Oxidative Stress and the Antioxidant Mechanisms in a Sample of Iraqi Patients with Polycystic Ovary Syndrome (POS)

Hasan F. Al-Azzawie* PhD

Esraa Hameed Humadi ** MBChB, FICOG, CABOG

Abstract:

Objective: The aim of this study was to investigate the levels of non enzymatic antioxidants glutathione (GSH), vitamin C and vitamin E and enzymatic antioxidants glutathione peroxidase (GPx), glutathione reductase (GRx), superoxide dismutase (SOD), catalase (CAT), glutathione s- transferase (GST) in patients with polycystic ovary syndrome.

Patients & Methods: Fifty women with PCOS and fifty healthy, age and body mass index (BMI) matched controls were evaluated in this controlled clinical study. Erythrocyte GSH, plasma vitamin C, vitamin E, and activities of antioxidant enzyme SOD, GPx, GRx, catalase and GST were estimated. Oxidant status was evaluated by determination of erythrocyte malondialdehyde (MDA) concentration.

Results: The women with PCOS had significant increase in the erythrocyte MDA levels compared to controls ($P < 0.05$). The activities of erythrocyte antioxidant enzymes SOD, GPx, GR_X and plasma GST were significantly increased in PCOS patients compared to healthy subjects, while levels of erythrocyte GSH, Vitamin C, vitamin E and catalase activity were significantly decreased in patients with PCOS compared to healthy subjects.

Conclusion: An increase in oxidant status was found in women with PCOS.The results of this study suggest higher oxygen free radical production, evidenced by increased a marker of lipid peroxidation (MDA) and decreased GSH, vitamin C & E, and catalase activity, support to the oxidative stress in PCOS patients. The increased activities of antioxidant enzymes may be a complementary regulation in response to increased oxidative stress.

Keyword: antioxidant / PCOS / MDA / enzymatic and non enzymatic antioxidant

Introduction

Polycystic ovary syndrome (PCOS) is the most commn endocrinopathy in women of reproductive age, affecting 5-10% of population, and it is the leading cause of female infertility.[1].

It is a heterogeneous disorder of chronic anovelation and hyperandrogensim and it is often associated with obesity and insulin resistance [2]. Women with PCOS are at greater risk of developing type 2 diabetes mellitus and may also demonstrate other features of the metabolic syndrome; including dyslipidemia, hypertension, impaired fibrinolysis and vasodilation, all of which increase the risk of cardiovascular disease [3,4].

In the pediatric age group, there is no universally accepted definition of the metabolic syndrome, although the aforementioned features cluster in a similar fashion as in the adult population [5-6].

At recent Joint European society human reproduction and embryology/American society for human reproductive medicine ESHRE/ASRM consensus meeting a refine definition of PCOS was agreed namely the presence of 2/out of the following three criteria: oligo and /or anovulatory, hyperandrogenism (clinical parameter) polycystic ovaries(Rotterdam ESHRE sponsor PCOS consensus work shop group 2004) .

The morphology of the polycystic ovary has been redefined as an ovary with 12 or more follicle measuring 2-9 mm in diameter and increase overran volume > 10 cm^3 on trans vaginal ultrasound [7].

Free radicals are formed in both physiological and pathological conditions in mammalian tissues [8].The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies [9]. Oxidative stress due to damage brought about by free radicals is also known to influence the response of these patients to therapy. Moreover the body's defense mechanisms would play a role in the form of antioxidants and try to minimize the damage, adapting itself to the above stressful situation. Antioxidants are compounds that dispose, scavenge, and suppress the formation of free radicals, or oppose their actions [10] and two main categories of antioxidants are those whose role is to prevent the generation of free radicals and those that intercept any free radicals that are generated [11].

They exist in both the aqueous and membrane compartment of cells and can be enzymes or non-enzymes. Studies suggest that polycystic ovary syndrome may increase risk for several conditions like type 2 diabetes, dyslipadaemia, endometrial cancer and hypertension [12,13].

So, the present study was undertaken to assess oxidative stress and antioxidant status in Iraqi patients with polycystic ovary syndrome.

Patients & Methods:

The study was conducted in the section of Obstetrics and Gynecology at al Yermouk Teaching Hospital between April 2007 and June 2009. Fifty patients with PCOS (study group) and fifty healthy women (control group0 included in the study.

