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Evaluation of Oxidative and Antioxidant Markers in Unexplained Infertile Women: A Case-Control Study

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ABSTRACT

Background: Unexplained infertility (UI) affects approximately 30% of infertile couples. Recent data indicate that oxidative stress (OS) may contribute to idiopathic reproductive failure in women. This study aims to assess oxidative stress markers and antioxidant enzyme levels in women with UI as compared to fertile controls.

Methods: This case-control study was conducted from April 2023 to March 2025 at King George's Medical University, Lucknow, India, involving 200 women (100 diagnosed with UI and 100 age-matched fertile controls). After clinical evaluation and routine infertility workup, blood samples were analyzed for oxidative stress marker malondialdehyde (MDA) and catalase (CAT) by ELISA kit respectively, and three antioxidant enzymes: glutathione peroxidase (GPx), superoxide dismutase (SOD), and glutathione reductase (GR) by Assay kit as per manufactured kit by using a microplate reader.

Results: There was an insignificant association found in age between UI and control. MDA levels were significantly elevated in UI patients (96.7±41.14 ng/ml) compared to controls (201.14±92.16 ng/ml) ($p<0.05$), indicating increased oxidative stress. CAT levels were also significantly higher in the UI group (18.02±4.61 ng/ml vs. 12.35±3.18 ng/ml), GR levels were significantly lower in UI patients than control (1.306±0.58 mU/ml vs. 1.89±0.979 mU/ml), SOD and GPx levels showed no significant differences ($p>0.05$).

Conclusion: Women with unexplained infertility exhibit an altered oxidative-antioxidative balance characterized by elevated MDA and CAT, and reduced GR levels. These findings support the role of oxidative stress in the etiology of UI and highlight the potential utility of oxidative stress biomarkers in diagnosis and management.

Key-words: Unexplained infertility, Oxidative stress, Marker, antioxidant, Women, World Health Organization

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INTRODUCTION

The condition where a couple is unable to conceive a child after a year of regular unprotected intercourse is known as infertility [1]. Recent studies by the World Health Organization indicate that infertility rates are increasing, with approximately one in six individuals worldwide experiencing infertility at some point in their lives in 2022 [2]. It is estimated that around 30% of infertile couples are diagnosed with UI when no

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abnormalities are found in the reproductive systems of both partners [3].

The diagnosis of UI is usually made after thorough investigations fail to reveal any specific cause. However, there is currently no standardized approach to diagnosing this condition. According to the International Committee for Monitoring Assisted Reproductive Technologies, UI is defined as the inability to conceive in couples with normal reproductive functions, adequate coital frequency, and no detectable abnormalities in the male and female reproductive organs [4].

Research has shown that oxidative stress (OS) plays a significant role in female infertility. OS happens when the body's antioxidant and ROS (reactive oxygen species are out of balance, leading to cellular damage. This imbalance can be caused by an excess of pro-oxidants overwhelming the natural detoxification of body's repair mechanisms [5].

It is believed that during ovulation, inflammatory cells in the ovary release ROS when the follicle ruptures. To protect gametes from the harmful effects of ROS, glutathione reductase and antioxidants convert them into harmless substances [6,7]. The body's antioxidative enzymatic system, which includes SOD (superoxide dismutase) and GR (glutathione reductase), kicks in to counteract oxidative stress caused by ROS [8].

Studies have shown that levels of antioxidants are lower in infertile patients compared to fertile individuals. Furthermore, infertile patients tend to have higher levels of malondialdehyde (MDA), a byproduct of lipid peroxidation and a type of ROS, in their peritoneal fluid [9]. Imbalance in the antioxidative enzymatic system, including enzymes like catalase (CAT) and glutathione peroxidase (GP), can lead to redox imbalance and decreased fertility [10]. In this study, we aim to investigate the oxidative stress parameters in women with unexplained infertility.

MATERIALS AND METHODS

Study Design- This case-control research was done at the Department of Obstetrics and Gynaecology in collaboration with the Department of Biochemistry at King George's Medical University in Lucknow, Uttar Pradesh, India, from April 2023 to March 2025.

Selection of subjects- The study samples were selected from women of reproductive age group attending the outdoor Patients (OPDs) at the Infertility clinic at Queens Mary Hospital, Department of Obstetrics & Gynecology, KGMU, Lucknow, Uttar Pradesh. Cases and controls were chosen based on clinical history, physical examination, and normal husband semen analysis. Subjects meeting the inclusion/exclusion criteria and providing written informed consent were enrolled as cases and controls.

Selection of Cases

Inclusion Criteria for Cases

- Age between 18 - 40 years.
- Females with unexplained infertility for at least one year.

