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Original Research Article

The Relationship between Prostate Specific Antigen (PSA) and Oxidative Stress in Iraqi Women with Polycystic Ovary Syndrome (PCOS)

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Abstract

PCOS is one of the commonest endocrine disorders among the women during their reproductive age .The world-wide prevalence of the disease ranges between (5- 10 %). Those patients were classified in this study into three classes according to Rotterdam criteria which states that PCOS is defined as at least two of three of the following criteria:Obigomenorhea or anovulation, Biochemical signs of increased androgen levels (such as hirsuitism), and Detection of polycystic ovary (Ten or more cysts of 2-10 mm in diameter and in the absence of other endocrine disturbances).

This study included 90 women with PCOS divided into three groups , infertile , hirsute , and combined (both infertile and hirsute) thirty patients of each group, with forty women apparently healthy as a control group.

The measurement of Prostate Specific Antigen (PSA) has been done by using cobas e 411 analyzer.

The oxidative stress has been determined depending on Malondialdehyde (MDA) as a biomarker by spectrophotometric method, the concentrations of total testosterone and sex hormones (LH, FSH) were measured by using ELFA technique.

Total Antioxidant Capacity (TAC), Super Oxide Dismutase (SOD1), Dehydroepiandrosterone (DHEA) concentrations have been measured by using ELISA technique.

The results of BMI showed that highly significant differences between all patient groups compared with control group.

The results revealed a significant differences in levels of (PSA, MDA, TAC, SOD1, Testosterone and LH/FSH Ratio) ($P<0.05$, $P< 0.01$) in all patient groups in comparison with control.

The results demonstrated negative correlation between PSA and MDA in all patient groups, as well as positive correlation between PSA and both of testosterone and LH/FSH Ratio.

From the above results and their correlations we can conclude that PSA considered to be a valuable biomarker for Androgens activity in PCOS patients.

The results also showed that no significant changes in DHEA level this indicates that no contribution of the pituitary-adrenal axis in these changes.

Key words: PCOS, PSA, ELISA, Iraqi Women.

الخلاصة

تعتبر متلازمة تكيس المبايض (PCOS) واحدة من اضطرابات الغدد الصماء الأكثر شيوعا و تواترا في النساء اللاتي تتراوح أعمارهم بين ١٢-٤٥ سنة ، مما يؤثر على ٥-١٠ ٪ من النساء.تم تصنيف هؤلاء المرضى في هذه الدراسة إلى ثلاث فئات وفقا لمعايير Rotterdam التي نصت على أن متلازمة تكيس المبايض تشخص او تعرف بوجود على الاقل اثنين من المعايير الثلاثة التالية :عدم انتظام الدورة الشهرية، علامات بايو كيميائية لزيادة مستويات الاندروجين، الكشف عن تكيس المبايض باستخدام جهاز السونار(عشرة أكياس أوأكثر يتراوح قطرها بين ٢-١٠ ملم في غياب اضطرابات الغدد الصماء الأخرى).

تم تضمين (٣٠) مريضة يعانون من مشاكل عدم الإنجاب (Infertile) و (٣٠) مريضة يعانون من زيادة الاندروجين (Hirsute) و(٣٠) مريضة يعانون من كلا الحالتين (Combined) بالإضافة إلى (٤٠) امرأة سليمة (Control).

تم قياس مستويات الانتجين النوعي للبروستات (PSA) باستخدام جهاز cobas e 411 analyzer. تم تقدير الجهد التأكسدي وحسابه بالاعتماد على (MDA) كعلامة كيميائية لقياسه, بالإضافة إلى قياس مستوى التستوستيرون والهرمونات الجنسية (LH, FSH) باستخدام تقنية

ELFA.تم قياس تركيز مضادات الأكسدة الكلية (Total Antioxidant Capacity) ,تركيز(SOD1), وتركيز (DHEA) باستخدام تقنية ELIZA.

بينت قيم BMI وجود فروق ملحوظة إحصائيا (P<0.01) بعد إجراء مقارنة بين مجاميع المرضى ومجموعة السيطرة.أوضحت النتائج وجود تغيرات ملحوظة في مستويات (PSA, MDA, TAS, SOD1, Testosterone and LH–FSH Ratio) (P<0.05 ,P< 0.01) بعد المقارنة بين مجاميع المرضى مع مجموعة السيطرة.

