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Orexin hormone and serum lipids in polycystic ovary patients

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Abstract

In the current research, (60) cases of polycystic ovary patients, whose ages ranged from (22-45) years, were studied. A questionnaire was used for each patient, in which special information was recorded for each of them . The study sample was divided according to age into three groups: the first group (22-29) years, the second group (30-37) years, and the third group (38-45) . Also, it was classified according to body mass into two groups, the first being women of normal weight (24-29.9)and the second is for women with an above-normal weight (30 and over).The findings demonstrated a statistically significant drop in orexin hormone levels in PCOS individuals compared to the control group at the level of probability ($p \leq 0.01$) .In accordance with the body mass index (BMI) differences between the two groups, the results also revealed a significant decrease ($p \leq 0.01$) in the levels of the orexin hormone in the PCOS group of women compared with the control group.comparing the amount of orexin hormone in the polycystic ovary syndrome group of women who had children to the group of women with the condition who had no children show significantly decrease at ($p \leq 0.01$) .

In terms of blood lipids, the results revealed a significant increase in the level of triglycerides ,cholesterol,low-density lipids ,and very low-density lipids women with polycystic ovary syndrome when compared to the control group at($p \leq 0.01$).In contrast with the levels of high-density lipids for cholesterol were significantly lower ($p \leq 0.01$)in women with polycystic ovary syndrome compared to the control.The results in terms of age showed a significant increase ($p \leq 0.01$) in triglycerides ,cholesterol,low-density lipids ,and very low-density lipids women with polycystic ovary syndrome when compared to the control group at($p \leq 0.01$), while there was a significant decrease ($p \leq 0.01$) in the levels of high-density lipids for cholesterol in women with polycystic ovary syndrome compared with the control group , In terms of body mass index (BMI), the findings revealed a significant rise in triglyceride,cholesterol ,low-density lipids ,and very low-density lipids levels in women with polycystic ovary syndrome when compared to the control group at ($p \leq 0.01$) . While the levels of high-density lipids for cholesterol in women with polycystic ovary syndrome were significantly lower ($p \leq 0.01$)compared to the control group,. According to the findings regarding children, there was no discernible difference between the groups of women with polycystic ovary syndrome who had children and those who did not have children in terms of their levels of triglycerides, high-density lipoproteins for cholesterol (HDL-C), low-density lipoproteins for cholesterol (LDL-C), and very low-density lipoproteins for cholesterol (VLDL-C).

Key Word

Lateral hypothalamic area	LHA
Cholesterol	Cho
Ethylenediaminetetraacetic acid	EDTA

1- **Introduction :**

Polycystic ovary syndrome :

Polycystic ovary syndrome (PCOS) One of the prevalent disorders affecting the function of the endocrine glands in women who have polycystic ovary syndrome (PCOS) is PCOS. The excessive production of androgens and gonadotropins that characterizes this condition. Previous studies have shown that the percentage of women with this disease ranges between 6-20% [(2-1)] for women. In childbearing age and the first to reach a description of this disease are the two researchers (Stien and Leventhal), as this disease was named after that Leventhal and Stein syndrome in the year 1935 [(3)].

Orexin Hormone

It is a neuropeptide that is also known as hypocretin and is primarily produced by nerve cells in the lateral hypothalamic region (LHA) and the perifornical area (4).

Aim Of Study

1. A clinical study of the hormone Orexin in polycystic ovary patients and finding a relationship with other clinical variables and risk factors in patients and comparing it with the control group.
2. Measurement of body mass index (BMI) and its relationship with other biochemical variables that have been studied to know its effect on women with polycystic ovary syndrome.
3. Measuring some other vital variables, including lipids (cholestrol, HDL-C, LDL-C, trigyceride VLDL-C,) and comparing them with non-infected women to find a difference in the concentration of these variables between the two groups.
4. Determine the correlations between a number of variables that have been studied.