Exclusion Criteria for Cases

- Pregnancy.
- Subjects with endocrine disorders, diabetes mellitus, genital tuberculosis, or chronic medication.
- Subjects with mullerian anomalies.

Selection of Controls

Inclusion Criteria for Controls

- Age between 18-40 years.
- Females with at least one conception.

Exclusion Criteria for Controls

- Hormonal contraception uses within the past year.
- Subjects with illness, endocrine disorders, autoimmune/ immunocompromized conditions, or on chronic medication.

Clinical characteristics, history, and dietary history of patients for data collection- After the complete brief questionnaire to collect data on demographic characteristics, occupational status, living style, medical history, infertility history, family history, contraceptive history, lifestyle factors, dietary habits, smoking history, BMI assessments, A detailed physical examination, including general, systemic, local and gynaecological examination (per speculum and vaginal examination) was done.

Infertility work-up (case)- Infertility work-up was done as per a previous study [11]. Infertile women attending the infertility clinic were recruited in this study after a written informed consent. A detailed history was taken, including Personal history, and previous obstetrics history, including duration of infertility, parity, abortion

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and stillbirth. Menstrual history including menarche, length of cycle, duration, flow, dysmenorrhea and associated symptoms. Previous treatment history for Infertility.

General appearance, including Blood pressure, cardiovascular system, respiratory system, height, weight, BMI, WHR, secondary sexual characteristics, breast examination, galactorrhea, and abnormalities on pelvic examination, is also being taken.

Medical history of mumps, Hormonal investigation status, which includes PRL, thyroid profile, LH, FSH, Estradiol, Testosterone, SHBG, FAI (free androgen index), fasting G/I, and AMH, was being taken.

General investigations like Hb%, TLC DLC, ESR, ABO & RH, Blood sugar fasting and PP, VDRL, HBsAg, HCV, HIV, Platelet count, ANA, and APTT were noted.

Other investigations include HSG for Tubal patency, diagnostic laparoscopy and hysteroscopy (if required) and cervical swab C/s, Baseline USG on D2 of menses (AFC, ovarian volume, stromal flow). Husband evaluation including personal history, medical history, semen analysis with quantity, liquefaction time, total count, morphology, motility, and pus cells. Epithelial cell sperm aggregation, previous treatment history, general investigation and family history were also taken. (*Management of infertility was done as per standard hospital protocol.

Sample Collection- This present case-control study was done after the approval by the Institutional Research Ethics Committee of KGMU. 6 ml Venous Blood sample is being collected on 2nd to 3rd day of the menstrual period from each participant after the written consent form. 2 ml sample in EDTA vial for plasma isolation and Erythrocyte lysate sample for oxidant and antioxidant markers. The entire sample was stored in -20°C deep freezer for further analysis.

Estimation of Oxidative stress and its scavenging mechanism- The assessment of oxidative stress levels was done by using specific kits. MDA and CAT were measured by the Abcam ELISA Kit, respectively, in the Plasma samples according to the manufacturer's instructions. SOD, GPx, GR has been done by Abcam assay kit respectively in the Erythrocyte lysate sample according to the manufacturer kit by using a microplate reader.

Statistical Analysis- IBM SPSS statistical software for Windows, version 22.0, was used to conduct the statistical analysis (IBM Crop, Armonk, NY, USA). Continuous variables were evaluated for normality using the Kolmogorov-Smirnov test. Using the Mann-Whitney U test. Group comparisons were performed for data that did not have a normal distribution. The Pearson Chi-Squire test was used to analyze the relationships between the categorical variables and case/control status after they were reported as frequencies and percentages. Additionally, unadjusted odds ratios (ORs) and their 95% CIs were estimated using univariate binary logistic regression. p-value below 0.05 was regarded as statistically significant. Software such as SPSS and Graph Prism was used to construct the visual representations.

Ethical approval- This present study has been approved by the Institutional Research Ethics Committee of KGMU (2217/Ethics/2023 dated 04/03/23, ref code: 118th ECM IIA/P6).

RESULTS

The study involved a total of 200 participants, split into two groups of 100 each: one group consisted of women with UI while the other group comprised age-matched controls. The average age of the UI patients was 30.02 years with a standard deviation of 4.02, whereas the controls had an average age of 31.15 with a standard deviation of 5.19. Despite the UI group having a slightly lower average age, a t-test showed no significant age difference between the two groups (30.02±4.02 years vs. 31.15±5.19 years, t=1.72, p=0.08). Additionally, there were no significant variations in the distribution of residence between the two groups (p>0.05), indicating successful matching based on age and residence.

