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Original Article

Elevated serum malondialdehyde (MDA), insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and thyroid-stimulating hormone (TSH), and reduced antioxidant vitamins in polycystic ovarian syndrome patients

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Abstract

Elevated oxidative stress and hormonal imbalance have been suggested to associate with polycystic ovarian syndromes (PCOS), a causal factor for unsuccessful pregnancy outcomes and other associated complications in women. The aim of this study was to compare the oxidative stress markers and different relevant hormones between pregnant women with and without PCOS. The levels of malondialdehyde (MDA), insulin, follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), vitamin A and vitamin C were measured in 80 pregnant women with PCOS and 80 healthy pregnancies. The mean MDA and insulin levels were significantly elevated in pregnant women with PCOS compared to healthy controls (1.98±0.07 *vs.* 1.06±0.02 nmol/mL and 11.15±0.25 *vs.* 6.67±0.25 mIU/L, respectively with $p<0.001$ for both). Compared to healthy controls, the mean concentrations of FSH (3.65±0.16 *vs.* 1.75±0.10 IU/L) and LH (15.67±0.63 *vs.* 3.65±0.16 IU/L) were significantly higher in pregnant women with PCOS, $p<0.001$ for both comparisons. Similarly, the concentration of serum TSH was also higher in PCOS cases compared to controls (2.79±0.22 *vs.* 2.34±0.06, $p=0.048$). In contrast, the levels of vitamin A and C were lower in PCOS cases compared to healthy pregnancy group, 0.45±0.01 *vs.* 1.05±0.01 and 0.26±0.01 *vs.* 0.53±0.02, respectively with p-values <0.001 for both comparations. In conclusion, in PCOS cases, serum MDA, insulin, FSH, LH and TSH levels were found to be elevated while the levels of antioxidant vitamins were lower compared to healthy pregnant women. Unusual hormonal imbalance and increase of oxidative stress markers during the pregnancy might be important to establish the PCOS diagnosis.

Keywords: PCOS, pregnancy, oxidative stress, MDA, hormone

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Introduction

Polycystic ovarian syndrome (PCOS) is characterized as an endocrine disorder that occurs during reproductive age and occurs due to oligo-anovulation and hyperandrogenism [1,2]. PCOS is now considered as a metabolic disorder where the patient suffers from complexity like insulin resistance, impairment in glucose tolerance, dyslipidemia, and increase in various cardiovascular risk factors [3]. Several studies found that insulin resistance with compensatory hyperinsulinemia is closely associated with PCOS development [4-7]. In addition, women with PCOS possess higher body mass [8-11]. PCOS's initial hypothesis described the elevated level of intrauterine androgen as a hallmark of the disease. Oxidative stress in the follicular environmental fluid may cause pathological condition such as inadequate oocyte development, impaired embryo development, and overall consequences of the pregnancy period [12]. PCOS can be explained as a genetic predisposition and environmental influence characterized by the excessive yield of androgens [13].

The mechanism of how oxidative stress has role on PCOS is not clearly understood, but studies suggest that insulin resistance plays a crucial role in the PCOS pathogenesis, accelerating oxidative stress [14]. A very close association between oxidative stress and PCOS has been observed [15,16]. MDA is a significant aldehyde formed by the metabolism of lipid hydroperoxides, which turns out to be a vital biomarker to determine lipid peroxidation level [17]. Malondialdehyde (MDA) is a critical biomarker for determining oxidative stress under any clinical circumstances [19]. It is a very reactive and potentially toxic compound and reacts with thiobarbituric acid that has been explicitly used as a potential biological marker for the peroxidation of lipid of omega-3 fatty acids [18,19]. MDA is a significant indication of lipid peroxidation, which may be generated due to the oxidation of lipid [20]. A previous study proved the role of circulating oxidative stress markers in inducing PCOS and the levels of the markers were elevated in PCOS patients compared to healthy individuals [21] but never observed in pregnant women with PCOS.