2-**Materials and methods of work**

Patient group :

Between 2021/12/1 and 2022/4/1, this research was carried out in the Kirkuk General Hospital and Public Health Laboratory's labs. Those who suffer from polycystic ovary syndrome, through the diagnosis of the specialized doctor for them based on the clinical symptoms that exist for each patient, in addition to an ultrasound examination (sonar). A questionnaire form has been approved for each patient in which special information is recorded for each of them.

Control group :

The thirty women who participated in the current research were chosen at random from the Kirkuk Governorate's population, were healthy weighted and uninfected, and ranged in age from 22 to 45 years.

Collection Of Blood Samples:

A needle (syringe) of size 5 was used to draw the blood from the vein. The blood was then put in a vacuum tube filled with GEL (Tube gel), a non-EDTA anticoagulant, and left in the tube for several minutes.The tube was centrifuged at a speed of 3000 revolutions per minute for 30 minutes while being maintained at room temperature to separate the blood serum. (20 minutes). After that, it was put in a simple plastic container and kept at -20 oC. Through a unique questionnaire for each patient, the patients' precise queries were recorded.

Determination of Orexin Concentration in blood serum by ELISA method:

Basic principle:

The orexin hormone was estimated in the blood serum using a measuring kit prepared by the Chinese company Bioassay Technology laboratory. The enzyme-linked immunosorbent assay serves as the foundation for this test. (ELISA). The antigen (Orexin) in the sample attaches to the pre-coated antibodies on the pits of the pre-coated antibody-coated plate. The method depends on On the use of a plate that contains pits containing the antibody of the substance to be measured, where

standard solutions and samples are added to the pits, as the antigen unites with the Biotin-Conjugated Antibody of the Biotinylated antibody present in the pit, and then Strepavidin linked to Horseradish Peroxidase is added. (HRP) to each hole for a period of time. After the washing procedure is finished, the Substrate solution is added to the etching, causing a change in color. Next, the Reaction Stop Solution is added, and it is measured at a wavelength of 450 nm. The concentration of the Reaction Stop Solution in the sample is determined by comparing the absorbance of the Sample with the Standard Solution by drawing the curve between them (5).

calculation:
The average of the repeated readings was taken for each standard solution, the control and the samples, and the average density of the equivalent solution was subtracted, and a standard curve of absorbance versus concentration was established as in Figures (1-2). The graph process was done using the O.D values for each concentration, where the absorbance, that is, the O.D values, is on the Y axis, and the concentration (the concentration of the standard solution) is on the x axis.

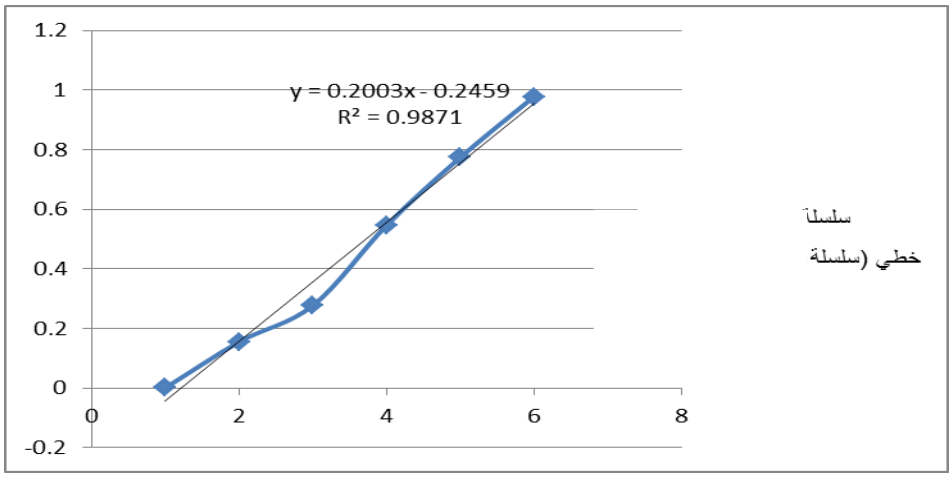

Figure (1-2): The standard curve of orexin

3-Results and discussion:
Results and discussion:
Women with polycystic ovary syndrome (PCOS) had their orexin hormone levels tested, and those levels were compared to those of healthy women. Table (3-1), Table (3-2), and Figure (3-1).

