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LEVELS OF ENZYMATIC ANTIOXIDANT SUPEROXIDE DISMUTASE AND NON-ENZYMATIC ANTIOXIDANT URIC ACID IN POLYCYSTIC OVARY SYNDROME PATIENTS IN A TERTIARY CARE HOSPITAL OF ASSAM

Biochemistry

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is one of the most common hormonal disorders, occurring in 5–10% women in reproductive ages. Despite a long history of studies on PCOS, its aetiology is still unknown. Oxidative stress is now recognized to play a central role in the pathophysiology of many different disorders, including PCOS. Although intracellular reactive oxygen species (ROS) production and propagation are controlled by highly complex antioxidant enzymatic and non-enzymatic systems, understanding of mechanisms that oxidative stress is important to develop strategies for prevention and treatment of PCOS. **Objectives:** To estimate the serum levels of superoxide dismutase and uric acid in PCOS patients. **Methodology:** A case-control study was conducted in a tertiary care hospital of Assam, with 60 PCOS patients attending the outpatient Department of Obstetrics and Gynaecology, Gauhati Medical College and Hospital (GMCH), Guwahati, Assam along with 60 healthy controls of same age group. The samples were evaluated by ELISA and VITROS 5600 Autoanalyser using standard kits and reagents in the Department of Biochemistry, GMCH. **Results:** The results were statistically analysed and the mean and SD of SOD were found to be 969.95 + 62.96 among the cases and 1298.8 + 137.8 among the controls with p value <0.001, whereas the mean and SD of uric acid was 5.41 + 0.70 in cases and 3.76 + 0.74 in controls with p value <0.001.**Conclusions:** Significantly lower serum levels of SOD and significantly higher levels of Uric acid were found in cases with PCOS compared to the control group.

KEYWORDS

Antioxidant, PCOS, SOD, Uric acid

INTRODUCTION

Polycystic ovary syndrome is a complex multifactorial endocrinopathy. This affects a substantial population of reproductive aged women. It is the most common cause of infertility [1]. The main features of PCOS may be developed by genetics and lifestyle [2]. Women diagnosed with PCOS are usually presented with hyperandrogenism, ovulatory dysfunction and polycystic ovaries [3]. Obesity and insulin resistance frequently occur in women with PCOS, increasing the risk for metabolic complications such as type2 diabetes, hypertension, hyperlipidemia, fatty liver and encourages oxidative stress [4].

It occurs in 5 to 10% of women of reproductive age [5]. There is increasing evidence in literature suggesting the role of oxidative stress in PCOS leading to mitochondrial and genomic DNA damage, thereby leading to reduced fertility [6]. Oxidative stress is commonly referred to as the condition in which imbalance between the anti-oxidants occurs which leads to the formation of an excessive quantity of reactive oxygen species (ROS). Several studies have shown that change in oxidative stress markers in women with PCOS as compared to normal women and play an important role in the pathogenesis of PCOS.

Superoxide dismutase (SOD) has been commonly identified as an enzymatic antioxidant that plays an important role in the elimination of superoxide anions. It is a dismutase enzyme that catalyses the conversion of superoxide to hydrogen peroxide and elemental oxygen and help protect oocyte and embryo [7].

Uric acid (UA) is an organic acid which is produced during the period of purine nucleotide metabolism. Elevated serum uric acid (SUA) is closely associated with metabolic disorders, including insulin resistance, obesity and metabolic syndrome. Serum UA accumulation is closely related to ovarian ovulation disturbances. High uric acid in patients with PCOS was also observed to be accompanied by significant levels of oxidative stress, especially in the presence of obesity [8]. These patients tend to have significant elevations in oxidative stress biomarkers, including NO, malondialdehyde, and many adaptive changes in antioxidative stress markers [9]. The imbalanced oxidation state caused by the accumulation of UA exacerbates the occurrence of inflammation. In the inflammatory state of PCOS, monocytes release more ROS due to hyperglycaemia, which then reactivates cytokines such as TNF-α and inflammatory transcription factors. Finally, in this vicious cycle, UA mediates the development of insulin resistance and hyperandrogenaemia [10] In order to address the problems with PCOS diagnosis and to better understand its pathophysiological mechanism, we performed this study to investigate the biomarker potential of serum superoxide dismutase and uric acid in the clinical identification of PCOS.

Literature suggests conflicting results in association between the level of SOD and uric acid in PCOS. Few of the studies have shown increased levels of SOD in patients with PCOS as compare to control [11] in contrast to this finding, some studies have shown a lower level of SOD in subjects with PCOS [12,13]. Some studies have shown increased level of uric acid in PCO--S as compare to control [14].The differences between the methodologies used in the biochemical measurement, selection of cases and control, differences in the study settings may explain the inconsistent results shown in the literature. It is important to determine the precise association between different parameter levels and women with PCOS as it could be used as an important biomarker for PCOS pathogenesis and also help in the optimal treatment of women with PCOS.