Table 1: Age description of the study group

Age	Case (n=100) (n, %)	Control (n=100) (n, %)	OR (95% CI)	p-value
Mean±SD	30.02±4.02	31.15±5.19	T stat: 1.72	0.08
≤25	15 (15)	17 (17)	Reference	
>25	85 (85)	83 (83)	0.86 (0.4-1.84)	0.69

OR: odds ratio; 95% CI: 95% confidence interval; p<0.05

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There was a significantly greater level of oxidative stress, as indicated by higher levels of MDA, observed in the UI case group compared to its respective control groups ($p<0.05$). The average MDA distribution was found to be 201.14±92.16 ng/ml (range: 374.22) in the control group, while it was 96.7±41.14 ng/ml (range: 230.8) in the control group (Fig. 1). The levels of antioxidant markers such as SOD, CAT, GR, and GP were measured in the study. It was observed that the CAT levels were higher in the UI group (18.02±4.61 ng/ml) compared to the control group (12.35±3.18 ng/ml).

On the other hand, the GR levels were significantly lower in the UI group (1.306±0.58 mMol/ml) compared to the control group (1.89±0.979 mMol/ml). The levels of SOD and GP did not show any significant difference between the two groups (SOD: 8.739±2.79 U/ml vs 9.367±2.16 U/ml; GP: 1.89±0.97 mU/ml vs 1.45±0.66 mU/ml) (all $p>0.05$). All the antioxidant parameters are visualized in Fig. 2.

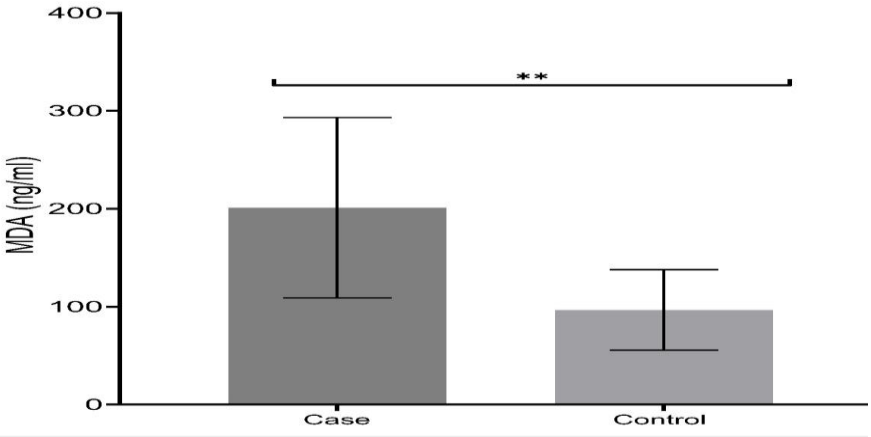

Fig. 1: Evaluation of oxidative stress in case (n=100) and control (n=100)