Table (1-3): Orexin level in the sera of polycystic ovarian patients compared to healthy subjects by age and body mass

Parameters / Groups	**Mean ± SD**		
	Control		**Patients**
O r e x i n ng/ml	**3.966±0.421**	**Total**	**2.93±1.75**
	4.045±0.265 a	**Age**	**3.339±2.695ab**
	3.852±0.550a		**2.592 ±0.243b**
	4.091±0.218a		**3.098 ±2.082ab**
	3.9939±0.4339a	**MBI**	**2.6085± 0.2258b**
	3.8280±0.3530a		**2.9760±0.6020a**
P value			

Parameters	(Total control/Total Patients)	Age	BMI
O r e x i n ng/ml	**0.0002**	**0.030**	**0.0003**

Table (2-3) Orexin hormone level in the sera of PCOS patients, according to the presence of children

Parameters / Groups	Mean ± SD	
	PatientsG1	**Patients**G0
O r e x i n ng/ml	**2.6393±0.2465b**	**3.8700±0.5190a**
P value		
Parameters	G1, G0	
O r e x i n ng/ml	**0.001**	

• G0: No children
• G1 There are children

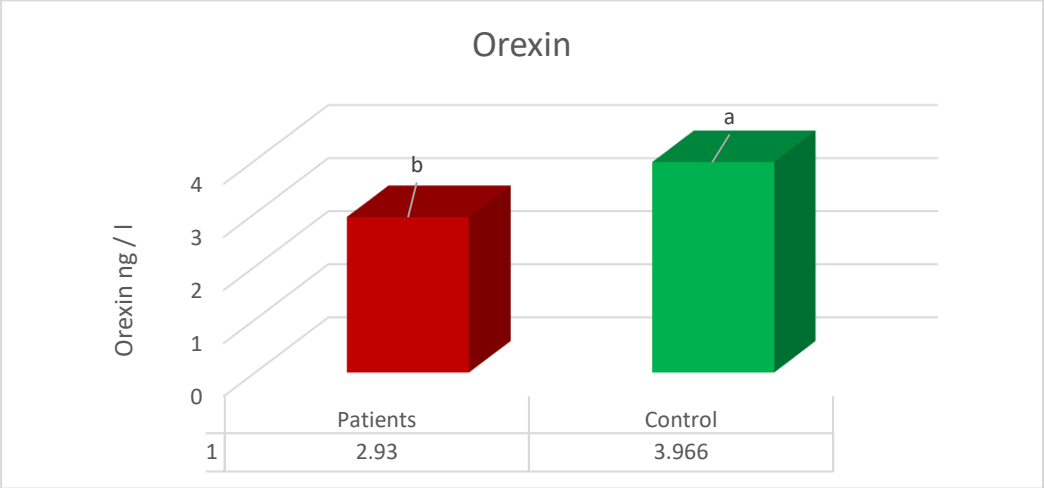

Figure (3-1) shows the level of orexin hormone in patients compared to healthy subjects

In comparison to the control group, the results revealed a statistically significant decline in the levels of the hormone orexin in PCOS patients ($p \leq 0.01$). ($p \leq 0.05$)in the amounts of the orexin hormone in polycystic ovary syndrome patients when compared to a control group.According to the body mass index (BMI) differences between the two groups, the findings in Table (3-1) revealed that there was a significant decrease (p0.01) in the group of women with PCOS when compared to the control group in terms of orexin hormone levels as well. in comparison to the group of women with polycystic ovary syndrome who did not have children, the amount of orexin hormone in the former group of patients.

These outcomes agree with Anna Valenzano 2020 and his team's conclusions. [6]and ErcanYilmaz 2013 and his group [7]who discovered a drop in the hormone orexin's concentration. [8] Anorexia nervosa.

