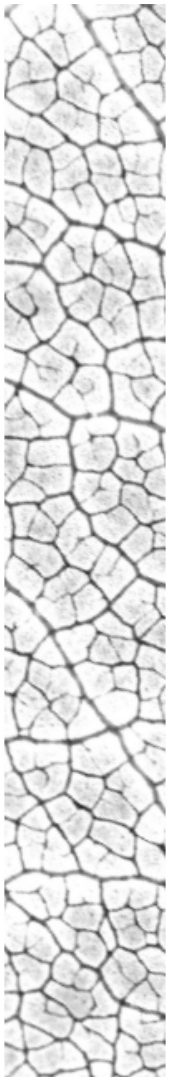

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Serum levels of Paraoxonase 1 activity and total antioxidant capacity relationship with anti-mullerian hormone in polycystic ovary syndrome

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Abstract
Polycystic ovary disease (PCOS) is a multifactorial disease and its prevalence rate increased significantly in the last few years. It is considered a cause of infertility and delayed reproduction, in addition to the many complications associated with the disease, which affect the lives of the patients in many aspects. During the study of the most important factors related to the disease it is clear that the rise in AMH is indicative of the degree of severity of the disease, where it reflects the number of small follicles in the ovary. Hyperinsulinemia and insulin resistance with hyper- androgenemia conditions associated with PCOS enters the patients in vicious circle of factors where there is an increase in oxidative stress and decrease in antioxidants, including PON-1 and TAOC as the results showed, this in turn more increases the resistance of insulin and high level of testosterone followed by various complications on the heart and fertility and obesity and others. Serum AMH and PON-1was measured using enzyme linked immunosorbent kit [Elisa], while FSH, LH, testosterone, prolactin and estradiol were determined by VIDAS kit method (enzyme linked fluorescent assay) and TAOC measured by colorimetric method.

Keywords: Anti-mullerian hormone (AMH), PON-1, TAOC, PCOS

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INTRODUCTION

Polycystic ovarian syndrome is multifactorial disorder affect women in reproductive age, its considered as systemic disease not only local disease exclusive in the ovary, this appellation come from its dire consequences on many systems of the females' body, since this syndrome associated with hyperinsulinemia and insulin resistance this lead to type 2 diabetes and central and/or general obesity and besides dyslipidemia in addition to fore mentioned factors all cause cardiovascular disease. Its frequently accompanies with oxidative stress, hyperandrogenemia and chronic inflammation (De Leo et al. 2016, Lim et al. 2012).

The mechanism hidden behind increased oxidative stress in PCOS not fully clear until now, there are many studied linked between increased oxidative stress in PCOS and insulin resistance that the most important feature in this disease. Oxidative stress is the condition of imbalance between oxidant and antioxidants. Antioxidants are chemical compounds that throw away, scavenge, and stifle the formation of free radicals, or suppress their actions (Elmasry et al. 2015, Zuo et al. 2016).

The HDL-associated paraoxonase 1 (PON1) enzyme is a glycoprotein composed of 354 a.a (Komoda 2013). Paraoxonase 1 in the serum secreted by the liver consider as antioxidant enzyme and is responsible for hydrolases organophosphate pesticides and neurotoxic compound (Fuhrman 2012, Mehdi and Rizvi 2012). It's also involve in increase macrophage associated-cholesterol efflux and degrades homocysteine thiolactone thereby preventing protein modification, and stabilizes free radicals, thus maintaining membrane integrity (Chistiakov et al. 2017). Furthermore, PON1 has been reported to stimulate insulin secretion and biosynthesis and boosted uptake of glucose by increased Glucose transporter type 4 (GLUT-4) expression, decrease in pon-1 activity may associated with increased insulin resistance as it happens in diabetes mellitus and AHD (Koren-Gluzer et al. 2011, 2013, Mehdi and Rizvi 2012). Studies in genetic aspect indicate that some genetic variation in pon-1 gene contribute to reduced expression of it (Dadachanji et al. 2018).

