

THE ROLE OF MELATONIN, REPRODUCTIVE HORMONES AND THYROIDISM IN POLYCYSTIC OVARY SYNDROME PATIENTS

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ABSTRACT :

This study was conducted at the Infertility Center at Al-Batool Teaching Hospital for Women and Children in Dyala Governorate from 08/22/2020 to 12/1/2020. It was followed up of 100 cases of women suffering from polycystic ovaries, and it was compared to the control group, which included 50 standard points and then confirmed. They are free of chronic diseases such as thyroid disease and diabetes .

The study aims to assess the clinical and hormonal status by measuring LH, FSH, melatonin, and the aetiology and risk factors for infertile women with polycystic ovary syndrome. . The concentration of hormones was measured for both women with the syndrome and those without the syndrome, including LH, as it was observed that the level of LH concentration increased significantly in women with the syndrome, as it reached a concentration of (17.61 ± 4.320) compared to the non-affected (4.53 ± 1.86) . The level of FSH hormone reached its concentration in women Female patients with the syndrome, as its attention reached (3.20 ± 1.57) compared with the control group (7.53 ± 1.68) and The melatonin hormone concentration was measured (64.85 ± 24.43) compared with the control group.

Key word : Poly cystic Ovary Syndrome, LH,FSH,melatonin

I. INTRODUCTION:

1-Poly Cystic Ovary Syndrome :-

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders in women of reproductive age (1), it is a condition associated with chronic anovulation, insulin resistance, and androgen excess(2). Affected individuals typically present to clinical attention during evaluation for infrequent menses, infertility, and/or hirsutism(3). The prevalence of PCOS among women of reproductive age in the general population has been estimated at 4% to 12%(4), the prevalence and characteristics of women with PCOS among broader, ethnically diverse populations and within usual care settings are less well understood, it appear to be higher (from 37% to 90%) in women with menstrual abnormalities and also is increased in the presence of certain diseases(5)

In addition to reproductive and hyper androgenic concerns, PCOS is also associated with a number of metabolic perturbations that ultimately may contribute to an excess risk for cardiovascular events. Women with PCOS are more likely to be insulin resistant, overweight, and obese, and several studies have demonstrated that PCOS is associated with an increased risk of glucose intolerance and type 2 diabetes mellitus, independent of body mass index (BMI)(6). Finally, a growing body of evidence suggests an association between PCOS and hypertension and markers of subclinical atherosclerosis and vascular dysfunction (5)

Excess insulin appears to increase production of androgen. Levels of androgen that are higher than normal can lead to acne, excessive hair growth, weight gain, and problems with ovulation(5). The cutaneous manifestations of increased androgenic hormones are hirsutism, acne, seborrhea, alopecia, obesity, and acanthosis nigricans(2).

On the basis of the National Institutes of Health (NIH) meeting in 2003, any two of the three are sufficient to confirm the diagnosis of PCOS, specific morphology of polycystic ovaries in ultrasonography findings, hyperandrogenism (biochemical or clinical), and oligo- or amenorrhea. (7)

2- Biomarkers of Polycystic ovary syndrome:

Follicle stimulating hormone (FSH)

Follicle-stimulating hormone is a glycoprotein (polypeptide hormone) that is manufactured and excreted by the basophilic cells gonadotropins of the anterior lobe of the pituitary gland. FSH adjusts the human body's development, growth, pubertal maturation and reproductive processes. In reproduction process, FSH and LH hormones are performed synergistically (8). FSH has β subunit consist of amino acid, which provides its particular biologic action and is responsible for FSH receptors interaction (9). In female, FSH starts follicle growth which directly influences granulosa cells with the accompanying raise of inhibin-B, then decreases FSH rates in late follicular phase. This looks essential in choosing only the most mature follicle for advance in ovulation. When the luteal phase ends, there is a small increase in levels of FSH which appears to be essential in initiating the subsequent ovulatory cycle. In the pituitary gland, the excretion of FSH is managed by the pulses of GnRH) and the pulses, in turn, are subjected to the feedback of estrogen from the gonads (10).

Luteinizing Hormone (LH)

Luteinizing hormone is renowned as lutropin, created and excreted in the anteriorly pituitary gland by gonadotropin (11). In women, an extreme increase in LH causes ovulation, and growth of corpus luteum (12). LH is a protein dimer that contains two glyco-peptides subunits which are termed as (α and β) subunits, and are non-covalently associated. α subunit of LH is identical, and contains (13) amino acids in human, while β subunits vary, and contain (14) amino acids which confer its specific biological activity which is responsible for the specificity of LH receptor interactions. The biologic half-life of the LH is twenty minutes, shorter than FSH that is (3-4 hours). At menstrual period, FSH initially begins follicular development (15). figure (1-8). As estrogens increase, LH receptors are activated on maturing follicles which contain an increasing level of estradiol. Finally at follicle maturation, the increase in estrogen contributes to the (positive feedback) impact through the hypothalamic interface, a releasing of LH over a period of (24-48) hour. Increasing LH leads to ovulation through not releasing the egg only, but also to start the change of the remaining follicle into a corpus luteum, which in effect generates progesterone to prepare the endometrium for probable implantation. Luteinizing hormone is required to maintain function of luteal during the first 2 weeks, and promotes ovarian theca cells that provide androgens and hormonal precursors for estradiol production (16).