أوضحت النتائج وجود ارتباط عكسي معنوي بين PSA ومستويات مضادات الأكسدة في جميع المرضى. بينما أشارت النتائج إلى وجود ارتباط طردي عالي المعنوية بين PSA ومستويات الهرمون الذكري والهرمونات الجنسية الأخرى وبذلك يمكن أن يعتبر PSAكعلامة بايوكيميائية للتغييرات الحاصلة في فعالية الاندروجين لمرضى متلازمة تكيس المبايض.كما أوضحت النتائج عدم وجود تغيرات ملحوظه في قيم DHEA وبذلك تدل النتائج إلى استبعاد وجود خلل في إفرازات الغدة الكظرية.

Introduction

Polycystic ovary syndrome (PCOS) is a syndrome of ovarian dysfunction, considered to be one of the most common and frequent endocrine disorders in reproductive aged women, affecting 5-10% of population [1, 2]. The aetiology of PCOS is still unknown, it was considered to be the leading cause of female infertility, menstrual irregularity along with hirsutism and androgen excess [3].

Prostate-specific antigen (PSA), a single chain glycoprotein exist as a 33-kDa serine protease with trypsin and chymotrypsin-like enzymatic activity, 7-10 % carbohydrate contents, isoelectric point at PH (6.8-7.5), 240 amino acids, five isoforms expressed by both normal and neoplastic prostate tissue is produced as a pro-enzyme by the secretory cells that line the prostate glands in males to liquefy the sperm entrapping seminal coagulum after ejaculation and increasing sperm motility and its concentration in serum is a valuable tumour marker in the diagnosis of cancer of prostate, where the pro-peptide is removed to generate active PSA [4].

Oxidative stress (OS) means an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defense system which produces the oxidative damage. Oxidative stress has been implicated in a number of diseases such as cardiovascular disease, neurologic disease, malignancies, renal disease, diabetes, skin diseases, aging, respiratory diseases, liver diseases and different types of viral infections [5].

Malondialdehyde (MDA), a carbonyl group produced as the end products of lipid peroxidation, is used as a marker of lipid peroxidation and increases in oxidative stress states. Products of lipid peroxidation reactions have been widely employed as biomarkers for OS. MDA, produced during the decomposition of poly unsaturated fatty acids, is one of the stable end products of lipid peroxidation that can serve as a good biomarker [6].

Total antioxidant status / capacity (TAS / TAC) are the ability of serum to quench free radical production, protecting the cell structure from molecular damage. It was sensitive to the changes in plasma antioxidant levels and degree of oxidative stress [6]. Recent studies have documented increased oxidative stress in patients with PCOS which may increase the risk of cardiovascular disease in such patients [7].

The responsibility of SOD is catalyzing the conversion of superoxide to elemental oxygen and hydrogen peroxide .This transformation is called dismutation, or it is a reaction between two identical molecules in which one is reduced and the other oxidized. Three forms of superoxide dismutase are present in humans. SOD1 is located in the cytoplasm, SOD2 in the mitochondria, and SOD3 is extracellular. The first is a dimer (consists of two units), whereas the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, whereas SOD2, the mitochondrial enzyme, has manganese in its reactive center [8].

Luteinizing hormone is a glycoprotein hormone like TSH with similar alpha subunit. It is produced by gonadotropic cells in the anterior pituitary gland[9]. In females, an acute rise of LH triggers ovulation and development of the corpus luteum[10]. In males, where LH had also been called interstitial cell-stimulating hormone (ICSH)[11], it stimulates Leydig cell production of testosterone[12]. It acts synergistically with Follicle-stimulating hormone (FSH), a glycoprotein hormone found in humans and other animals, synthesized and secreted by gonadotrophs of the anterior pituitary gland and regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH is a 35.5 kDa glycoprotein heterodimer, consisting of two polypeptide units, alpha and beta. Its structure is similar to that of luteinizing hormone (LH) [13].

Derived from cholesterol, testosterone is a steroid hormone from the androgen group and is found in humans and other vertebrates, which is responsible for the development and maintenance of masculine characteristics. In humans and other mammals, testosterone is secreted primarily by the testicles of males and, to a lesser extent, by the adrenal cortex and the ovaries of females. Small amounts are also secreted by the adrenal glands[14].

Obesity is strongly associated with PCOS and may be present in up to 50% of cases [15]. Obese women with PCOS are more likely than thin women with PCOS to suffer from anovulation. This effect on ovulation may be secondary to insulin resistance, which in turn results in hyperinsulinemia and stimulation of excess androgen production from the ovaries. Intra-ovarian hyperandrogenism in turn inhibits follicular maturation. Weight loss through exercise and diet has been proven effective in restoring ovulatory cycles and achieving pregnancy for many of these patients [16].