Hormone imbalances are associated with PCOS. In healthy women, follicle-stimulating hormone (FSH) detects ovarian follicles and stimulates the growth of small follicles (2-5 mm) and in the late follicular stage (6-8 mm) develop aromatase activity and enhance inhibin-B action which in turn reduce FSH levels [22]. In PCOS patients however these circles are disturbed leading to elevated luteinizing hormone (LH) and FSH leading to accumulating antral follicles that distinguish fast and undergo premature growth halt of follicle [23]. As sex hormones, female gonadotropins are interrelated, but their association in the procession of oxidative stress and lipid peroxidation has never been studied in pregnant women with PCOS. We aimed to determine any possible alteration in oxidative stress biomarkers along with female reproductive hormones and their role in the progression of PCOS, in comparison to normal healthy pregnant women.

Methods

Study design and blood sample collection

A case-control study was conducted at Department of Gynecology and Obstetrics, Shaheed Suhrawardy Medical College, Dhaka, Bangladesh, between January and July 2019. The pregnancy women with PCOS and healthy pregnant women as control groups were recruited. A control was kept as age and sex matched with the case. Both groups were well distributed from each trimester of pregnancy. Each PCOS case was diagnosed as PCOS before pregnancy and was further confirmed by measuring ovarian volume and ultrasound. All subjects did consume any antioxidants or antioxidant supplementation. Women with diabetes, hypertension, hypotension, weight loss, cancer, respiratory disease, thyroid disorder and those taking any medication were excluded from the study. Prior to enrolment and blood withdrawal, all PCOS patients and controls provided written informed consent. Subjects from both groups filled up a set of questionnaires collected some demographic information, biographical information (height, weight, age).

Five ml of venous blood was collected from each case and control after fasting overnight. The blood samples were preserved at room temperature unstirred for about 1 hour to clot and then centrifuged at 3000 rpm for 15 minutes for serum extraction. The serum samples were then stored at -80°C till used.

Determination of serum MDA level

The serum MDA was quantified according to the procedure described by Kei (1978) [24] with slight modification where thiobarbituric acid (Merck, Germany) was used. Briefly, 0.5 ml of serum was mixed with 2.5 ml of 20% trichloroacetic acid and endured for 10 min at room temperature. Then the mixture was centrifuged for 10 min at 3500 rpm and 2.5 ml of 0.05 M sulphuric acid was added. The supernatant's absorbance was analyzed at 530 nm by using a spectrophotometer, and the concentration of MDA was expressed in unit nmol/mL.

Determination of vitamin A and C

To determine the level of serum vitamin A, a UV-Vis detector was used at absorbance 291 nm as described previously [25]. To determine the vitamin C level, the serum was mixed with 5% trichloroacetic acid (Guangzhou HIRP Chemical Co., Ltd, Guangdong, China), then centrifuged at 3000 rpm for 10 min. The concentration of vitamin C was measured as described elsewhere [26]. The absorbance was quantified at 520 nm using phenylhydrazine-based UV-spectrophotometer (UV-1201, Shimadzu, Japan).

Determination of serum level of insulin, FSH, LH and TSH

To measure the insulin, the Insulin Human ELISA kit from Thermo Fisher Scientific (Catalog #KAQ1251) was used following the manufacturer' protocol. To determine serum FSH and LH, a Human FSH ELISA kit (Catalog #EH202RB, Thermo Fisher Scientific) and a human LH ELISA kit (Catalog #EHLH, Thermo Fisher Scientific) were used, respectively and the procedures were carried out according to the manufacturer protocols. Serum TSH level was measured using a previously established procedure [27].

Ovarian volume measurement

In all the subjects, the ovary size was measured using an ultrasound (both left and right) by calculating the highest plane of ovaries in 2D view and then positioning the vaginal probe at a 90° angle to obtain the third measurement. The ovary volume was determined using the formula [length × height × width × π/6] [28].

Statistical analysis

The levels of vitamin A, vitamin A, MDA, and hormone concentrations were presented as mean ± standard error of the mean (mean ± SEM) and the independent samples t-test was used to compare their levels between pregnant women with PCOS group and control group. A Pearson's correlation was employed to assess the correlation between two variables within PCOS group and control group. SPSS ver. 20.0 (Armonk, NY: IBM Corp.) was used for all statistical analyses.