The researcher (Matsumura) also indicated in 2013 that orexin is reduced in chronic obstruction pulmonary patients, and in patients with diabetes [9] and metabolic syndrome [10], as well as a decrease in its level in patients with early dementia, Alzheimer's disease. Perhaps the reduced

production of orexin receptors in women with polycystic ovary syndrome is the cause of the hormone orexin's drop in concentration[11], or because orexin acts as a pain reliever, as the orexin cells extend their axes to sites in the brain called the locus coeruleus, which are related to the perception of pain, as It contains adrenergic cells and participates in the physiological response to stress, pain and panic, and thus its level decreases as a result of the depletion of orexin receptors [12].
Age-related findings showed that the level of orexin A decreased significantly for patients compared to the control group, which is in line with findings by scholar Shoko Tsuchimine et al., 2019, and his team that the level of orexin A is unaffected by age[13], as well as BMI The findings of the study supported the assertions made by the author Chowdhury et al., 2019 and his team that women with polycystic ovary syndrome had significantly lower levels of the orexin hormone than did healthy individuals. [14] The level of orexin is inversely associated with body mass, which is caused by the decrease in the production of orexin receptors in women Obese people [15], or the reason may be due to the diet of obese people, which is represented by high-fat food, which leads to the programmed death of orexin cells in the hypothalamus.It also reduces spontaneous physical activity [16], as Orexin works to combat obesity by increasing spontaneous physical activity [17] and increases energy expenditure through thermal generation in the brown adipose tissue [18]Orexin is not only a neurotransmitter or regulator, but also Also, studies have indicated that OXA affects the secretion of hormones of the hypothalamus and pituitary gland [19] and has many functions, one of the most important of which is the regulation of appetite, sleep, wakefulness, energy balance, cognitive and perceptual processes [20], thermoregulation, regulation of cardiovascular functions, adipose tissue, motor activity, motivation and control Emotional and influencing a group of behaviors in addition to its important role in controlling the autonomic nervous system and controlling reproductionAnd that the diversity of functions of Orexin makes it a therapeutic target for the treatment of most pathological conditions such as mental disorders, infertility, cognitive dysfunction, insomnia, and polycystic ovary syndrome [21]. In accordance with the findings of the study by Roger Hart et al. (2004), the results also revealed a significant drop in the level of the hormone orexin in a group of patients with polycystic ovaries who had children compared to a group of patients with polycystic ovaries who had not. [18],Sometimes the cause may be related to high levels of the masculine hormone testosterone, obesity, hair growth, and irregular or infrequent menstruation. These findings point to a malfunction of the ovaries' hormone secretion, which has an impact on estrogen and progesterone levels[22].

Serum Lipids

According to the findings in Table 3-3, women with polycystic ovary syndrome had significantly higher levels of triglycerides ,cholesterol,low-density lipids ,and very low-density lipids at($p \leq 0.01$). However, they had significantly lower levels of high-density lipids for cholesterol ($p \leq 0.01$) than those in the control group Compared to the control group.

The results in terms of age,women with polycystic ovary syndrome had significantly higher levels of triglycerides ,cholesterol,low-density lipids ,and very low-density lipids at($p \leq 0.01$). However, they had significantly lower levels of high-density lipids for cholesterol ($p \leq 0.01$) than those in the control group Compared to the control group.
According to the body mass index BMI resultswomen with polycystic ovary syndrome had significantly higher levels of triglycerides ,cholesterol,low-density lipids ,and very low-density lipids at($p \leq 0.01$). However, they had significantly lower levels of high-density lipids for cholesterol ($p \leq 0.01$) than those in the control group. According to the findings regarding children, there was no discernible difference between the cholesterol, triglyceride, high-density lipoprotein (HDL-C), low-density lipoprotein (LDL-C), and very low-density lipoprotein (VLDL-C) levels in the polycystic ovary syndrome group of women who had children and the group of women with polycystic ovary syndrome who did not.

Table (3-3): Concentrations of Cho HDL, Tg, LDL, VLD in the sera of PCOS patients compared to healthy subjects by age and body mass .