METHODS AND MATERIALS

A case-control study was conducted in a tertiary care hospital of Assam, with 60 PCOS patients attending the outpatient Department of Obstetrics and Gynaecology, Gauhati Medical College and Hospital (GMCH), Guwahati, Assam along with 60 healthy controls of same age group. The samples were evaluated by ELISA and VITROS 5600 Autoanalyser using standard kits and reagents in the Department of Biochemistry, GMCH.

The study was carried out between July 2021 and June 2022. The diagnosis of PCOS was based on the Rotterdam criteriawith the presence of two of the three following features :hyperandrogenaemia, oligo or amenorrhea and polycystic ovaries by ultrasound imaging .

Inclusion Criteria

Patients attending outpatient department of Obstetrics & Gynaecology Department of Gauhati Medical College and Hospital, Guwahati.

Clinically and Ultrasonographically diagnosed cases of Polycystic ovarian syndrome were included in this study.

Exclusion Criteria

IncludePregnancy, Congenital adrenal hyperplasia, Adrenal/ovarian tumors, Diabetes mellitus, Hypothyroidism, Cushing disease, Oral contraceptive use etc.

This study was approved by GMCH Ethical committee and informed consent was obtained from patients.

Chemicals And Reagents

Superoxide dismutase ELISA kit were used product of Elab science and uric acid reagent were used which was product of Ortho clinical diagnostic, USA.

Equipment

Centrifuge machine (REMI T_8), Micropipettes: fixed and variable volume were used, VITROS 5600, Thermo Scientific MultiscanFC, well wash Versa ELIZA,Microtitre plate washer,Vortex type Mixer,Distilled water.

Collection Of Serum Samples

About 5 ml blood was collected from the ante-cubital vein after proper aseptic and antiseptic precautions.The sample was collected in sterile evacuated vial (SEV).

Content of SEV was allowed to clot for 30 – 40 minutes. After clot formation, the sample was subjected to centrifugation at 5000 rpm for 5 minutes. After centrifugation the serum portion was collected and the relevant tests were performed.

Storage Of Sample

The vials containing serum samples were labeled properly and stored at – 20 degree celsius, if estimations were not done within 8 hours of collection of the blood.

Biochemical Analysis:

Estimation Of Superoxide Dismutase (SOD)

The ELIZA kit used in this study uses the competitive ELIZA principle. The micro ELIZA plate provided in this kit is precoated with Human SOD1. During the reaction, Human SOD1 in the sample or standard competes with the fixed amount of Human SOD1 on the solid phase supporter for sites on the Biotinylated Detection Ab specific to human SOD1. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horse Radish peroxidase (HRP) are added to each microplate well and incubated. TMB substrate solution is then added to each well. The enzyme substrate reaction is terminated by addition of stop solution and the colour change is measured spectrophotometrically at a wavelength of 450nm ± 2nm. The concentration of human SOD1 in the samples is then determined comparing with the optical density of samples of the standard curve.

Estimation Of Uric Acid

The standard orthoclinical Diagnostic reagent kit used in this study in VITROS 5600 Fully automated autoanalyser based on reflectance spectrophotometry.

Statistical Analysis

Data were collected and handled as per standard guidelines. The data obtained from the case and control subjects were compared for their serum levels and were analyzed further to differentiate between the serum levels of SOD and uric acid between the case and control groups with a maximum sensitivity and minimum false positivity. Data were first analysed for their pattern of distribution and were found to follow normal distribution pattern as determined by the Smirnov Kolmogorov test. For the comparison between two data groups student t test was performed. All statistical analyses were performed using MedCalc software version 20.115 for Windows. For all statistical analysis P value was considered to be significant at a value of less than 0.05 for a 95% confidence interval.

RESULT

Table 1: Levels Of Sod And Uric Acid In Control And Pcos Patients.

Parameter	Control	PCOS
SOD(pg/ml)	1298.8 + 137.83	969.95 + 62.95
Uric acid(mg/dl)	3.76 + 0.74	5.41 + 0.70

Values Are Presented As Mean ± SD.