Results

Characteristisc of the samples

This study recruited 80 pregnant women with PCOS and 80 healthy pregnant volunteers as control group. The characteristics patient and control group are presented in **Table 1**. The mean age of patients and of control was 27.17 years and 27.90 years, respectively. There was no different of gestational weeks and hemoglobin concentration between PCOS and control group. There was a slight difference in body mass index (BMI) between case and control group (28.08±0.53 *vs.* 26.01±0.29, $p<0.05$). The ovarian volume was significant larger in PCOS patients compared to healthy group (14.52 cm^3 *vs.* 8.22 cm^3).

Comparation of oxidative stress markers and hormonal

The mean level of serum MDA was significantly higher in pregnancy women with PCOS compared to healthy pregnancy control group (1.98 *vs.* 1.06 nmol/mL, $p<0.001$). The level of

vitamin A (0.45 *vs.* 1.05 µmol/L), vitamin C (0.26 *vs.* 0.52 mg/dL), however, were significantly lower in PCOS patients in comparison to the normal healthy individuals (p<0.001 for both comparations) (**Table 1** and **Figure 1**).

Table 1. Sociodemographic and clinical characteristics, biochemical, and hormonal parameters of pregnant control and pregnant PCOS patients

Parameters	PCOS patients (mean ± SD)	Healthy control (mean ± SD)	p-value
Number of subjects	80	80	-
Age, years	27.17 ± 0.87	27.90 ± 0.79	0.538
Body mass index, kg/m^2	28.08 ± 0.53	26.01 ± 0.29	0.001*
Hemoglobin, mg/dL	10.11 ± 0.24	10.73 ± 0.22	0.067
Gestational weeks	28.47 ± 1.31	29.42 ± 1.35	0.617
Ovarian volume, cm^3	14.52 ± 0.35	8.22 ± 0.15	<0.001**
Malondialdehyde (MDA), nmol/mL	1.98 ± 0.07	1.06 ± 0.02	<0.001**
Vitamin A, µmol/L	0.45 ± 0.01	1.05 ± 0.01	<0.001**
Vitamin C, mg/dL	0.26 ± 0.01	0.52 ± 0.02	<0.001**
Follicle-stimulating hormone (FSH), IU/L	13.03 ± 0.39	1.75 ± 0.10	<0.001**
Luteinizing hormone (LH), IU/L	15.67 ± 0.63	3.65 ± 0.16	<0.001**
Thyroid-stimulating hormone (TSH), mIU/L	2.79 ± 0.22	2.34 ± 0.06	0.048*
Insulin, mIU/L	11.15 ± 0.25	6.67 ± 0.25	<0.001**