Parameters / Groups	Mean ± SD		
	Control		Patients
Cho mg/dl	173.7± 11.1	Total	240.0± 33.9
	177.56 ± 7.16b	Age	234.29 ± 16.2a
	171.86 ± 14.4b		245.52± 12.9a
	172.29 ± 6.82b		236.40± 17.0a
	173.16± 11.90b	MBI	238.81± 17.82a
			244.31 ± 12.06a
	176.20 ±16.10b		
TgmEq/L	110.78± 6.25	Total	226.07± 6.07
	113.50 ± 5.97b	Age	226.99 ± 6.42 a
	110.54 ± 6.03b		225.84 ± 6.70a
	107.74 ± 6.38b		225.71 ± 5.05a
	110.44 ± 6.04b	MBI	224.91 ± 5.95a
	112.48 ± 7.76b		230.23 ± 4.65a
HDL mEq/L	47.79± 6.64	Total	24.85± 2.86
	49.060 ± 7.160a	Age	25.186 ± 2.454b
	45.910 ± 6.800a		24.433 ± 3.070b
	49.930 ± 5.320a		25.189 ± 2.899b
	48.060± 7.070a	MBI	24.600± 2.806b
	46.460 ± 4.110a		25.746± 2.991b
LDLmEq/L	43.43± 4.63	Total	156.90± 5.34
	44.16 ± 8.34b	Age	157.67 ± 5.74a
	45.01± 8.26b		156.77 ± 5.75a
	39.31 ± 9.63b		156.51 ± 4.63a
	43.000 ± 9.15b	MBI	156.466 ± 5. 293a
	45.540 ± 5.50b		158.450± 5.45a
VLDL mg/dl	22.18 ± 1.34	Total	45.36± 1.88
	22.522 ± 1.230b	Age	46.079± 3.192a
	22.264 ± 1.371b		45.156 ± 1.370a
	21.557 ± 1.401b		45.111 ± 0.992a
	22.068 ±1.223b	MBI	44.962 ± 1.206a
	22.720 ± 1.904b		46.785 ±2.996a
P value			
Parameters	(Total control/Total Patients)	Age	BMI

Cho	**0.0006**	**0.0007**	**0.0009**
Tg	**0.00002**	**0.000002**	**0.000002**
HDL	**0.0007**	**0.000005**	**0.00006**
LDL	**0.00001**	**0.000007**	**0.000004**
VLDL	**0.00002**	**0.00009**	**0.000008**

Table (3-4): Concentrations of Cho HDL, Tg, LDL, VLD in the sera of PCOS patients, according to the presence of children

Parameters / Groups	**Mean ± SD**	
	PatientsG1	**Patients G0**
Cho mg/dl	**237.52±18.12a**	**248.14±19.77a**
TgmEq/L	**225.36±6.21a**	**228.39±5.14a**
HDL mEq/L	**24.733±2.981a**	**25.229±2.486a**
LDLmEq/L	**156.652±5.46a**	**157.700±5.04a**
VLDL mg/dl	**45.167±2.067a**	**45.650±1.062a**
P value		

Parameters	**G1 , G0**
Cho	**0.0008**
Tg	**0.000004**
HDL	**0.00007**
LDL	**0.000006**
VLDL	**0.000005**

- G0: No children
- G1 There are children

The serum of women with polycystic ovary syndrome has higher levels of total cholesterol, triglycerides TG, VLDL-C, and LDL-C compared to the control group, while HDL levels are lower. These findings are in line with those of Rhea Jabbour 2020 and his team. [(23)]As shown in Table 3-3, women with polycystic ovary syndrome had significantly higher cholesterol levels in their sera than the control group, and this difference was significant at the level of likelihood ($p \leq 0.01$).The significant increase in the concentration of cholesterol in patients with polycystic ovary syndrome can cause obesity, which is associated with insulin resistance to cause many diseases, including infertility. In addition, the increase in the concentration of cholesterol can come through a decrease in the concentration of steroid hormones and the lack of use of the base substance (cholesterol) in the process of its construction [(24)].