The patient's ages ranged from 25-45 years. The diagnosis of PCOS was based on the recommendations' of Rotterdam consensus conference on PCOS by at least two of the following three features: (i) Oligo-or anovulation,(II) clinical and / or biochemical signs of hyperandrogenism and (III) polycystic ovaries

(The Rotterdam ESHRE/ASRM sponsored PCOS Consensus Workshop Group 2004).Some patients had clinical hyperandrogenism (presence of acne, Ferriman-Gallwey score of >8, oligomenorrhea(fewer than six menstrual periods in the preceding year).And the presence of 12 or more subcapsular follicles in each ovary criteria comprised infectious diseases, hypertension, family history of cardiovascular disease, chronic liver diseases ,endocrinopathies including diabetes, Cushing syndrome, androgen secreting tumors .non-classical 21 hydroxylase deficiency, thyroid dysfunction, hyperprolactinemia,smoking,alachol consumption and use of all medications' known to alter sex hormones, lipoprotein, carnitine metabolism or insulin secretion or action.

The women in the control group had regular menstrual cycles that were defined as cyclic uterine bleedings with duration of 4-5 days and a frequency of 25-30 days.

The healthy state of the control subjects was determined by medical history, physical and pelvic examination and the whole blood chemistry.

None of the fifty healthy women in the control group met any exclusion criteria mentioned above. This work was approved by the local medical ethics committee and all participants gave informed consent before the consent of study

The heparinized venous blood samples obtained under aseptic conditions, from these subjects in fasting state were used for the analysis. Plasma was separated by centrifugation at 1,500 g for 15 minutes. Separated plasma was used for the estimation of vitamin E, Vitamin C, MDA and for the measurement of GST activity.

Follicle-stimulating hormone (FSH), luteinizing hormone (LH), Prolactin and testosterone analyses were carried out with a radioimmunometric assay by using the gamma counter 1275 LKB analyzer (Diagnostic Products, Los Angeles, Calif, USA)

The buffy coat was removed and the packed cells were washed three times with physiological saline. The erythrocyte suspension was prepared by the method of [14]

The packed cells were used for the analysis of GSH, SOD, Catalase, GPx and GRx. Plasma Vit. C levels were estimated by the method of Lin [15]. Plasma vitamin E levels were estimated by the method of Bieri[16]. SOD activity was determined in the hemolysate by the method of Misra & Fridovich based on to inhibition of auto oxidation of epinephrine to adenochrome at alkaline pH [17].

Catalase activity was measured by the method of Beers and Sizer [18], activity of GST was measured by using 1-Chloro-2, 4-Dinitro Benzene [19].All reagents used were of analytical reagent grade. DTNB, CDNB and Thio Barbituric Acid were obtained from sigma chemicals.

Laboratory Measurements:

Measurement of MDA: Lipid peroxidation (LPO) is frequently investigated in biomedical research, and the assays for thiobarbituric acid-reactive substances (TBARSs) are more widely used than any other index of LPO in biological samples. Thiobarbituric acid reacts with LPO aldehydes, such as malondialdehyde (MDA).

Therefore, assessment of TBARS is a useful index of oxidative deterioration and LPO determination in body fluids. MDA levels were determined at 532nm by the method of Ohkowa et al. [20]. MDA formed a colored complex in the presence of thiobarbituric acid, which was detectable by measurement of absorbance at 532 nm. Absorbance was measured with Shimadzu UV-160 spectrophotometer.1,1-3,3-tetraethoxypropane was used as a standard. Levels were calculated as nmoles/gm of Hb.

Measurement of GSH: glutathione content was measured according to the method of Beutler [21]. In brief, 0.5mL sample or standard solution was mixed with 0.25mL of 1mol/L sodium phosphate buffer (pH 6.8) and 0.5mL 5-5-dithiobis-(2-nitrobenzoic acid) (DTNB, 0.8 g/L in the phosphate buffer) for 5 minutes.

Then, the absorbance was measured at 412nm using a Shimadzu UV-160 spectrophotometer.

The GSH concentration was determined using standard aqueous solutions of GSH. Results were expressed as mg/dl

Measurement of GRx: Glutathione reductase was assayed by following the oxidation of NADPH at 340nm at 37◦C [22]. The reaction was initiated by the addition of 50 μ**L** supernatant to 1mL of assay mixture containing 50 mmol/L Tris, pH 7.6, 100μmol/L Na_2EDTA, 4 mmol/L GSSG, and 120 μmol/L NADPH. A blank cuvette was prepared in which the sample was replaced with water. The reaction was linear for 2 to 3 minutes. Hemoglobin in erythrocyte lysates was estimated using the method of Drabkin. Activity in samples was normalized for hemoglobin content and is expressed in U/gm Hb [22].