* Statistically significant at p=0.05
** Statistically significant at p=0.001

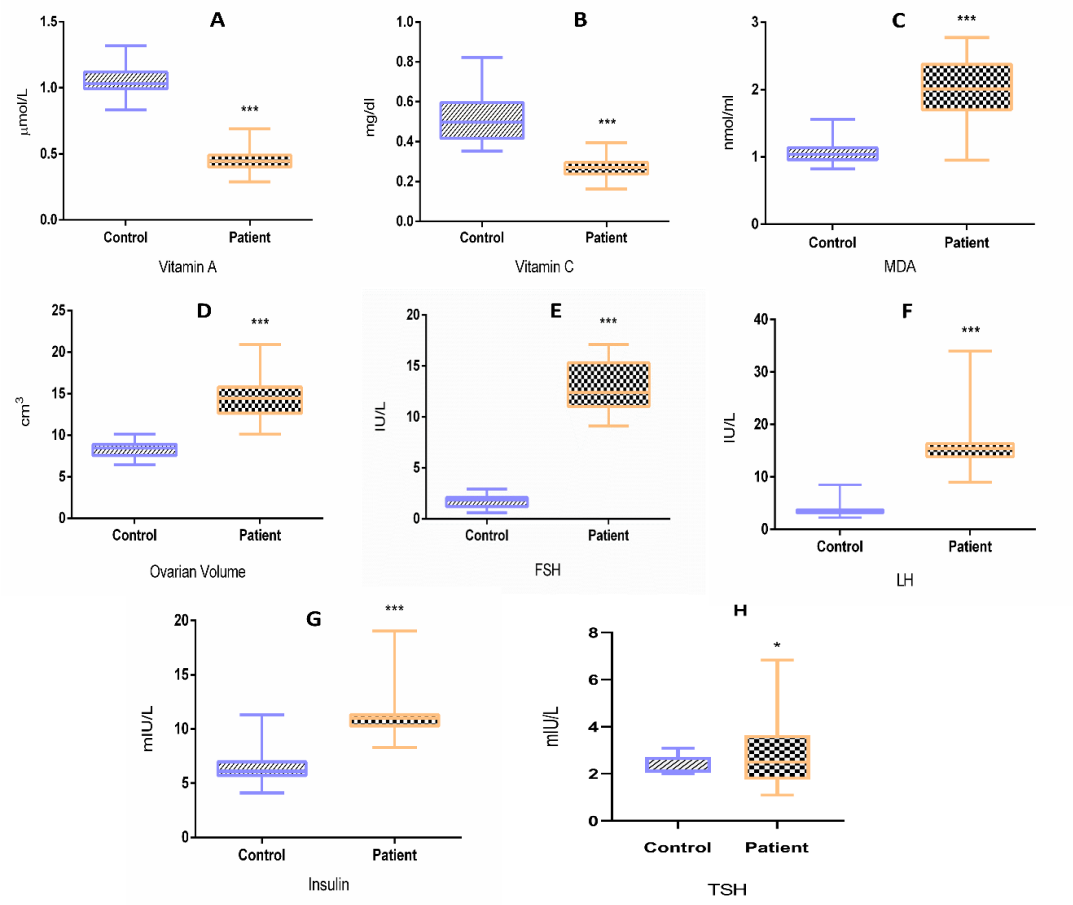

Figure 1. Comparation of serum oxidative stress marker, antioxidant and hormonal between pregnancy women with polycystic ovarian syndromes (PCOS) and healthy pregnancy. Compared to healthy control, in PCOS patients there is a reduction of vitamin A level (A), reduction of vitamin C level (B), elevated of MDA level (C), increased of ovarian volume (D), elevated of FSH level (E), elevated of LH level (F), increased of insulin level (G) and decreased serum of TSH level. * Statistically significant at p=0.05; *** Statistically significant at p=0.001.

Our data suggested that the level of FSH (13.03 *vs.* 1.75 IU/L), LH (15.67 *vs.* 3.65 IU/L), TSH (2.79 *vs.* 2.34 mIU/L) and insulin (11.15 *vs.* 6.67 mIU/L) were statistically significant higher in pregnancy women with PCOS compared to healthy pregnancy control group and all with $p<0.05$ (**Table 1** and **Figure 1**).

Correlation analysis

To understand the inter-parameter relationship, a Pearson correlation test was performed within PCOS patient and control group. The results of the correlation analyses are presented in **Table 2**. In PCOS group, there was a statistically significant positive correlation between the ovarian volume and the level of FSH ($r=0.796$, $p<0.001$). As expected, there was a negative correlation between the level of MDA and vitamin C in PCOS group but not in control group. There was no other significant correlation between parameters within PCOS group (**Table 2**).

Table 2. Correlation study among various study parameters for control and patient groups