Also, the high total cholesterol is due to the nutritional pattern, as nutrition is one of the most important factors that cause high concentrations of fat in the body. When eating foods containing a high percentage of saturated fats, this causes an increase in cholesterol levels. And that the increase in the level of cholesterol has a significant impact on the woman's body, as it may lead to heart disease and arteries, and the high cholesterol may be a result of an imbalance in the distribution of fats (dyslipidaemia), and this condition was observed by 70% among women with polycystic ovary syndrome [(25)]These results vary according to the nature of the body, the weight of the woman, and her family history, because heredity and the nature of the body affect the distribution of fats, and the reason for the imbalance in the distribution of fats in the body may be a result of resistance to the hormone insulin, as well as a rise in the hormone insulin (Hyperinsulinemia), especially for patients with diabetes of the second type, as this could be the cause of the disorder A change in the activity of the hepatic lipase enzyme may be the cause of the imbalanced distribution of fats in the body, which leads to a rise in cholesterol.[(26)].

Additionally, women with polycystic ovary syndrome had higher triglyceride concentrations than women in the control group; this difference was significant, as indicated by the likelihood value of P 0.01 in Table 3-3. The cause of this is due to eating a lot of foods high in fat, which increases the production of chylomicrone minutes in the intestine. When these minutes decompose, they release a lot of fatty acids, which the liver then absorbs. This causes the liver to release a lot of triglycerides, such as VLDL and T.G. [(27)].

As shown in Table (3-3) ,when compared to the percentage of the control group, the serum of women with polycystic ovary syndrome has higher levels of low-density lipoproteins (LDL).. With cholesterol coming from the food intake, where large quantities of cholesterol reach the liver, and that low-density lipoproteins are the ones that transfer cholesterol from the liver to the bloodstream, as well as the receptors on the surface of the cells when a defect occurs in them, they hinder the binding and this increases the concentration of LDL ,Low-density lipoproteins (LDL) may filter through the walls of blood vessels and then transform into oxidized low-density lipoproteins (Oxi-LDL) by deposition of LDL on the wall of the artery, and this exposes the body to atherosclerosis, or they collect among themselves, forming a thrombus (clot) [(25)].

Through our study, we noticed a decrease in the level of concentration of high-density lipoproteins (HDL), as shown in Table (3-3), and this decrease is a significant decrease, and the probability value was $P < 0.01$. This decrease can be explained by the fact that high-density lipoproteins are considered one of the most variable types of fats in women Women with polycystic ovary syndrome, and the reason for the decrease may be hyperandrogenism, which has an important role in this change, and hyperinsulinemia (insulin resistance) has a significant impact on the change in the level of HDL, as women with polycystic ovary syndrome suffer from a weakening of fibrin activity, which is responsible for clot formation bloodyIn this case, it is closely related to the condition of insulin resistance, and there is an explanation for the decrease in HDL, which is that the high levels of fatty acids and their lack of conversion into esters, this will lead to an increase in triglyceride levels and reduce HDL, as it is associated with the high percentage of fat intake, as the factors that lower the concentrations HDL are the same factors that reduce the degradation of very low-density lipoproteins (VLDL), and they deplete HDL molecules by blocking the transport of Apoprotein,Also, lipoproteins, especially HDL, have a possible role in causing female infertility [(24)]. In terms of age, the results were at a probability level of $P \leq 0.01$, but these differences are not significant because the age groups under study are of similar ages, and this is consistent with what the researcher Cindy Meun 2019 and his group [(20)] stated. Perhaps the reason for this increase is due to Slowing of metabolic processes, as there is no equivalence between demolition and construction processes with age [(27)]. The findings for BMI were also at the level of probability ($p \leq 0.01$), which is consistent with what researcher 2021 Andrea Enrquez Guerrero and his team claimed.[(28)]The

findings of Tables (3-4) regarding the presence of children revealed that there was no discernible difference between women with polycystic ovary syndrome who had children and women with polycystic ovary syndrome who did not, and this was ascribed to diet and exercise[29].

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