Measurement of GPx: Glutathione peroxidase activity was determined by a minor modification of the method of Paglia and Valentine [23]. A 50 μ**L** supernatant was transferred to a 1mL quartz cuvette, containing 950 μL of the reaction mixture (Tris buffer, 50 mmol/L, pH 7.6, containing per liter, 1 mmol of Na_2EDTA, 2 mmol of reduced glutathione, 0.2 mmol NADPH, 4 mmol sodium azide, and 1000U of glutathione reductase). The mixture was incubated 5minutes in 37◦C. Then, the reaction was initiated by adding 25 μL of H_2O_2, 8.8 mmol/L (%30). The decrease in NADPH absorbance at 340nm was followed for 3 minutes. The nonenzymic reaction rate (blank) was

determined by substituting water for the supernatant. The decrease in NADPH absorbance was recorded. Hemoglobin was evaluated as described above. Activity in samples was normalized for hemoglobin content and is expressed in U/gm Hb [23].
Statistical analysis between group 1 (controls) and group 2 (study patients) was performed by the student t-test. The data were expressed as mean ± SD. $p < 0.05$ was considered as significant.
Multiple regression analysis was conducted with antioxidants, antioxidant enzymes and lipid peroxidation, all using patient group as outcome and BMI, Testosterone, additional independents. Multiple regression analysis was carried out by using SPSS Version 10

Results: Compared with control subjects, women with PCOS exhibited higher ($P < 0.05$) circulating levels of testosterone (0.95 ± 0.24 vs. 0.46 ± 0.25 ng/dl). Androgen results were similar when subjects were grouped by body mass (data not shown). The levels of FSH and Prolactin were not changed in comparison with controls while levels of LH was highly significant increased ($p<0.001$) in PCO syndrome 77 ± 12 IU/L vs. 14 ± 3.5 IU/L as showed in **table 1.**

As depicted in **table 2** there was a statistically significant increase in the erythrocyte MDA levels in patients with polycystic ovary syndrome compared to controls($p<0.05$).The activities of erythrocyte antioxidant enzymes SOD, GP_X, GR_X and plasma GST were significantly increased in group 2 (patients) compared to group1 (controls). The levels of erythrocyte GSH, vitamin C, plasma vitamin E and catalase activity were significantly decreased in patients with polycystic ovary syndrome compared to controls.

.**Table 1: The mean ± SD values of testosterone, LH, FSH and Prolactin in controls and patients with Polycystic Ovary Syndrome**

Parameter	Group1 (Controls)(mean ± SD) n=50	Group 2 (PCOS) (mean ± SD) n=50	P-value
Testosterone (ng/ ml)	0.46 ± 0.25	0.95 ± 0.24	<0.01
LH (IU/L)	14 ± 3.5	77 ± 12	<0.001
FSH (IU/L)	10 ± 2.2	12 ± 2.7	NS
Prolactin (ng/ml)	7.8 ± 2.2	10.2 ± 1.7	NS

Table 2: Values of glutathione(GSH), malondialdehyde (MDA), vitamin C, vitamin E, superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) glutathione peroxidase (GRx), glutathione - S-transferase (GST) in patients with Polycystic Ovary Syndrome and controls.(Mean ± SD)

parameter	Group 1 (Controls) (Mean ± SD) n=50	Group 1(PCOS) (Mean ± SD) n=50	P value
GST (µmoles /dl)	*8.01 ± 1.21*	*9.41 ± 1.25*	***< 0.05***
GPx (U/gm of Hb)	*54.32 ± 1.50*	*63.42 ± 2.78*	***<0.001***
Glutathione (mg/gm Hb)	*23.22 ± 1.33*	*10.41 ± 1.63*	***<0.001***
Vitamin C (mg/dl)	*5.72 ± 1.28*	*5.01 ± 1.27*	***< 0.05***
Vitamin E(µmoles/L)	*10.23 ± 1.43*	*3.45 ± 1.63*	***<0.001***
MDA(nmoles/gm of Hb)	*14.32 ± 2.43*	*37.31 ± 2.46*	***<0.001***
SOD(U/gm of Hb)	*733.59 ± 35.54*	*825.56 ± 69.82*	***<0.001***
Catalase(U/gm of Hb)	*9.89 ± 1.13*	*7.05 ± 1.36*	***<0.05***
GRx (U /gm of Hb)	*6.83 ± 0.34*	*9.05 ± 0.39*	***<0.05***

Discussion

In the present study a significant decrease in the levels of erythrocyte glutathione (GSH), vitamin C and plasma vitamin E (non enzymatic antioxidant defense system) in patients with PCOS when compared to the controls was observed .The decrease in the levels of these non enzymatic antioxidant parameters may be due to the increased turnover.