Melatonin Hormone

Melatonin (N-acetyl-5-methoxytryptamine) is an indolamine hormone that was firstly recognized in the 1950s (17). Melatonin levels are regulated by photoperiod as its production and secretion are promoted at night in response to darkness since light can suppress its secretion (18). Melatonin is also produced in other organs such as the gastrointestinal tract, skin, retina, bone marrow, and lymphocytes (19). It seems that mitochondria are the site of melatonin synthesis within cells. Moreover, the female reproductive organ, including the follicular cells, oocytes, and cytotrophoblasts are also among the melatonin production sites (20). Several studies have shown the involvement of melatonin in the pathogenesis of diabetes cancer Alzheimer's disease immune and cardiac diseases (21). Melatonin has been identified to have different pharmacological properties such as antioxidant immunomodulatory anti-angiogenic and oncostatic effects (22) Melatonin acts as an inhibitory factor on the hypothalamic pituitary gonadal axis (1745) Melatonin receptors are transmembrane G-protein-coupled receptors including melatonin receptor 1 (MT1; *MTNR1A*) and melatonin receptor 2 (MT2; *MTNR1B*) (23).

The effects of melatonin on female reproductive physiology are mediated via its receptors in hypothalamic, pituitary, and ovarian sites, Melatonin is also a potent free radical scavenger that exerts protective effects in female reproductive organs; for instance, it is involved in the protection of the oocyte against oxidative stress, particularly at the time of ovulation. It can also be used to protect the developing fetus from oxidative stress can be happened by melatonin (24). The levels of melatonin in follicular fluid are higher than its levels in the blood. The concentration of melatonin in follicular fluid significantly rises as the follicles become mature (25).

II. MATERIAL AND METHODS

1- Chemicals:-

All chemicals and reagents that were used in this study are illustrated in Table (2-1).

Table (1): List of chemicals and reagents used.

No	Chemicals	Company
1	LH (luteinizing hormone) Kit	Roche-USA
2	FSH (follicle stimulating hormone) Kit	Roche-USA
4	Melatonin ELISAKit	Bioassay

2- Instruments:-

All our instruments that used showed in Table (2-2).

Table (2): List of Laboratory Equipment .

No	Instruments	Company
1	Cooling centrifuge	Eppendorf-Germany
2	Centrifuge	KOKUSAN-Japan
3	Deep Freezer	Karl Kolb- Germany
4	ELISA Microplate Reader &Washer	Bio Tek-USA
5	ELISA system	Bio test-Germany
6	Water bath	Memmert-Germany
7	Cobas 411 system	Roche-USA

3-Samples Collection and Preparation:

-Patients and Control Groups:

One hundred of Polycystic ovary syndrome (PCOS) patients, throughout the study for the period from December 2019 to March 2020 in Baquba teaching Hospital, The medical history was taken, body weight , height were measured as well as body mass index (BMI). The age of these patients ranged from (18 to 40) years. All patients were subjected to a personal interview using a specially designed questionnaire format full history with detailed information (Appendix I). The diagnoses of the disease were confirmed by biochemical examination and ultrasound. Which was carried in the laboratories of the hospital mentioned above for comparison, fifty healthy were included in the study as control with matched body weight, height and their body mass index (BMI).

Blood Samples Collecting:

Ten milliliter of blood were collected in to test tubes without anticoagulants. After coagulation, the samples were centrifuged for ten min and the serum stored on an ice bath and was used in the same day for the enzymatic activity assays, Liver function test and mineral determination. The remainder of the sera were stored at -20°C, to be used for other parameters estimation. The sera samples should be unhemolyzed and non-jaundice to avoid any interference in the obtained results.

- Determination of Some Biochemical Parameters in Serum:

- Hormones:

- Luteinizing hormone(LH) and follicle stimulating hormone (FSH):-

- Sandwich principle. Total duration of assay: 18 minutes

-Melatonin Hormone

-This kit is an Enzyme-Linked Immunosorbent Assay (ELISA).

III. RESULTS:

Description of the Study Groups:

The total number of (150) individual samples were included in the present study, the control group consists of (50) healthy individual samples, while the PCOS patients were (100) individual samples.

1-Hormonal profile

Table (3) shows comparison of the mean value of selected hormonal profile between PCOS patients and controls group containing (FSH, LH ,Melatonin).