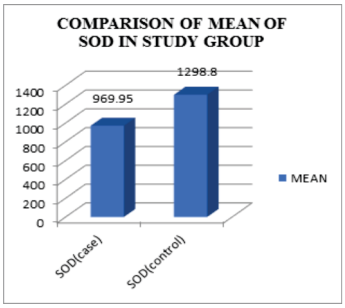

Fig1: Comparison Of Mean Of Superoxide Dismutase (SOD) In Study Group

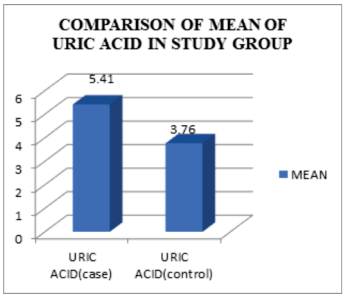

Fig 2: Comparison Of Mean Of Uric Acid In Study Group

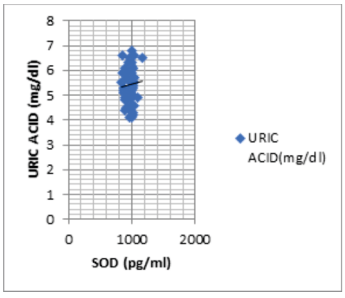

Fig 3: Correlation Between Sod And Uric Acid

Table 2: Correlation Between Antioxidant Parameter Of Pcos Patients.

	Uric acid
SOD	0.061

Values Are Presented As Pearson Correlation®.

Table 1 showed significant decrease ($p<0.05$) in SOD concentration in PCOS patients while there were significant increase ($p<0.05$) in the level of uric acid in PCOS patients compared to the healthy controls. However, the age of the PCOS patients showed no significant difference ($p>0.05$) when compared with the healthy controls.

Table 2 showed there was no correlation between SOD and uric acid with $p>0.05$.

DISCUSSION

PCOS is a common endocrine andreproductive syndrome that is frequently associated with obesity, insulin resistance, metabolic disorders, and cardiovascular risk factors . The etiology of this syndrome remains largely unclear, but increasing evidence suggests that PCOS might be that of a complex multigenic trait in which environmental influences play an important role, such as organic compounds and heavy metals. Polycystic ovary syndrome is a major cause of anovulatory infertility. Oxidative stress can have detrimental effects on female fertility by affecting ovulation, fertilization, embryo development and implantation.

The present study was conducted among the sixty diagnosed cases of polycystic ovary syndrome and apparently normal healthy individual attending the Tertiary care hospital Assam. The levels of Superoxide dismutase and uric acid were evaluated and the possible correlations of Superoxide dismutase and uric acid were evaluated.

In the present study, the mean ± SD of SOD in the case and control

groups were 969.95 ± 62.95 pg/ml and 1298.8 ± 137.83 pg/ml respectively(Table1). This was also represented as bar diagram (Figure 1). The mean SOD level in case group is significantly decreased than in the control group with the p value <0.05. The difference is statistically highly significant.

Our result matched with the findings of Zhang et al. who expressed that the serum SOD levels in PCOS patients were significantly lower than those of controls.The decreased in SOD activity in this study may be connected to increased utilisation of SOD to scavenge produced ROS.Not matched with findings of a study conducted by Sabuncu et al, they showed that women with PCOS had higher SOD levels than normal subjects.

Higher level of SOD may be due to compensatory increased of antioxidant against high oxidative stress.

The mean±SD of uric acid in the case and control groups were 5.41±0.70 mg/dl and 3.76±0.74 mg/dl respectively(Table 1).This was also represented as bar diagram (figure 2). The mean uric acid in case group is significantly increased than in the control group with p value <0.05. The difference is statistically highly significant.This outcome was matched with the findings of Liangshan Mu et al. who found that the serum uric acid levels in PCOS patients were significantly higher than those of controls.Our findings are also matched with findings of Yan-NanLiu et al.

The high level of uric acid is linked to reproductive health in PCOS patients, mainly through oxidative stress, inflammation, insulin resistance and mitochondrial dysfunction.

There was no correlation between SOD and Uric acid with p>0.05.

CONCLUSION

It was found that patients with PCOS have lower level of Superoxide dismutase than healthy control. It was also found that patients with PCOS have higher level of uric acid than healthy control.

An attmpt was made to find the correlation of superoxide dismutase and uric acid where we have found that there was no correlation between SOD and uric acid.

A more elaborate study would have been desirable to precisely establish the role of superoxide dismutase and uric acid in diagnosis and treatment of PCOS , but due to paucity of time , resources and due to conduction of the study in a single institution it was not implementable. However we made a model effort to fulfil the same through whatever resources accessible to us . And the results of the study tally with most of the studies conducted in foreign countries as well as with the few conducted in india. Hence it is hoped that the present study will encourage further studies on the present topic in a bigger way.

A humble attempt has been made , which if followed would prove useful to the polycystic ovary syndrome patients. Further studies for generating novel ideas and designing studies to address queries related to its real significance have to be carried out.

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