Glutathione, a tripeptide present in millimolar concentrations in all the cells, is an important antioxidant, glutathione normally plays the role of an intracellular radical scavenger and is the substrate of many xenobiotic elimination reactions .A marked decreased level of reduced glutathione is reported in the plasma of PCOS patients. GSH systems may have the ability to manage oxidative stress with adaptation changes in enzymes regulating GSH metabolism. Indeed, in hyperglycemia conditions due to insulin resistance associated with PCOS patients, glucose is preferentially used in polyol pathway that consumes NADPH necessary for GSH regeneration by the GSH-Red enzyme.

Hyperglycemia is therefore indirectly the cause of GSH depletion. As GSH is an important

antioxidant molecule, its depletion leads to the increase of oxidative stress.

Vitamin E is the primary *in vivo* chain breaking, lipid soluble antioxidant in human serum and is particularly effective in lipid peroxidation inhibition. Enhanced lipid per-oxidation increases the need for lipid soluble antioxidant such as vitamin E thus, plasma vitamin E levels in the PCOS patients were less than from those of healthy group.

Several explanations for reduced serum vitamin C concentrations in PCOS patients might be considered to (i) renal reabsorption of vitamin C may be reduced by hyperglycemia, insulin resistance which may be associated with PCOS patients (ii) blood glucose may compete with vitamin C for uptake into certain cells and tissues, (iii) cellular regulation of vitamin C may be impaired, and (iv) increased oxidative stress may deplete antioxidant reserves.

For preventing oxidative damage in these patients suggesting an increased defense against oxidant damage in polycystic ovary syndrome.

The lipid peroxidation product i.e. MDA levels have been increased significantly in erythrocytes of the patients with polycystic ovary syndrome compared to controls. Rise in MDA could be due to increased generation of reactive oxygen species (ROS) due to the excessive oxidative damage generated in these patients. These oxygen species in turn can oxidize many other important bimolecular including membrane lipids. Similar reports of elevated MDA levels have been reported in patients with PCOS [24].

On the other hand, the erythrocyte antioxidant enzymes i.e. SOD & GPx activities have been increased significantly in patients with PCOS compared to controls.

SOD is the important antioxidant enzyme having an antitoxic effect against super oxide anion. The over expression of SOD might be an adaptive response and it results in increased dismutation of superoxide to hydrogen peroxide. GPX, an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes [25].

The rise in the activity of GPX could be due to its induction to counter the effect of increased oxidative stress.

The Glutathione –S– Transferase is a group of multifunctional proteins, which play a central role in detoxification of electrophilic chemicals & the hepatic removal of potentially harmful hydrophobic compounds from blood [26]. We have observed a significant increase in the GST activity in patients with polycystic ovary syndrome compared to controls.

The rise in the activity of GST could be due to its induction to counter the effect against increased oxidative stress.

In the present study, we have observed a significant decrease in the activity of catalase in patients with PCOS compared to controls. Catalase is the enzyme, which protects the cells from the accumulation of hydrogen peroxide by dismutating it to form water and oxygen or by using it as an oxidant in which it works as a peroxidase [27].

With multiple regression analysis the reduction in antioxidants along with significant increase in the lipid peroxidation, antioxidant enzymes was calculated to be independent from the BMI, Testosterone and FAI (Free Androgen Index) in patients with Poly Cystic Ovary Syndrome (PCOS) compared to Controls ($p > 0.05$) and these parameters were considered as the independent determinants significantly increased oxidative stress levels in patients with PCOS.

In conclusion, oxidative stress is increased in patients with polycystic ovary syndrome. The results of this study have shown higher oxygen free radical production & decreased catalase activity, support to oxidative stress in PCOS.

The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress.

A decrease in antioxidant capacity may contribute to the increased risk of cardiovascular disease in women with PCOS, in addition to known risk factors such as insulin resistance, hypertension, central obesity, and dyslipidemia.

Reactive oxygen species may play a role in the regulation of growth of ovarian mesenchyme.

Under pathological condition, such as those encountered in PCOS, excessive oxidative stress and depletion of antioxidant may contribute to ovarian mesenchymel hyperplasia [28].So, the treatment with antioxidants in the initial stages of the disease may be useful as secondary therapy to prevent the oxidative damage.

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Dept of Biotechnology, College of science, University of Baghdad
Lecturer in Al-Mustansyria Medical College
Senior Officer in Obstet.and Gyneco. Department in Al-Yermouk Teaching Hospital