Parameters	Healthy pregnant control		PCOS patients	
	Correlation co-efficient	p-value	Correlation co-efficient	p-value
Ovarian volume and MDA	0.026	0.875	-0.139	0.392
Ovarian volume and Vitamin A	0.195	0.228	-0.109	0.505
Ovarian volume and Vitamin C	0.126	0.439	0.009	0.954
Ovarian volume and FSH	-0.214	0.185	0.796	<0.001**
Ovarian volume and LH	-0.066	0.688	0.309	0.052
Ovarian volume and TSH	-0.220	0.172	0.004	0.982
Ovarian volume and Insulin	0.199	0.219	0.301	0.059
MDA and Vitamin A	-0.87	0.593	-0.307	0.054
MDA and Vitamin C	-0.114	0.485	-0.611	<0.001**
MDA and FSH	-0.322	0.043*	0.023	0.889
MDA and LH	0.132	0.418	-0.248	0.122
MDA and TSH	-0.077	0.638	-0.060	0.711
MDA and Insulin	-0.018	0.912	0.049	0.762
Vitamin A and Vitamin C	0.086	0.597	0.079	0.626
Vitamin A and FSH	0.017	0.917	0.040	0.805
Vitamin A and LH	-0.194	0.231	-0.111	0.497
Vitamin A and TSH	-0.134	0.411	-0.066	0.684
Vitamin A and Insulin	0.022	0.893	-0.170	0.293
Vitamin C and FSH	0.122	0.452	-0.126	0.439
Vitamin C and TSH	0.253	0.115	0.052	0.749
Vitamin C and LH	-0.278	0.083	0.275	0.086
Vitamin C and Insulin	-0.060	0.715	0.080	0.624
FSH and LH	-0.193	0.234	0.138	0.396
FSH and TSH	-0.269	0.093	0.001	0.995
FSH and Insulin	-0.150	0.356	-0.187	0.248
LH and TSH	-0.108	0.506	-0.006	0.968
LH and Insulin	0.112	0.491	-0.088	0.591
TSH and Insulin	0.293	0.066	0.090	0.582

* Statistically significant at p=0.05
** Statistically significant at p=0.001

Discussion

PCOS is responsible for anovulatory infertility [29] where oxidative stress plays a negative impact on women's fertility by preventing implantation, embryo development, fertilization, and ovulation [30]. Studies have found that oxidative stress is more predominant in PCOS women than normal or control cases [31,32]. Our present study suggested elevated levels of MDA and low levels of vitamin A and vitamin C in pregnant PCOS patients compared to normal subjects. Our study also found the elevated level of essential hormones in PCOS patients such as LH, FSH, and insulin in comparison to control individuals. The development of PCOS is very complex and PCOS is found to responsible for early pregnancy loss (EPL) comparing to normal pregnant women [33]; even more pregnant PCOS women are at a high risk of premature delivery, gestational diabetes mellitus, and pregnancy related hypertensive disorders [34].

MDA, the most common metabolic end-product that is generated at the time of lipid peroxidation, plays a vital role in oxidative stress. Despite being harmful, ROS sometimes can provide a beneficial effect on the physiological process [35]. For instance, releases of pro-inflammatory cytokines and cellular damage are triggered due to oxidative stress [36]. Further, when the MDA level rises then it may help to stimulate the phospholipase A2 and interrupt cellular membrane integrity. In our study, we have observed that the MDA level was elevated in PCOS patients in comparison to controls. Similar results have been reported where increased MDA level also associated with hyperglycemia and insulin resistance [37]. Our results also are similar with previous studies [38–40]. However, a study has reported no significant difference [41]. In our observation, we also found elevated of insulin level, suggesting that PCOS patients are at risk of developing type-2 diabetes and insulin resistance [42].

Vitamin C plays a significant role in human physiological process such as inhibiting lipolysis, modulating lipid accumulation, interfering with adipocyte-macrophage crosstalk, and finally scavenging reactive oxygen species [43]. Both peritoneal fluid and endometrial tissue of PCOS patients contain a low level of vitamin C in comparison to normal pregnant women indicating, PCOS patients are more prone to oxidative stress [44]. It has been found that oxidative stress is responsible for more than 50-60% of recurrent pregnancy loss [45]. Our current study demonstrated that the serum vitamin C levels in PCOS were significantly lower than that of the control group, which is similar to that of the previous results [46-48]. A statistically substantial negative correlation has been observed between MDA and vitamin C, which indicates that when MDA level rises, then vitamin C level depletes, and our data are in line with the previous studies [16,49,50].