Total antioxidant capacity (TAOC) is defined as the ability of serum to quench free radical production and

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Table 1. Demographic features with hormonal levels in patients and control

Characteristics	Samples		P value
	Patient (80) Mean± SD	Control (60) Mean ± SD	
Age	24.24 ± 5.78	25.68 ± 6.30	0.161 NS
BMI(k/m²)	30.83 ± 5.50	25.80 ± 4.22	0.0001**
Waist :hip:	0.85 ± 0.06	0.79 ± 0.040	0.0001**
FSH (mIU/ ml)	4.25 ±1.58	5.90 ±1.50	0.0001**
LH (mIU/ ml)	7.09 ± 4.37	3.37±1.00	0.0001**
LH/FSH	1.84 ± 1.56	0.58 ± 0.165	0.0001**
Prolactin (ng/ml)	22.63 ±11.80	9.02 ± 4.10	0.0001**
Testosterone(ng/ml)	1.30 ± 0.65	0.42 ±0.12	0.0001**
E2 (Pg/ml)	52.49 ± 23.33	49.65 ± 24.80	0.489 NS
AMH(ng/ml)	14.65 ±4.14	5.02 ± 2.02	0.0001**

** (P<0.01), NS: Non-Significant.

thus protecting the cell structure from molecular damage. Studies on this marker and its relation with PCOS is conflicting, can be measured its value in various method but the most common one its spectrophotometric assay (Hyderali and Mala 2015, Jeelani et al. 2017). Anti-Mullerian hormone (AMH), a dimeric glycoprotein and member of the transforming growth factor-b family, is produced by granulosa cells in preantral and early antral follicles within the ovaries and is correlated with ovarian reserve. In PCOS, AMH reduce sensitivity of small follicles to FSH and that lead to anovulation (Piltonen et al. 2005, Rzeszowska et al. 2016). This study was designed to evaluate the serum paraoxonase (PON1) activity and total antioxidant capacity in Iraqi women with PCOS and its correlation with Anti-mullerian hormone level and other hormone and PCOS pathogenesis.

MATERIALS AND METHODS

This study comprised 80 woman diagnosed with PCOS according to Rotterdam criteria by specialist gynecologists, and 60 healthy woman have normal regulatory menstrual cycle as control, where women who were diagnosed with PCOS had at least two of these criteria: 1- oligo ovulation and /or anovulation 2- hyperandrogenemia clinically or biochemically, and 3- polycystic ovaries on ultrasound, their age ranged from 16 to 40 year. The blood samples were collected from Al-Shatrah hospital and Alhussien teaching hospital in Thi-Qar governorate/Iraq during the period from April-September/ 2018. We excluded the patients with endocrine disease and those with hormonal therapy that effect on the hormones level and also excluded pregnant and smoking woman.

Blood samples (5 ml) was collected during the follicular phase (2-3) day of menstrual cycle) for both groups.

Body Mass Index (BMI) was determined by the following equation; BMI = weight kg/ height m2 for both patient and control. Measure waist circumference in centimeters, measured at the smallest circumference of the waist, above the belly button and the hip circumference measured at the widest part of the buttocks, then calculated the WHR by dividing waist/hip.

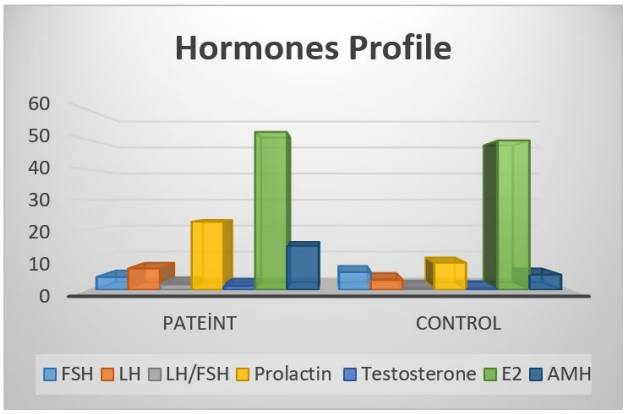

Fig. 1. Hormones profile for the studied groups

PON-1 enzyme level and AMH were measured using Enzyme linked immunosorbent assay (ELISA) method, [Elabscience Biotechnology Inc and Anshlab, (both of USA origin) respectively]. While serum total antioxidant capacity was estimated by Colorimetric method (Elabscience Co.) and serum level of FSH, LH, Testosterone, prolactin and E2 were determined by VIDAS which is full automated technique using principle of enzyme linked Fluorescent Assay.

RESULT

The data in **Table 1** showed that there was no significant difference in the mean of age in women with PCOS and control, while there was a significant difference in the BMI between those groups. Additionally, table revealed that there was a significant difference in the mean of hormones (FSH, LH, FSH/LH, PRL, testosterone, AMH) between patients and control, while the value of E2 did not reach to the statistically significant level (**Fig. 1** & **2**).

