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Serum levels of Anti-Mullerian Hormone, Leptin, T3, T4 and TSH in Women with Polycystic Ovary Syndrome in Iraq

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ABSTRACT

Background: Endocrine abnormalities associated with polycystic ovarian Syndrome (PCOS) are essential problems. PCOS is a frequent trouble inflicting menstrual irregularity and infertility among women regarding effective age. Increased level of Anti-Müllerian Hormone (AMH) is presently considered an essential marker for PCOS. Aims of current study were to evaluate serum levels of AMH and to correlate them with age, BMI and serum levels of TSH, T3, T4 and leptin in females with PCOS as compared to normal controls. . **Methods**: Current study involved 120 females with PCOS belonging to age group (18-35) years from Misan City, Iraq. These patients were divided into three equal groups as follows: G1(controls), G2 (females having PCOS and hyperthyroidism) and G3 (females having PCOS and hypothyroidism).

Results: Data from current study showed highly significant ($P< 0.001$) increase in age, BMI in G2 and G3 when compared with G1. Also, results showed non-significant increase in AMH in group G3 and G2 when compared with G1. The results also revealed significant increase in TSH, T3, and T4 in G2 and G3 compared with G1. In addition, there was highly significant increase in leptin hormone in G2 and G3 when compared G1. The results showed AMH was not related with age in G1 and patients groups. Also, there were non-significant correlations between BMI, TSH, T3 and T4 in G1 and patients groups. Also AMH showed negative correlation with leptin in G2, while non-significant positive correlation in control and G3. **Conclusion**: PCOS women of fertile age have higher AMH,TSH, T3, T4 and leptin levels in patients groups than that of control. It can be considered as an important marker for the diagnosis of PCOS.

Keywords: *AMH, PCOS, Thyroid hormones, Leptin, BMI, Pearson correlation.*

INTRODUCTION

Polycystic ovary syndrome (PCOS), the most important endocrinopathy of females within the reproductive age, has a prevalence of 22% in accordance with 26% [1]. It is characterized by irregular unovulatory menstrual cycles, features ofr hyperandrogenism, and then PCOS [2]. Multiple endocrine derangements were described among patients with PCOS including abnormally excessive levels of FSH, LH, FSH/LH, prolactin, testosterone, estradiol and insulin resistance [3]. The patients with PCOS have risks of infertility, miscarriage and complicated pregnancy. A previous study revealed that the occurrence of oligo-ovulation or unovulation in patients with PCOS ranged from 65% to 80%. The most common complaint reported by patients with PCOS was infertility due to chronic unovulation [4].

Hyperandrogenism is the key factor of the pathophysiology responsible for the interruption of physiologic feedback mechanisms that is fundamental for the establishment of ovulatory cycles, which leads to chronic unovulation. An interesting recent review and meta-analysis confirmed that females of all ages with PCOS have an elevated risk of endometrial cancer but the risk of ovarian and breast cancer were not significantly elevated [5]. However, primary ovarian dysfunction, disorders of production, action of growth elements and Anti-Mullerian hormone (AMH) are other mechanisms related to possible changes in follicular recruitment and development [6]. Anti-Mullerion hormone AMH is a 140 KDa diametric glycoprotein hormone of transforming growth factor- β family. Its level in serum shows little fluctuations at some point of the menstrual cycle. Serum AMH level is represents a reliable marker on ovarian follicular pool. . Since AMH level reflects the number

of growing follicles, its measurement may be used as a marker regarding ovarian follicular pool impairment in PCOS. In unovulatory females with PCOS, the follicular development is halted at 6-9 mm diameter. These elevated follicles contribute to the elevated AMH, as AMH production per granulose cell is also elevated [7].

PCOS shares common characterized with hypothyroidism like abnormal menses, unovulatory cycles, obesity, dyslipidemia and psychological disturbances. Recent research has shown an association between PCOS and thyroid dysfunction including changes in thyroid gland size [8]. PCOS with thyroid disease creates independent risks of ovarian malfunction as well as pregnancy-associated problems. Abnormal thyroid hormones concentrations could trigger alterations in ovulation and then menstruation. Initial levels of thyroid malfunction can result in delicate changes in ovulation as well as endometrial receptivity, which consequently may have drastic influence on fertility [9]. Thus, to reach the real diagnosis and to assess the AMH and thyroid function, free fraction of this hormone is essential.

