to inability of the ovary to form and produce ovarian hormones by the granules of the estrogen and progesterone and this leads to lower their levels in the blood while, there was an increase in the level of testosterone produced by the ovary .

Progesterone level showed a significant decrease in patients compared with the control group [13] the reason for this was mentioned earlier.

The testosterone level showed a significant increase in the group of patients compared with the control group. This is consistent to study[14] . Who attributed the increase of this hormone to the weakness of

fertility and reproduction in women, Another study has proved that androgen plays an effective role in the fertility of women, Androgen and its receptors are the main factors to regulate the normal growth of follicle and functional decline in the reserve of ovaries and primary ovarian insufficiency. The incidence of polycystic syndrome is accompanied by an increase in the level of testosterone and obesity, the emergence of acne in the face and below the chin and the lack or scarcity of menstruation in patients and this is consistent with the study of (Thomas, Rebar) [15] who indicated a disturbance in other hormones produced from the ovary whish effect on the concentrations of estrogen and progesterone .This indicates the effect of the level of testosterone on women with the polycystic ovarian syndrome .

Table (1) Effect of the level of hormones on 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	0.0002
	patients	60	918±168	
FSH mlU\ml	control	30	5.22±1.81	0.139
	patients	60	4.64±1.59	
LH mlU\ml	control	30	4.07±1.70	0.080
	patients	60	3.38±1.76	
PRL ng\ml	control	30	15.48±7.06	0.057
	patients	60	18.73±8.31	
Progesterone ng\ml	control	30	6.80±2.24	0.025
	patients	60	5.75±1.46	
E2 Pg\ml	control	30	93±113	0.047
	patients	60	56.8±17.80	
Testosterone ng\ml	control	30	0.59±0.25	0.005
	patients	60	0.79±0.38	

The results of the current study (Table 2) showed an increase in the level of T3 hormone in the group of patients compared with the control group. This is consistent with the study of [16] This is due to iodine retention due to hypothyroidism and iodine balance is necessary for thyroid activity. The study showed that the increase in the level of thyroid hormone and the decrease in the level of T3 can be explained by a decrease in the function of the thyroid gland to form hormones, T3 This decrease in thyroid stimulating is caused by T4 in women with multiple ovarian syndrome. The incidence of hypothyroidism in some women is associated with increased secretion of the thyroid hormone to hypothyroidism from hypothyroidism (TRH) [17] .

Table (2) The effect of the level of thyroid hormones on the group of women with polycystic ovarian syndrome and control group

parameters	Groups	N	Means±SD	P-Value
TSH µIU\ml	control	30	2.09±1.66	0.852
	patients	60	2.16 ±1.28	
T3 nmol \l	control	30	1.20±0.40	0.010
	patients	60	1.42±0.29	
T4 nmol \l	control	30	88.50±14.80	0.056
	patients	60	94.70±14.80	

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 results of the study Table (3) showed a difference between the first group and the third group, while the second group has no differences between them and the two groups of the level of prolactin. This was in disagreement with [18] who indicated that the obesity factor and BMI, has no effect on the hormone. While the rest of the hormones did not notice any significant difference between the groups and the reason is that the body mass index has no effect on these hormones.

Table (3) The level of hormones for BMI groups of G1, G2, G3

parameters	Group (BMI)	N	Means±SD
HEP Pg\ml	G1	22	931.80^a± 19.20
	G2	26	919.20^a± 13.70
	G3	12	866.70^a± 20.00
FSH mlU\ml	G1	22	4.49^a± 1.91
	G2	26	4.59^a± 1.29
	G3	12	4.66^a± 1.14
LH mlU\ml	G1	22	3.12^a± 1.74
	G2	26	3.46^a± 1.98
	G3	12	4.01^a± 1.32
PRL ng\ml	G1	22	20.78^a± 3.60
	G2	26	18.50^{ab}± 2.35
	G3	12	15.48^b± 2.32
Progesterone ng\ml	G1	22	5.83^a± 1.92
	G2	26	5.84^a± 1.14
	G3	12	5.43^a± 1.17
E2 Pg\ml	G1	22	53.69^a± 6.06
	G2	26	57.22^a± 8.43
	G3	12	61.36^a± 9.67
Testosterone ng\ml	G1	22	0.78^a± 0.35
	G2	26	0.81^a± 0.42
	G3	12	0.82^a± 0.33