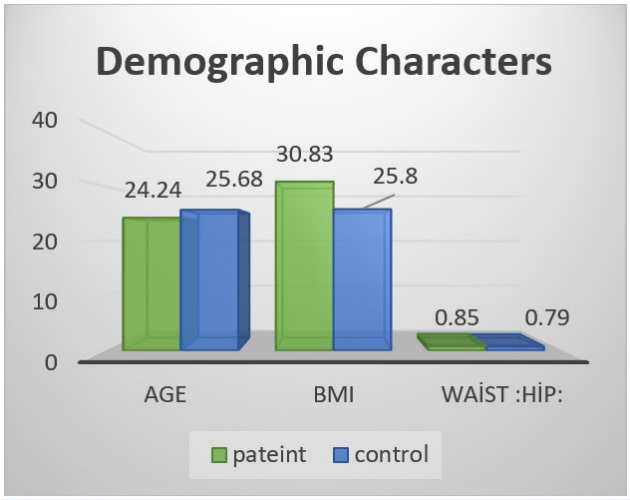

Fig. 2. Demographical Properties for the studied groups

Table 2. Serum antioxidants level in patient and control

	Samples		P value
	Patient (80) Mean ± SD	Control (60) Mean ± SD	
TAOC(*U/mL*)	15.77 ± 8.34	38.16 ± 11.96	0.0001**
PON-1(ng/mL)	2.79 ± 0.82	5.33 ± 1.58	0.0001**

** (P<0.01)

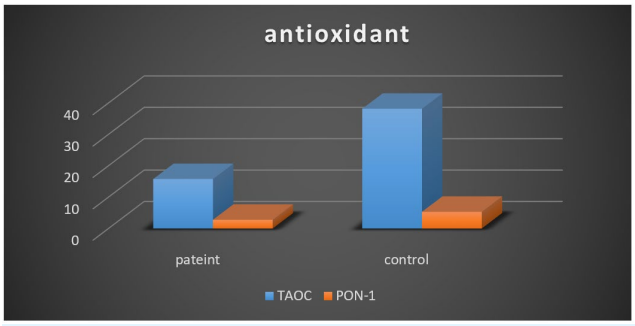

Fig. 3. Concentration of Antioxidants in patients and controls

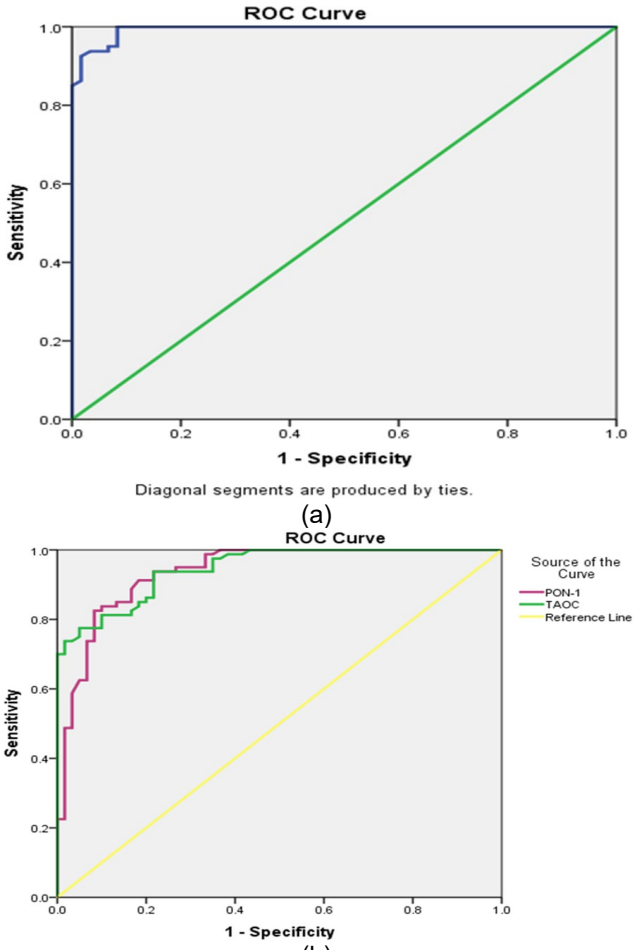

Fig. 3. ROC curve of AMH, PON-1 and TAOC (a) ROC curve of AMH (AUC 0.994, CI 0.99-1.000) larger test result indicates more positive result. (b) ROC curve of PON-1 (AUC 0.937, CI 0.89-0.97) and TAOC (AUC 0.945, CI 0.91-0.97) smaller test result indicates more positive result all parameters have significant >0.0001

Table 3. (ROC) curve analysis for the AMH, PON-1 and TAOC with sensitivity and specificity

Parameters	AUC	Optimal cut off value	Sensitivity	specificity	p- value	95% Confidence Interval	
						Lower Bound	Upper Bound
AMH	0.994	7.48	100%	92%	**0.0001	0.99	1.000
PON-1	0.937	3.90	91%	82%	**0.0001	0.896	0.977
TAOC	0.945	25.34	85%	82%	**0.0001	0.913	0.978

** (P<0.01)

Table 2 and **Fig. 3** illustrated that the serum level of total antioxidant capacity and paroxonase -1 were significantly decreased in the PCOS patients in comparison with control.

