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Lipid peroxidation, glutathione, ascorbic acid, vitamin E, antioxidant enzyme and serum homocysteine status in patients with polycystic ovary syndrome

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Abstract

The exact pro-oxidant and antioxidant status in patients with polycystic ovary syndrome is still not clear. Many studies suggest that polycystic ovary syndrome may increase risk for several conditions like type 2 diabetes, dyslipidemia, endometrial cancer and hypertension. To add a new insight to the question, changes in the erythrocyte lipid peroxidation products (MDA), glutathione (GSH), ascorbic acid, plasma vitamin E and activities of antioxidant enzymes super oxide dismutase (SOD), glutathione peroxidase (GP_X), catalase in erythrocyte, plasma glutathione – S – transferase (GST) and serum homocysteine levels were measured in patients with Polycystic Ovary Syndrome. This work was undertaken to assess oxidative stress and antioxidant status in patients with Polycystic Ovary Syndrome and its contribution to the risk of cardiovascular disease. The study was conducted in fifty-six patients & compared to controls. Erythrocyte MDA, GSH, ascorbic acid, plasma vitamin E and activities of antioxidant enzymes SOD, GP_X, catalase in erythrocytes, plasma GST and serum homocysteine were estimated in Polycystic Ovary Syndrome patients. These parameters were measured in fifty-six patients and compared to controls. It was observed that there was a significant increase in erythrocyte MDA levels, SOD, GP_X and plasma GST activities and a significant decrease in erythrocyte GSH, ascorbic acid, plasma vitamin E levels and catalase activity in patients with polycystic ovary syndrome when compared to controls. Serum homocysteine levels were significantly higher in polycystic ovary syndrome patients than in the controls. The results of our study suggests higher oxygen free radical production, evidenced by increased MDA and decreased GSH, ascorbic acid, vitamin E and Catalase activity, support to the oxidative stress in polycystic ovary syndrome. The increased activities of antioxidant enzymes may be a compensatory regulation in response to increased oxidative stress. Increased homocysteine levels and decreased 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.

Keywords: Malondialdehyde, Homocysteine, oxidative stress, antioxidants, cardiovascular risk, polycystic ovary syndrome.

Introduction

Polycystic ovary syndrome, also known as Stein-Leventhal syndrome is a common health problem that affects teenage girls and young women. Although no one really knows what causes PCOS, it seems to be related to an imbalance in hormones. It is one of the most common endocrine disorders of women in the reproductive age group, with a prevalence of 4-12% (Aziz R et al 2004, Asuncion M et al 2000). It occurs amongst all races and nationalities and is a leading cause of infertility (Carmina E, Lobo RA 1999). The principal features are weight problems, lack of regular ovulation and /or menstruation, and excessive amounts or effects of androgenic hormones. While the causes are unknown, insulin resistance, diabetes and obesity are all strongly correlated with PCOS. Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage. Free radicals are formed in both physiological and pathological conditions in mammalian tissues (Tas F et al 2005). The uncontrolled production of free radicals is considered as an important factor in the tissue damage induced by several pathophysiologies (Yeh CC et al 2005). Alteration in the oxidant -antioxidant profile is known to occur in polycystic ovary syndrome (Fatma Ferda Verit, Ozcan Erel 2008). 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 (Sie H 1988) 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 (Cotgreave I et al 1988). 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, dyslipidemia, endometrial cancer and hypertension (E. Mor et al 2003, Susmeeta T et al 2006). So, the present study was undertaken to assess oxidative stress and anti oxidant status in patients with polycystic ovary syndrome and its contribution to the risk of cardiovascular disease.

In the present study, the following parameters were assessed in the erythrocytes and plasma to elucidate the oxidant-antioxidant status in patients with Polycystic Ovary Syndrome. Erythrocyte malondialdehyde (MDA) levels were measured as thiobarbituric acid reacting substances (TBARS), which serves as an index of extent of lipid peroxidation. Erythrocyte glutathione (GSH), ascorbic acid and plasma vitamin E serves as non enzymatic antioxidant parameters. The activities of antioxidant enzymes superoxide dismutase (SOD), catalase, glutathione peroxidase (GP_X) in erythrocytes, glutathione-S -transferase (GST) in plasma and serum homocysteine levels were estimated. GST is an enzyme involved in antioxidant defense and also involved in detoxication. The present work is an attempt to determine alteration in oxidant – antioxidant status and its contribution to the risk of cardiovascular disease in polycystic ovary syndrome patients.