Significant association between abdominal fat mass and insulin resistance evaluated by the euglycemic hyperinsulinemic clamp. And found that a highly significant correlation between free fatty acids (FFA) concentration and insulin resistance which support the concept that an increase of FFA flux from the highly lipolytic abdominal fat to the liver and muscle may represent an important link between upper–obesity and insulin resistance [17].

The aims of the study was to determine serum levels of PSA in different subgroups of PCOS patients, determine antioxidant status and oxidative stress in PCOS patients in comparison with healthy control group, find the relationship between the changes in oxidative stress and PSA levels in the corresponding patients, and finally to correlate this mentioned changes with the levels of androgen in different subgroups of PCOS patients.

Materials and Methods

Subjects

This study included 90 patients divided into three groups, infertile, hirsute, and combined (both infertile and hirsute) thirty patients of each group, with forty apparently healthy women as a control group, as in (Table 1).

Table 1 : Groups of women with PCOS

PCOS Women				Total
Infertile	Hirsute	Combined	Control	
30	30	30	40	130

All samples were collected from Advisory clinic in Babylon teaching hospital in Hilla city. All samples were collected from December 2014 until March 2015. The practical side of the study was performed at the laboratory of biochemistry department in College of Medicine/University of Babylon.Questionnaires were designed to obtain the information from patients and control.

All patients underwent medical examinations as well as biomedical investigations, were done by gynaecologist.

Sample collection

Blood sample were obtained from subject groups at second to fourth day of menstrual cycle.Blood put in gel tube and separated at 3000 xg for 10 min to obtain serum.Consequently, serum was divided into aliquots in eppendorf and stored at (-20ºC) until time of analysis.

Method of Estimation

LH, FSH and total testosterone were measured by ELFA technique using VIDAS (BioMerieux/ France), while total PSA were measured by Chemilumine-scenceusing Cobas E411, also SOD1 was estimated by using ELISA technique.

Results

Table 2:BMI in all groups of patient and comparison with control group

Parameter	Groups	N	Mean ± SD	P value
BMI	Infertility	30	27.89± 4.14	0.000
	Hirsute	30	30.88± 3.65	0.000
	Combined	30	30.11± 4.36	0.000
	Control	40	24.51± 3.44	

Table 3: Result of PSA in PCOS subgroups and control

Parameter	Groups	N	M ± SD	P value
PSA (pg/ml)	Infertility	30	14.13± 6. 75	0.318
	Hirsute	30	30.40 ± 20.98	0.000
	Combined	30	26.66 ± 19.22	0.000
	Control	40	10.52± 9.05	

Table 4: Relationship of MDA in PCOS groups and control

Parameter	Groups	N	M ± SD	P value
MDA (µmol/ml)	Infertility	30	4.78± 0.33	0.000
	Hirsute	30	5.34 ± 0.43	0.000
	Combined	30	5.26 ± 0.45	0.000
	Control	40	2.75 ± 0.49	

Table 5: Result of SOD in PCOS subgroups and control

Parameter	Groups	N	M± SD	P value
SOD (pg/ml)	Infertility	30	661.34± 117.21	0.000
	Hirsute	30	548.57± 114.79	0.000
	Combined	30	562.73± 120.08	0.000
	Control	40	1298.50± 238.34	

Table 6: Result of serum Testosterone in PCOS subgroups and control

Parameter	Groups	N	M± SD	Range
Testosterone (ng/ml)	Infertility	30	0.98± .31	0.27- 1.46
	Hirsute	30	1.18± .36	0.34- 1.63
	Combined	30	1.17± .28	0.48- 1.56
	Control	40	0.65± 0.26	0.12-1.21

Table 7: Result of LH/FSH Ratio among all studied groups and control

Parameter	Groups	N	M ± SD	P value
LH/FSH ratio	Infertility	30	1.33± 0.53	0.000
	Hirsute	30	2.00± 0.83	0.000
	Combined	30	1.49± 0.73	0.000
	Control	40	0.56 ± 0.10	

Discussion

PCOS is one of the commonest endocrine disorders among the women during their reproductive age.The world-wide prevalence of the disease ranges between (5- 10 %) [1,2]. Those patients were classified in this study into three classes according to ESHRE/ARSM criteria which states that PCOS is defined as at least two of three of the following criteria:

a. Obigomenorheaor anovulation.
b. Biochemical signs of increased androgen levels (such as hirsuitism).
c. Detection of polycystic ovary (Ten or more cysts of 2-10 mm in diameter and in the absence of other endocrine disturbances) [18].