Therefore this study was planned to assess AMH, TSH, T_3 and T_4 in patients with PCOS.

On the other hand, leptin is a cytokine that is produced by the white adipose tissue. It is a key hormone that participates in retaining energy homeostasis and weight through the determination of food intake and an elevated energy output [10]. Leptin decreases appetite, increases energy expenditure, and reduces the manufacturing of neuropeptide Y from the hypothalamus. Leptin may also have a major role in reproductive function, acting at many levels of the hypothalamic-pituitary-ovarian axis [11]. Circulating leptin levels have been positively related with body fat independent from PCOS according to some studies [12]. However, some studies have not shown significant differences in serum leptin concentrations in female with PCOS when compared with age- and BMI-matched controls [13].

AIMS OF STUDY

The aims of this study were to evaluate serum levels of AMH, in relation to age and BMI, leptin concentrations as well as thyroid hormones levels. In addition, the study will investigate the correlation between AMH levels and levels of the measured hormones.

MATERIALS AND METHOD

Current study recruited 120 females with PCOS belonging to age groups of (18-35) years from the city of Misan, Iraq. The detailed information regarding family background, personal history, dietary habits and physical activity of each subject was recorded. These patients were divided into three groups as follows: G1 (controls) consisted of 40 female and G2 consisted of 40 PCOS patients with hyperthyroidism and G3 consisted of 40 PCOS patients with hypothyroidism.

Venous blood sample (5 ml) was taken from each participants, allowed to clot, centrifuged and stored at -20C° till the assay for AMH, using immunoassay analyzer (RD-Ratio Diagnosis GmbH/Germany) and following the manufacturer's instructions. On the other hand, TSH, T3, and T4 were measured using enzyme linked fluorescent assay EIFA (BioMericux SAF/France) kits.

Statistical analysis

Statistical analysis of data was executed by SPSS (version 20). Student *t*-test was employed for calculating comparisons between groups. Results were considered statistically significant and highly significant at $P<0.05$ and $P<0.001$, respectively. Pearson correlation analysis was used to determine liner correlations between the variables and relationships between all measured parameters. Results were presented as Mean±Standard Deviation (SD).

RESULTS

Results of current study showed highly significant increase in age and BMI of participants in G2 and G3 when compared with G1 (controls). However, AMH levels were not significantly different among the three groups of study (Table 1, Figures 1 &2).

The results also revealed highly significant differences in serum levels of TSH, T3, T4 and leptin in G2 and G3 as compared with G1 (Table 1, Figures 1&2). In addition, there was highly significant increase in leptin hormone levels in G2 and G3 when compared with G1.

The relationships between variables measured in this study were studied using Pearson's correlation analysis. The analysis revealed no significant correlation between AMH levels and age, on the one hand, and between

AMH and BMI, on the other hand, within the three groups of study (Table 2, Figures 4).

In addition, data from current study revealed non-significant negative correlations between serum levels of AMH and those of TSH, T3, and T4 within the three studied groups (Table 2, Figures 3, 5 &7).

On the other hand, current study showed non-significant negative correlation of AMH serum levels with those of leptin within G2 as compared with G1 (Table 2) However, there was non-significant positive correlation of AMH serum levels with those of leptin within G3 as compared with G1 (Table 2).