Table (3) Comparison between PCOS patients and controls groups according to the selected Hormonal profile

Groups		N	Mean \pm SD	P value
LH	Patients	100	17.61 \pm 4.32	0.002**
	Controls	50	4.53 \pm 1.86	
FSH	Patients	100	3.20 \pm 1.57	0.001***
	Controls	50	7.53 \pm 1.68	
Melatonin	Patients	100	64.85 \pm 24.43	0.001**
	Controls	50	19.28 \pm 9.20	

Luteinizing Hormone (LH)

Result of current study shows the mean value was high for LH hormones patients (17.61 \pm 4.32) than control (4.53 \pm 1.86) with high significant different (P<0.05) between study groups as shown in figure (3).

The finding result is in line with Hashemi *et.al* in 2016(26), Moustafaet *al* in 2019 (27) and Abdulwahid *et al* in 2019 (28) since they found that mean values of serum-LH for patients were significantly higher than those of controls.

In PCOS, a unique trait is the unsuitable GnRH excretion with high levels of LH and relatively low levels of FSH(29). By their aromatization to the estrogens, the androgens are able to alter (gonadotropin release), and there are some theories which tried to clarify the causes of elevate LH in PCOS, as primary irregularity of hypothalamic organization of LH as an elevate LH pulse frequency (30).

Follicle Stimulating Hormone (FSH)

Result of current study shows the mean value was high for FSH hormones patients (3.20 \pm 1.57) than control (7.43 \pm 1.68) with high significant different (P<0.05) between study groups as shown in figure (3).

The finding result is in line with AL-Deresawi *et.al* in 2015 (245), Shoaib *et al* in 2015 (31) and Oyebanji *et al* in 2018 (32) since they have observed that the mean values of patients serum-FSH was significantly lower than those of controls.

In usual menstruation, the elevation of plasma-FSH through the (luteal-follicular change) is crucial for develop follicle and consequently ovulation. In hyperandrogenemic female who destined to improve PCOS, different from natural early pupery, decrease levels of FSH is probably due to the fact that the nocturnal elevate in the ovarian steroid hormones may be not sufficient to repress the GnRH pulse generator, that leads to a continuous increases rapid pulse frequency of LH, and decrease FSH manufacture which causes insufficient follicular growth(33),

Melatonin

Result of current study shows the mean value was high for FSH hormones patients (64.85 \pm 19.28) than control (24.43 \pm 9.20) with high significant different (P<0.05) between study groups as shown in figure (3).

The finding result is in line with (34) since they concluded that mean values of patients serum- Melatonin were significantly higher than those of controls. Melatonin levels in serum and follicular fluid of PCOS patients are different from healthy women. In PCOS patients, melatonin level in serum is usually higher than in healthy women, which is considered as a sign of diagnosing PCOS (35). But a reverse condition occurs in melatonin level of follicular fluid. Due to fewer uptakes of melatonin in ovarian follicle in PCOS patients against healthy women, follicular fluid contains lesser melatonin level compared to the healthy condition (36). Melatonin seems to promote follicular maturation and ovulation through the protection of follicles against oxidative stress and their rescue form atresia (37). Furthermore, melatonin showed protective effects on corpus luteum against ROS via its antioxidant effects (38).

2-Phenotypic Patterns & Pathogenic of PCOS:

The results of mean anthropometric are indices in sera of all studied groups. Result of current study shows the percentage of patients have BMI>25 show (83.0%) was more than BMI<25 or 25 with high significant different (P<0.001), while the percentage for patients have Acne 88.0% was more than no have Acne (12.0%) with high significant different (P<0.001)

Table (4) Comparative current study parameters between study groups.

		Count	Percent	P value
BMI	<25	7	7.0%	0.001***
	25	10	10.0%	
	>25	83	83.0%	
Acne	Yes	88	88.0%	0.001***
	No	12	12.0%	
hair show	Yes	94	94.0%	0.001***
	No	6	6.0%	
DM	Yes	56	56.00%	0.23
	No	44	44.00%	
infertility	Firstly	76	76.00%	0.001***
	Secondly	24	24.00%	
Genetics	Yes	78	78.0%	0.001***
	No	22	22.0%	
menstruation	Yes	96	96.0%	0.001***
	No	4	4.0%	

One of the clinical features of PCOS is obesity with menopause or irregular menstruation and abnormal hair growth in some parts of the body (hirsutism) and infertility. However, not all females with polycystic ovary disease are obese and not all obese females have polycystic ovary syndrome (39).

the percentage for patients have hair show was 94.0% more than no have hair show (6.0%) with high significant different ($P < 0.001$) Hirsutism can be defined as excessive (unwanted) hair outgrowth in some areas usually associated with male sexual characteristics, i.e. in the chest area, chin area of the face, lower abdomen, buttocks and up thighs. Hirsutism appears due to the effects of androgen on the grease unit and is usually associated with oily skin and acne. This is often due to the increased production of androgen from the ovaries, Androgen overproduction in females with PCOS causes a series of skin changes such as acne, hirsutism and androgenic alopecia, One of the factors that contribute to appear some symptoms of PCOS such as hirsutism, acne, and infertility, the high concentrations of testosterone(40)The results noticed by this study were in agreement with Azziz et. al (41) and Ovalle and Azziz (42).

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