Retinaldehyde is the initial metabolite of vitamin A that consists of the heme and rhodopsin [51]. The other metabolite is retinoic acid, a lipid-soluble biomolecule that helps in gene expression with a receptor-mediated process. During pregnancy, the recommended dietary vitamin A boost is 770 mcg per day. Our present study found a significant lower of serum vitamin A levels in PCOS patients compared to normal pregnant which may be due to the oxidative stress in the pregnant PCOS patients. Previous studies found no relationship between low vitamin A level and intrauterine growth retardation [52–54]. However, a British study has observed a correlation between anthropometric indexes and birth weight [55]. Therefore, there might be a critical association with vitamin A levels with PCOS's progression and etiology during pregnancy requiring further investigation.

Generally, plasma FSH levels are minimized during pregnancy for elevated levels of estrogen and progesterone are observed but abnormally elevated levels of FSH are observed in the case of pregnant PCOS patients. After ovulation, there is a minor role of FSH and ovarian FSH receptor (FSHR) for a successful outcome in pregnancy, but studies suggest the role of extra ovarian FSHR on the female reproductive tract [56]. For pregnant PCOS patients, elevated FSH are found to be positively correlated for the increment of ovarian volume. Our study revealed elevated FSH and LH levels in pregnant PCOS patients where they have decreased conception rate, and the increment of miscarriage were observed for PCOS women compared to normal women with standard LH levels [57]. A study also showed that women who have elevated levels of LH are more prone to miscarriage comparing to normal healthy women and women with PCOS [58]. Restored LH levels in PCOS women could increase the ovulation rates and pregnancy induction, proving the debilitating role of elevated LH for women [59]. Considering the above evidence, LH concentration is associated with healthy pregnancy outcomes and a critically important risk factor diagnosis and treatment target for pregnant PCOS women.

As increased LH surge is observed in PCOS patients, this hyperandrogenic condition in theca cells of the ovary is augmented by increased insulin production, which is a response to PCOS [60]. Women with PCOS mostly have type-2 diabetes, insulin resistance, elevated inflammation, and obesity, where all and/or either of this evidence is prevalent [61]. In our study, increased levels of serum insulin were observed, and this hyperinsulinemia condition contributes to obesity development in PCOS patients [62]. Impairment in glucose uptake

resulted in blastocyst apoptosis, and this impaired glucose uptake responsible for Insulin-like growth factor 1 receptor downregulation [63].

Moreover, elevated risk of preeclampsia and gestational diabetes are closely linked with PCOS condition [64]. TSH levels are generally raised in women with PCOS condition where TSH levels of above 2.5 mIU/L can significantly increase spontaneous pregnancy loss at the first trimester with complications in mother and child [65]. PCOS patients express their prevalence of metabolic problems, dyslipidemia, and insulin resistance [66–68]. Elevated TSH levels in PCOS women were also positively correlated with systolic blood pressure, fasting insulin, HDL, and total-cholesterol/HDL ratio compared to PCOS women with normal TSH values [69]. Our data support previous evidence of increased TSH levels in PCOS subjects [70] and for pregnant PCOS women [70], resulting in an increased risk of pregnancy loss, insulin resistance, and type 2 diabetes, suggesting further clinical investigation over other biochemical parameters. PCOS women with pregnancy must be under the intervention of hormonal monitoring, and also increment in MDA in pregnant PCOS patients influence the progression of PCOS where these parameters might be used as a pathological lead. Dietary supplementation with antioxidant is suggested for minimizing clinical conditions and complications of pregnant PCOS patients.

There are some limitations of this study. Our study did not correlate any metabolic diseases with PCOS patients and the role of nutrition in disease prognosis should be carried out. Moreover, we did study on a small number of population and we did not interlink racial differences with PCOS. For in-depth understanding of the disease, a large number of study-subject and samples from various races might be important.

Conclusion

There is an increase in oxidative stress and gonadotropin hormones in pregnant PCOS patients, including some metabolic dysfunction. Increased MDA, insulin, TSH, LH, FSH, and minimized vitamin A, C levels are evident and highly associated with pregnant PCOS women.

Ethics approval

The research protocol study was approved by the Ethical Committee of Manarat International University (BPM-14340/2019). The principles of the Helsinki declaration were strictly followed in this study.

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Conflict of interest

The authors declare that they have no competing interests.

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Underlying data

Derived data supporting the findings of this study are available from the corresponding authors on request.

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