Table 1 Age, BMI and serum levels of AMH, TSH, T3, T4, and Leptin for participants of current study

Parameter	**Mean±SD**		*P*-value	**Mean±SD**	*P*-value
	G1	**G2**		**G3**	
Age/ year	23.824 ± 4.270	33.021 ± 5.346	HS	36.187 ± 4.937	HS
BMI (kg/m^2)	22.807 ± 3.546	35.494 ± 10.604	S	31.476 ± 7.450	HS
AMH (ng/ml)	7.029 ± 3.391	13.208 ± 3.580	NS	8.238 ± 3.291	NS
TSH (µUI/L)	1.872 ± 1.505	0.388 ± 0.314	HS	28.157 ± 3.128	HS
T3 (nmol/L)	2.159 ± 0.869	**5.164 ± 2.194**	**HS**	**0.727 ± 0.517**	**HS**
T4 (nmol/L)	**114.118 ± 19.225**	**153.92 ± 22.926**	**HS**	**54.255 ±9.195**	**HS**
Leptin (ng/mL)	10.382 ± 3.070	14.498 ± 2.734	HS	11.309 ± 2.703	HS

S : Significant (P< 0.05). HS: Highly Significant (P< 0.001). NS: Non-Significant (P>0.05).

Table 2 Pearson correlation between AMH and the study parameters within each of the three studied groups

Parameter	**G1**	***P- value***	**G2**	***P-* value**	**G3**	***P- value***
Age	-0.299	P>0.27	-0.136	P>0.515	-0.488	P>0.185
BMI	0.0435	P>0.181	0.175	P>0.487	-0.469	P>0.188
TSH	-0.304	P>0.347	-0.255	P>0.24	-0.027	P>0.1
T3	-0.075	P>0.794	-0.281	P>0.169	-0.502	P>0.121
T4	-0.271	P>0.45	0. 98	P>0.592	-0.483	P>0.165
Leptin	0.295	P>0.4	-0.118	P>0.114	0.173	P>0.94

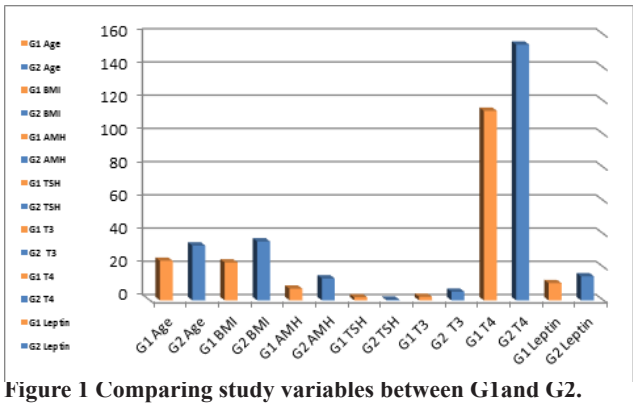

Figure 1 Comparing study variables between G1and G2.

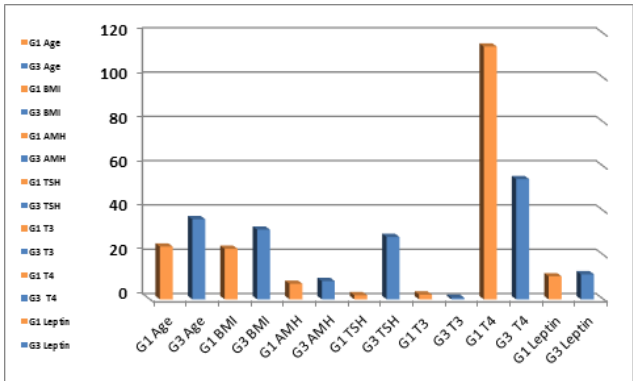

Figure 2 Comparing study variables between G1and G3.

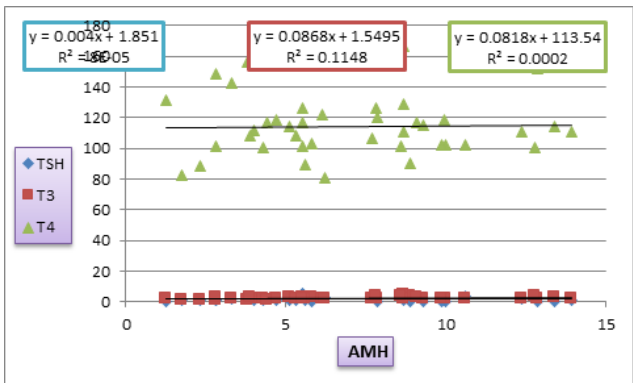

Figure 3 Correlation between serum levels of AMH and TSH, T3 and T4 within G1.