In Table (4), there was no change in TSH and T4 values for BMI and for all three groups in patients with polycystic ovarian syndrome. However, there was a significant change in the value of T3 for the first group from the third group, while the second group showed no significant change Between them and between the two groups .

Table (4) Effect of thyroid hormone level for body mass index (BMI) groups for G1, G2, G3

parameters	Group (BMI)	N	Means±SD
TSH µIU\ml	G1	22	2.32^a± 1.13
	G2	26	1.89^a± 1.52
	G3	12	2.04^a± 1.06
T$_3$ nmol \l	G1	22	1.51^a± 0.28
	G2	26	1.48^{ab}± 0.34
	G3	12	1.21^b± 0.23
T$_4$ nmol \l	G1	22	96.21^a± 5.05
	G2	26	96.22^a± 5.34
	G3	12	88.88^a± 2.58

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, Table 5 showed that there was a significant increase in hepcidin with age and G1, G2 and G3. As shown, FSH also showed significant increase in age and G1, G2, G3, LH showed a significant increase among the three groups with age, while estradiol showed a significant decrease with the age of the group while a rise was observed in the second and first groups.

Table (5) Effect of hormones level for the age groups of G1, G2, G3

parameters	Group (Age)	N	Means±SD
HEP Pg\ml	G1	24	879.20^b± 84.10
	G2	21	947.60^a± 77.80
	G3	15	946.70^a± 45.70
FSH mlU\ml	G1	24	3.87^b± 1.40
	G2	21	4.82^{ab}± 1.38
	G3	15	5.51^a± 1.63
LH mlU\ml	G1	24	2.84^c± 1.58
	G2	21	3.38^b± 1.65
	G3	15	4.72^a± 1.72
PRL ng\ml	G1	24	21.85^a± 1.34
	G2	21	16.74^b± 4.77
	G3	15	16.07^b± 4.60
Progesterone ng\ml	G1	24	5.89^a± 1.53
	G2	21	5.97^a± 1.35
	G3	15	5.50^a± 1.47
E2 Pg\ml	G1	24	54.77^b± 9.24
	G2	21	66.28^a± 6.97
	G3	15	46.60^c± 7.62
Testosterone ng\ml	G1	24	0.72^a± 0.35
	G2	21	0.90^a± 0.42
	G3	15	0.80^a± 0.32

The study showed in Table (6) that there was no significant difference between the three groups of hormones TSH, T3, T4. This indicates that the age factor did not affect these hormones for thyroid gland.

Table (6) Level of thyroid hormones for age groups G1, G2, G3

parameters	Group (Age)	N	Means±SD
TSH µIU\ml	G1	24	2.15^a± 1.23
	G2	21	2.15^a± 1.43
	G3	15	2.06^a± 1.17
T$_3$ nmol \l	G1	24	1.43^a± 0.23
	G2	21	1.48^a± 0.40
	G3	15	1.31^a± 0.18
T$_4$ nmol \l	G1	24	96.21^a± 13.33
	G2	21	94.10^a± 19.52
	G3	15	93.32^a± 8.85