Materials and Methods

Sixty-two patients (mean age: 36 $\pm$ 10 years) with clinically proven polycystic ovary syndrome were chosen for the study as study subjects (patients). Normal healthy age matched women volunteers were taken as controls. The women with PCOS and control subjects were selected as using the Rotterdam criteria to make the diagnosis of PCOS (The Rotterdam 2004). The written consents were also taken from the patients prior to study and the objectives of the study were fully explained. Six of the participants were dropped out at the end of the selection, as they did not like the idea of giving blood and we had limited our study to fifty-six patients only. An equal number of age matched healthy subjects were also investigated.

The complete clinical and personal history of the subjects was recorded. The subjects were ranging in age 26 – 46 years. All the patients in the study were clinically diagnosed as patients with polycystic ovary syndrome. Inclusion criteria for the study subjects (patients) were: chronic oligo- or amenorrhoea, hirsutism, plasma total testosterone concentrations of more than 0.6 ng/ml or free androgen index (FAI) of less than 5.0. All women were amenorrheoeic and anovulatory according to progesterone measurements and ultrasound examination. Characteristic ovarian morphology as detected by ultrasound was not considered as an inclusion criterion. None of these subjects were alcoholics or chronic smokers and did not suffer from any systemic diseases like hypertension or any diabetic complication. Patients suffering from disease of any origin other than polycystic ovary syndrome were excluded from the study. Fifty-Six healthy women, with normal cycles between 26 – 46 years old, acted as a control group. Each one had a history of regular 28 to 32 day menstrual cycles, absence of hirsutism and other manifestations of hyper androgenism, and thyroid dysfunction. They had normal hormonal status, were not receiving oral contraceptives or any drug therapy for at least 6 months before starting the study and had the antecedent of a normal term pregnancy with vaginal delivery of a healthy infant. Since Poly Cystic Ovary Syndrome can be diagnosed even in women with regular menses (Carmina E, Lobo R A 1999), only women with the antecedent of a normal term pregnancy were selected for the control group, in order to reduce a possible misleading effect of an inaccurate disease classification. Subjects who had no other diseases and subjects with normal nutritional habits without supplementing any vitamins during 6 months were included. Subjects with history of receiving anti-inflammatory drugs in last 6 months and history or present symptoms of any other stress induced disorder were excluded. Hyperprolactinemia, androgen secreting neoplasm, Cushing's syndrome and attenuated 21-hydroxylase deficiency, as well as thyroid disease, were excluded. Patients with Poly Cystic Ovary Syndrome and Control women were included in the study had no family histoy of diabetes.

The controls and patients were divided into two groups.

- Group 1 (Controls): Fifty-six healthy age matched women as controls.

- Group 2 (Study Subjects): Fifty-six patients with clinically proven polycystic ovary syndrome.

The Demographic details, clinical and metabolic characteristics of controls and patients with Polycystic Ovary Syndrome are shown in Table 1.

Table 1: The clinical and metabolic characteristics of controls and patients with Polycystic Ovary Syndrome.

Parameter	**Group1 (Controls)** **(mean ± SD)** **n=56**	**Group2 (Study Subjects)** **(mean ± SD)** **n=56**
BMI (Kg/m^2)	21.13 ± 2.34	24.83 ± 2.87 [a]
Waist Circumfrence	73.51 ± 2.95	76.62 ± 2.35
Testosterone (ng/ml)	0.49 ± 0.45	0.95 ± 0.84 [b]
Free Androgen Index	4.07 ± 2.51	7.80 ± 4.05 [a]
[a] P < 0.005 compared to controls [b] P < 0.01 compared to controls		

The heparinised venous blood samples obtained under asceptic conditions, from these subjects in fasting state were used for the analysis. Plasma was separated by centrifugation at 1,000 g for 15 minutes. Separated plasma was used for the estimation of vitamin E and for the measurement of activity of GST. 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 Dodge et al., (Dodge J F et al 1968) modified by Quist (Quist E H 1980). The packed cells were used for the analysis of GSH, ascorbic acid, MDA, SOD, Catalase, GP_X. Erythrocyte GSH was estimated by the method of Beutler et al (Beutler E et al 1963) using Di Thio Bis Nitro Benzoic acid (DTNB). Ascorbic acid levels were estimated by the method of Tietz (Tietz, N W 1986). Plasma vitamin E levels were estimated by the method of Baker H et al (Baker H et al 1968). MDA was determined as the measure of TBARS (Jain, S.K et al 1989). SOD (EC 1.15.1.1) activity was determined in the hemolysate by the method of Misra & Fridovich based on to inhibition of auto oxidation of epinephrine to adenochrome at Ph 10.2 (Misra, HP and Fridovich I 1972). Catalase (EC 1.11.1.6) activity was measured by the method of Beers and Sizer (Beers, R.F. & Sizer, I.W 1952). The activity of Glutathione Peroxidase (GP_X, EC 1.11.1.9) was measured as described by Paglia and Valentine (Paglia, D.E et al 1967) in erythrocytes and activity of GST (EC 2.5.1.18) was measured by using 1-Chloro-2, 4-Dinitro Benzene (CDNB) (Warholm, M et al 1985). Homocysteine was measured using a solid phase immunoassay system, which measures total homocysteine in plasma or serum using BioRad kit. All reagents used were of analytical reagent grade. DTNB, CDNB and Thio Barbituric Acid were obtained from sigma chemicals, St.Louis; MO.