Table 3 and **Fig. 4** Showed the Receiver Operator Curve (ROC) Analysis, to distinguish between patient and control by using the investigated testes, the ROC analysis was utilized and help to visually understand the impact of each parameters and quantify how well each test performs by finding the area under the ROC curve. The AMH ROC curve lies to the top left corner, the greater the area underneath it. Thus, a larger area under the ROC curve implies AMH is better test for PCOS with high sensitivity and specificity (100% and 92%) at the (7.48) optimal cutoff point which give 'highest sensitivity while maintaining high specificity'. The other test also shows large area under the curve (PON-1 (AUC 0.937) and TAOC (AUC 0.945) that indicated these test have importance in comparison between PCOS woman and normal women. The sensitivity and specificity (91% and 82%) for PON-1 and (85 % and 82%) for TAOC at the optimal cutoff point.

Table 4 manifested a significant positive correlation between PON-1 (pg/ml) with TAOC (r=0.564, p<0.0001), and also noted there is significant negative correlation

Table 4. Sample Table (one column) to put into column

Parameters	Correlation coefficient-r and PON-1	P value
FSH	-0.030	NS
LH	-0.202	NS
LH/FSH	-0.083	NS
Prolactin	-0.102	NS
Testosterone	-0.537	**
AMH	-0.421	**
E2	-0.079	NS
TAOC	0.564	**
BMI	-0.425	**

** (P<0.01), NS: Non-Significant.

between PON-1 with both AMH (r=-0.421, p<0.0001) and testosterone (r=-0.537, p <0.0001).

DISCUSSION

In polycystic ovaries, there is an increment in the number of small follicles and this enhanced the elevation in the concentration of the hormone AMH, as this hormone is excreted from the granulosa cell in these ova and this hormone has an inhibitory effect on the hormone FSH and thus prevents natural ovulation in these patients which explained the current results (Stracquadanio et al. 2018). The AUC of the serum AMH assay reached a value of 0.994 (95 % CI 0.98–1.000) with high sensitivity and specificity. This demonstrates the high diagnostic significance of AMH.

Many theories tried to explain the cause of elevation of LH in PCOS, as primary arising in LH pulse frequency (Hayes et al. 1998). But some suggested that it is due to abnormal feedback at hypothalamic level or sensitization of pituitary gland to GnRH by abnormal steroid feedback (Ehrmann 2005). Both increment in insulin and in LH, accompanied by insulin resistance, mediated high level of testosterone (Suresh and Vijayakumar 2015) Presence of hyperprolactinemia in PCOS could be clarified by many theories, one of them referred to the probable role of estrogen mainly estrone E_1 'from androgenic precursors' which prompts hyperprolactinemia or increased feedback of steroid on lactotrope (Mah and Webster 2002).

Elevation in lipid profile in PCOS, an increased LDL, with a decreased HDL (Kim and Choi 2013, Kiranmayee et al. 2017), and highness in inflammatory marker and other factors; all are reasons to oxidative stress in these patients (Alanbay et al. 2012, Blair et al. 2013). The oxidative stress status reflected by decrease in activity of PON-1 in serum of woman complaining from PCOS in addition to decrease in TAOC, the importance of these tests in explain the level of oxidative stress was further clarified by the ROC curve. The high level of oxidative stress rises the severity of PCOS disease which expressed in the high proportion of AMH, Pearson correlation demonstrate the strong correlation between AMH and pon-1 and TAOC. Moreover, increased in weight gaining or obesity of patient in turn results in increased in oxidative stress action (Azziz et al. 2016, Kaçar et al. 2015, Kayabasoglu and Kazıkdas 2015, Mardani et al. 2014, Suresh and Vijayakumar 2015, Zuo et al. 2016).

Finally, it could be concluded that in PCOS the reduction of PON-1 and TAOC serum level is associated with the increase in the AMH hormone, which reflects the severity of the disease and also with the increase in weight, the decrease in the rate of antioxidant factors and increase the oxidative stress, as weight plays an important role in all aspect of disease.

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