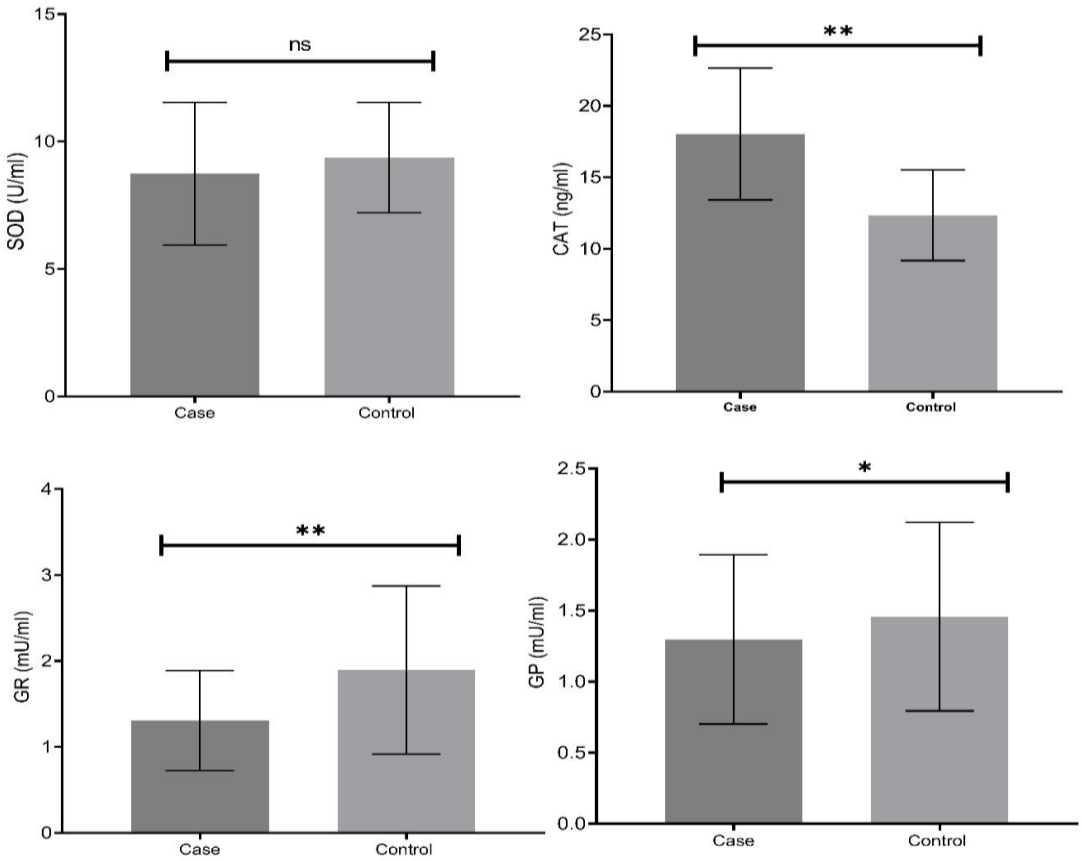

Fig. 2: Evaluation of oxidative stress in case (n=100) and control (n=100)

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DISCUSSION

The current study compared important oxidative and antioxidative indicators between UI patients and healthy controls who have at least one conception with regular menstrual periods to examine the function of oxidative stress (OS) in women with UI. The average age did not differ significantly between the two groups (p=0.08), indicating that age, a known factor influencing redox balance and fertility, did not skew the results, similar to the study done by Youssef *et al.* [12].

According to Verit, numerous etiological factors that may contribute to UI, including impaired oocyte quality, premature ovarian failure, minimal and mild endometriosis, tubal disease, pelvic adhesions, immunological and endocrinological abnormalities and defective endometrial receptivity, have been linked to OS [13]. The significantly higher level of MDA in the UI group when compared to controls was a notable finding in this present study. Similar results were found in some studies done by Rai *et al.* [14], Agarwal and Allamaneni *et al.* [15], Agarwal *et al.* [16], as well as Veena *et al.* [17].

MDA is an important indicator of oxidative damage to cells and lipid peroxidation. Its elevated concentration indicates that UI patients' reproductive environments are more burdened with OS. Similar results were reported by Pasqualotto *et al.*, who discovered that infertile women's serum and follicular fluid had higher MDA levels, indicating a decreased oocyte quality [18].

Given its clinical significance, elevated MDA levels have also been linked to decreased rates of fertilization and implantation in assisted reproduction [19]. Its increased levels in infertile women point to more severe oxidative damage to reproductive organs, especially the endometrial environment and oocytes. These findings are consistent with a previous research study by Agarwal *et al.* [16], which found that women with endometrial or idiopathic infertility had comparable increases in MDA levels [9]. The crucial part that MDA play in reducing follicular quality and embryo viability, which in turn leads to subfertility, was further highlighted by Kataki *et al.* [20].

Apart from MDA, our research revealed that women had considerably greater levels of catalase (CAT) (18±4.61 ng/ml) than controls (12.35±3.18 ng.ml).

A compensatory reaction to increased ROS generation may be reflected in the overexpression of catalase, which detoxifies hydrogen peroxide, a reactive oxygen species. This conclusion is consistent with research by Pasqualotto *et al.*, who found that infertile women had higher catalase activity and hypothesized that this could be a protective mechanism against OS [18].

In contrast, in this present study GR levels were considerably lower (1.306±0.58 mMol.ml) than the controls (1.89±0.979 mol/ml), suggesting a compromised antioxidant defence within the cell and impaired glutathione recycling. Infertility treatment results and decreased oocyte competency have been previously associated with decreased GR activity [13,14]. An accumulation of peroxides due to an improperly functioning glutathione system might exacerbate OS [18]. Some studies concluded that spontaneous conception had good antioxidant status [17,19,20].

GP and SOD levels did not significantly differ between the case and control groups. This could suggest that these enzymes either have little role in the pathophysiological cascade of UI or that regulatory mechanisms keep their levels within normal ranges. Nonetheless, some data indicate that these enzyme's activity may be post-transnationally modified or may change throughout particular menstrual cycle phases [20-25]. A study by Su *et al.* on the association between oxidative balance score and infertility from the National Health and Nutrition Survey 2013-2018 also suggested that increased antioxidants and decreased pro-oxidant exposure may lower female infertility risk [26].

CONCLUSIONS

Women with UI showed significantly elevated OS, as evidenced by higher CAT, MDA and decreased GR activity, compared to fertile controls. This study's finding suggests that OS may contribute to the pathophysiology of UI. Assessment of OS markers could aid in the diagnosis and management of UI for this, we need more interventional research, including a metacentric study with a large sample size.

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