Result indication that there was no correlation between hepcidin and the studied parameters but there was a positive correlation between the hormones of FSH and LH for the group of patients where (r = 0.427). There is also a positive correlation

between the hormones of the control group and (r = 0.485) as in Figs (1) and (2) , either hormonal Testosterone and FSH , Also have a positive correlation with (r = 0.392) for patients and a positive correlation between the hormones of the control group where the (r = 0.383) as in (3) and (4) . However, with the control group, the two hormones showed an inverse relationship and the value of (r = -0.416), as in (5) and (6) (r = 0.465) and control group (r = 0.866), as in Figs. (7) and (8) .
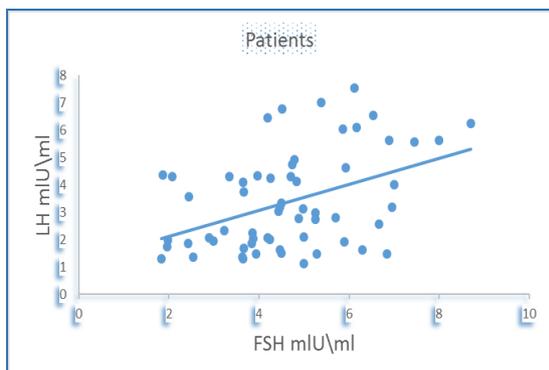

Figure (1) Correlation of FSH and LH, Hormones Of patients (r=0.427)
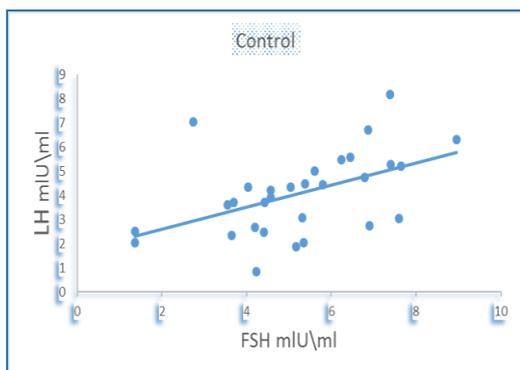
Figure (2) Correlation of FSH and LH , Hormones Of patients(r=0.485)
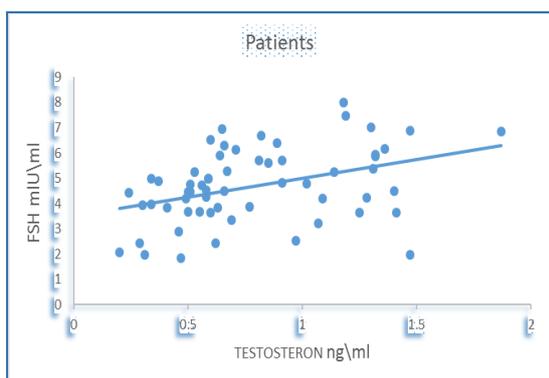
Figure (3) Correlation of the hormone, Testosterone and FSH for control amounted to amounted to (r = 0.383)
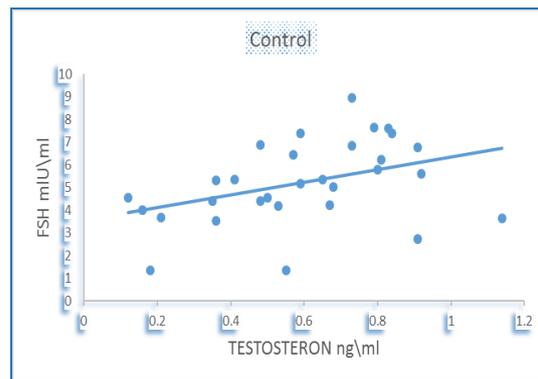

Figure (4) Correlation of the hormone, Testosterone and FSH for control amounted to (r = 0.392)
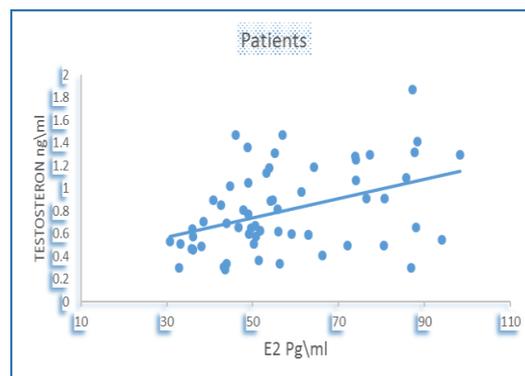