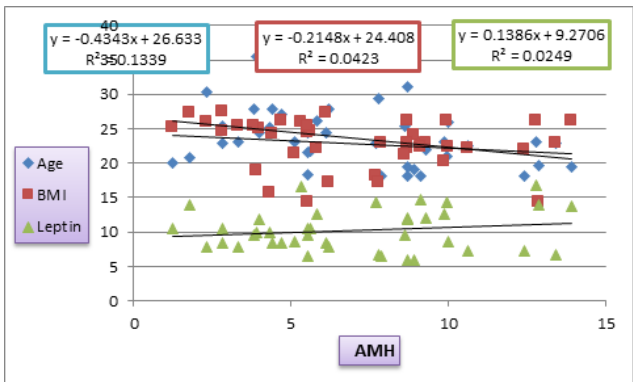

Figure 4 Correlation between serum levels of AMH and Age, BMI and Leptin level within G1.

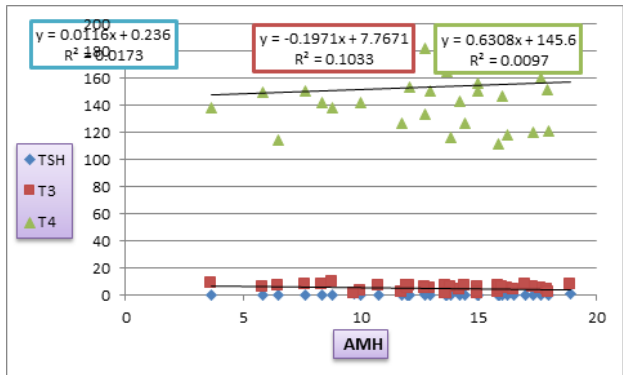

Figure 5 Correlation between serum levels of AMH and TSH, T3, T4 level within G2.

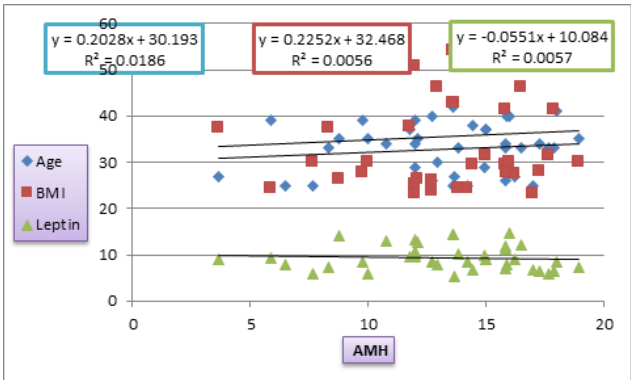

Figure 6 Correlation between Age and BMI and serum levels of Leptin level within G2.

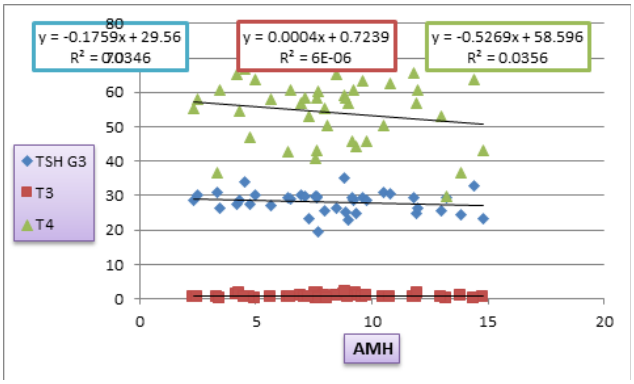

Figure 7 Correlation between serum levels of AMH and TSH, T3 and T4 level within G3.