For the above mentioned reasons PCOS patients were classified into three groups.

The selected patients showed a significant increase in BMI. Many previous studies revealed the role of hyperinsnlinemia associated with obesity in the etiology of ovarian steroidogensis and anovulatory mechanism [19].

These changes lead concomitant changes in pituitary ovarian axis leading to increased LH levels. This variation was conformed in our study by the significant correlation among BMI, testosterone, antioxidant status, PSA and oxidative stress in the selected patients and in both infertile patients group and hirsute group, Table (2) shows insignificant correlation between BMI and DHEA this indicates the non-important contribution of the adrenal axis in those changes.

Our results are in good agreement with those of diamantrietal and Azzizetal who reported that 30-40 % of PCOS patients were obese and overweight [20, 21].

Oxidative stress means the imbalance between the production of reactive

oxygen species and antioxidant defense mechanism (Total antioxidants level) [22].

Reactive oxygen species can be formed exogenously during aerobic metabolism as a result of various metabolic pathways of oocytes [23], and it can also arise from exogenous sources such as tobacco and various environmental pollutions.Also some investigators reported the ability of cycling a vices to product various markers of oxidative stress [24, 25]. Those studies indicated that the follicular fluid micro environ contains leukocytes, macrophages and cytokines which are known source for reactive oxygen species and reactive nitrogen species.

It is noteworthy to mention that moderate oxidative stress levels was shown to be an important factor that is required for ovulates [26].The results of those experiments reported that the final stages of oocyte maturation are associated with fluctuation in cytokine , prostaglandin, proteolytic enzyme ,nitric oxide and steroids. Those fluctuations leads consequently to increase in the level of ovarian blood flow that facilities follicle rupture.

A significant increase in oxidative stress values in this study led to harmful effects. Excess ROS induces infertility in women through different types of mechanisms. For instance, excessive amounts of ROS in the follicle may overwhelm follicular fluid antioxidant defense and leads to the damage of oocytes.The DNA of oocytes and spermatozoa may be also damaged resulting in detective fertilization. Even when fertilization was achieved apoptosis which was included by oxidative stress result in embryo fragmentation, implantation failure, abortion, impaired placentation and congenital abnormal-ities.

ROS is controlled and kept at physiological levels within the ovaries by different antioxidant systems. These include catalase, vit. E. glutathione and carotenoids [27]. Superoxide dismutase was shown to the most important enzymatic antioxidant which is capable of the protection of oocyte from excess ROS during its maturation and for this reason this enzyme was selected as an antioxidant in this study and the results revealed a good correlation between this enzyme and the other studied privative in different groups of PCOS patients.

The significant positive correlation between MDA (oxidative stress index) and LH/FSH ratio indicate the role of oxidative stress in increasing testosterone level.

Since the latter is also induce by increased LH levels. Increased testosterone lead consequently to increased PSA since the gene expression and protein production of PSA in non-prostatic tissues are under the regulation of steroid hormone via their receptors.

The significant correlation and regression results between testosterone levels and PSA levels indicate the fact that PSA can be considered as a good marker of androgen action in women.

The result also revealed the role of testosterone as a potent androgens in the induction of PSA in compassion with DHEA. This is accordance with some previous studies which indicated that most patients with PCOS showed increased testosterone levels but 25% of them have elevated DHEA.

The exact causes of adrenal androgen excess are not well-known. Some investigators reported that increased adrenocorticotropine hormone (ACTH) production , increased adrenal sensitivity to ACTH, altered steroidogenic enzyme activity (17,20 lyase, 3-beta hyroxysteriod dehydrogenase activity and overproduction of androgens in response hyperproduction have all been considered as a potential causes for this endocrine abnormality. Other investigators suggested a role for abnormal glucose metabolism (i.ehyperinsulinimia, insulin resistance) in the etiology of adrenal androgen excess.

From the above results and their correlations we can conclude that

oxidative stress and obesity leads to increased LH/FSH ratio (which is not necessarily more than two) and this consequently leads to hyperandrogenemia (mostly increased serum testosterone levels) with a concomitant increased in PSA level as a good marker in PCOS patients.

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