Statistical Analysis

Statistical analysis between group 1 (controls) and group 2 (study subjects) was performed by the student t-test using the Stat-View package. 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, FAI as

additional independents. Multiple regression analysis was carried out by using SPSS package.

Results

The mean ± SD of erythrocyte GSH, ascorbic acid, MDA, SOD, Catalase, GP_x , plasma vitamin E, plasma GST and serum homocysteine are indicated in the table1. There was a statistically significant increase in the erythrocyte MDA and serum homocysteine levels in patients with polycystic ovary syndrome compared to controls. The activities of erythrocyte antioxidant enzymes SOD, GP_X and plasma GST were significantly increased in group2 (study subjects) compared to group1 (controls). The levels of erythrocyte GSH, ascorbic acid, plasma vitamin E and catalase activity were significantly decreased in patients with polycystic ovary syndrome compared to controls.

Table 2: The mean ± SD values of malondialdehyde (MDA), glutathione, ascorbic acid, vitamin E, super oxide dismutase (SOD), catalase, glutathione peroxidase (GP_X), glutathione – S – transferase and serum homocysteine in controls and patients with Polycystic Ovary Syndrome.

Parameter	Group1 (Controls) (mean ± SD) n=56	Group2 (Study Subjects) (mean ± SD) n=56
Glutathione (mg/gm of Hb)	26.34 ± 1.54	11.52 ± 1.74 **
Ascorbic Acid (mg/dl)	5.66 ± 1.33	5.22 ± 1.30 ****
Vitamin E(μmoles/L)	10.53 ± 1.65	3.33 ± 1.88 *
MDA(nmoles/gm of Hb)	15.43 ± 2.59	39.51 ± 2.68 ***
SOD(U/gm of Hb)	733.59 ± 35.54	825.56 ± 69.82 ***
Catalase(U/gm of Hb)	9.79 ± 1.38	9.37 ± 1.36 **
GP_X (U/gm of Hb)	56.52 ± 1.71	61.20 ± 2.61 **
GST(micromoles / dl of plasma)	10.53 ± 1.02	11.03 ± 0.74 *****
Serum Homocysteine ((μmole/L)	18.96 ± 1.93	20.21 ± 1.99 **

* P < 0.0001 compared to controls ** P < 0.001 compared to controls

*** P < 0.0005 compared to controls **** P < 0.01 compared to controls

***** P < 0.05 compared to controls

Discussion

In the present study 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 biomolecules including membrane lipids. Similar reports of elevated MDA levels have been reported in patients with PCOS (Yildirim B et alv2007).

We observed a significant decrease in the levels of erythrocyte glutathione (GSH), ascorbic acid and plasma vitamin E (non enzymatic antioxidant defense system) in patients with PCOS when compared to controls. The decrease in the levels of these non enzymatic antioxidant parameters may be due to the increased turnover, for preventing oxidative damage in these patients suggesting an increased defense against oxidant damage

in polycystic ovary syndrome. Similar reports of decreased GSH, Ascorbic acid and Vitamin E levels in patients with polycystic ovary syndrome have been reported by various studies (Dinger Y et al 2005).

In our study the erythrocyte antioxidant enzymes i.e. SOD & GP_x 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. GP_X, an oxidative stress inducible enzyme plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membranes (Chandra R et al 2000). The rise in the activity of GP_X 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 (Smith GJ et al 1977). 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 (Lenzi A et al 1993).

Homocysteine has been recognized recently as a risk factor for vascular diseases. In our study, Serum Homocysteine levels were significantly increased in patients with PCOS and compared to controls. An increased serum homocysteine level is associated with the formation of atherosclerotic plaques and myocardial infarction. The sulfhydryl groups in homocysteine were oxidized to disulfide catalyzed by the transition metals by which several reactive oxygen species and hydroperoxides were produced and initiates lipid peroxidation which is responsible for endothelial injury. Similar reports of increased levels of homocysteine in polycystic ovary syndrome were reported by Ahmed Badawy et al (Ahmed Badawy et al 2007). With multiple regression analysis the reduction in antioxidants along with significant increase in the lipid peroxidation, antioxidant enzymes and homocysteine levels 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 and increased Homocysteine levels in patients with PCOS.

In conclusion, oxidative stress is increased in patients with polycystic ovary syndrome. The results of our 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. Increased homocysteine levels and decreased 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. 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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