Figure (5) Correlation of the hormone, E2 and Testosterone for patients amounted to (r = 0.404)
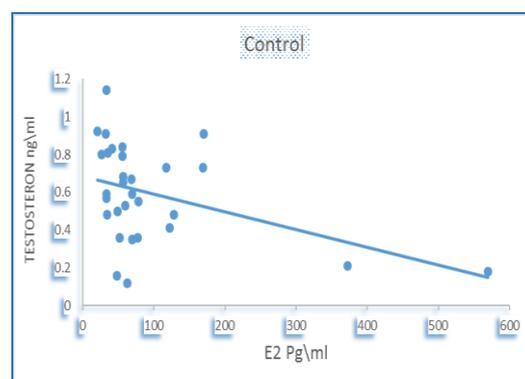

Figure (6) Correlation of the hormone , E2 and Testosterone for control amounted to (r = -0.416)
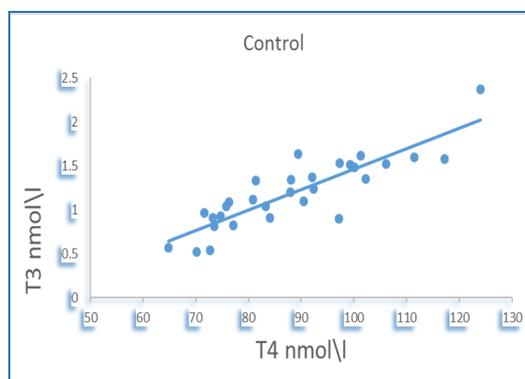

Figure (7) Correlation of the hormone. T4and T3 for patients. amounted to (r = 0.465)
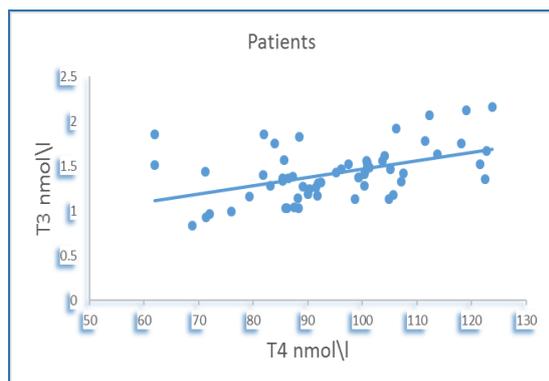
Figure (8) Correlation of the hormone, T4and T3 for patients , (r = 0.866)

Conclusion

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

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

There was a positive correlations between FSH with LH and Testosterone. E2 with Testosterone and between T4 with T3 .

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تقدير مستوى الهيبسيدين وعدد من المتغيرات الكيموحيوية في مصل دم المريضات المصابات بمتلازمة المبيض متعدد الاكياس في مدينة كركوك

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الملخص

متلازمة المبيض متعدد الاكياس تعد من الاضطرابات الشائعة في الغدد الصم ومن اهم نتائجه عدم الانجاب، اذ يتميز المبيض بتجمع أكياس صغيرة محاطة بطبقات الجريب الداخلي المتضخمة للجريبات المتكيسة.

مستوى الهيبسيدين وعدد من المتغيرات الكيموحيوية تضمنت (60) مريضة بالإضافة الى (30) عينة الدم للسيطرة. واظهرت نتائج الدراسة الحالية انخفاض معنوي في مستوى هرمون الهيبسيدين وكذلك في المتغيرات (Progesterone ، E2) بينما أظهرت متغيرات أخرى ارتفاع في مستوياتها وهي (Testosterone ، PRL) في مرضى متلازمة المبيض متعدد الاكياس مقارنة مع مجموعة السيطرة ، كما لوحظ عدم وجود علاقة ارتباطية بين هرمون الهيبسيدين مع باقي المتغيرات لكن يوجد علاقة ارتباطية ايجابية بين هرمونات FSH مع LH و Testosterone وكذلك بين FSH ، E2 و Testosterone وبين T4 و T3 .