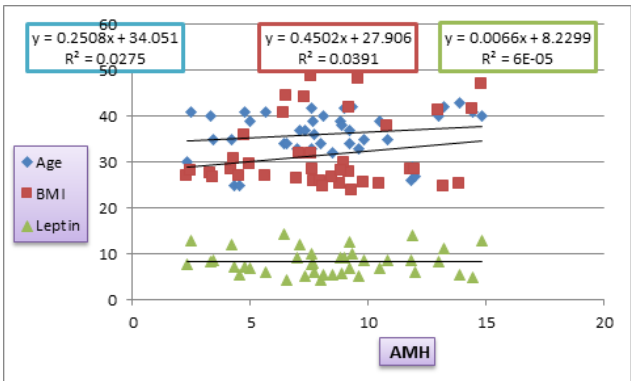

Figure 8 Correlation between Age and BMI and serum levels of Leptin level within G3.

DISCUSSION

This investigation was aimed to provide additional data on PCOS and contribute to improve understanding of the process of how serum AMH changes with age, ratio of BMI, hormones of thyroid gland and leptin in PCOS. Such an effort would seem interesting as AMH has been suggested as a beneficial marker in the diagnosis of PCOS [14]. A large prospective study of youthful population showed that although AMH serum levels were increased in youthful with PCOS, the hormone was not verified to be a reliable predictor of PCOS [Reference]. In a recent study it was established that AMH production per granulosa cell was elevated by up to 75% in females with PCOS compared to controls [Reference]. Several studies had demonstrated elevated levels of AMH in adult women with PCOS [15]. The results of present study showed that serum levels of AMH were significantly higher in PCOS patients with autoimmune hyperthyroidism than in control group while the results showed that AMH was non-significantly higher in PCOS patients with hypothyroidism when compared with control group. This study was in agreement with study of Cristina et al. [16], who found that hypothyroidism/ thyroid autoimmune disease was increased in women with PCOS. Also, it was in agreement with Kowalczyk et al. [17].

The adipocytokines (leptin and resistin) serve as causative or protective elements in the improvement of thyroid function disorders. Abnormal concentrations of adipocytokines (leptin and resistin) in hypothyroidism and hyperthyroidism have been reported with controversial results [18]. Thyroid dysfunction is also related with female infertility. Therefore, it has been proposed that women with hypothyroidism and ovulatory dysfunction infertility or have a desire to become pregnant, must be treated [19].

In present study, serum AMH levels were considerably correlated with leptin in both G2 and G3 groups as compared with G1. A correlation between thyroid hormone and leptin has been demonstrated in several studies. TSH stimulates leptin secretion by a direct influence on adipocytes, probably via TSH receptors on the surface of adipocytes [20]. PCOS is characterized by chronic unovulation and hyperandrogenemia. These features become evident with advancement of age and gradual increase of adipose tissue [21] which are often linked to leptin and its receptor [22]. Leptin seems to be directly related with obesity by preserving homeostasis of energy with reduced food intake and elevated energy spending [23]. In present study, relationship between leptin and BMI in females with PCOS was investigated. It was increased with BMI, but weak correlation with endocrine parameter [24]. Our results indicated that serum leptin was significantly highin PCOS with hyperthyroidism when compared with control group, however, it was significantly low in PCOS patients with hypothyroidism when compared with G1. This result was in agreement with Nasrin et al. [25], who showed elevated levels of serum leptin in patients with PCOS.

Moreover, a recent study found that serum leptin levels, among premenopausal females with hyperthyroidism, hypothyroidism or normal thyroid function, were similar before and after treatment of their abnormal thyroid status [21].

Furthermore, negative correlation was observed between serum AMH concentrations and leptin levels in hyperthyroidism group while positive correlation between serum AMH and leptin levels in hypothyroidism group.

In conclusion, PCOS women of fertile age have higher AMH, TSH, T3, T4 and leptin levels, regardless of their thyroid gland function, as compared with healthy control subjects. AMH can be considered as an important marker for the diagnosis of PCOS.

Ethical Clearance: Obtained from the Research Ethics Committee at College of Dentistry/ University of Misan, Iraq.

Source of Funding: Self-funded.

Conflict of Interest: Nil.

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