Correlation of oxidative stress and serum gonadotropins in female infertility - A cross sectional study in a tertiary care hospital in Eastern Zone of India

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ABSTRACT

Background: Infertility is a very important issue to family and society. Oxidative stress (OS) may affect ovulation, fertilization, embryo development, and implantation resulting in infertility in women. Gonadotropins are required for follicle development and estrogen production, hence low levels of these hormones may result infertility. Aim and Objectives: Our aim was to study OS and serum gonadotropins level in infertile women and to study whether the OS has any effect on gonadotropins level in infertile women. Materials and Methods: It is a hospital-based cross-sectional study. The study group included 50 infertile women in the age of 20–45 years. Age-matched 50 women without a history of infertility were selected as control. Serum samples were collected on the third day of the menstrual cycle and assayed for carbonylation of serum protein, a marker of OS by Levine’s method and Serum Follicle-stimulating hormone and luteinizing hormone by Chemiluminescence Immunoassay method in ADVIA, Centaur CP (SIEMENS) autoanalyzer. Statistical analysis of data was done by SPSS software. P < 0.05 was considered statistically significant. Results: From our study, we observed that OS was significantly high in infertile women than control (P < 0.05). Serum gonadotropins levels were significantly low in infertile women than control (P < 0.05). Our study shows significant negative correlations between OS and serum gonadotropins level in infertile women (P < 0.001). Conclusion: It can be concluded that both OS and low serum gonadotropin levels may be etiological factors for infertility in women. Oxidative can cause infertility by direct effect on reproduction physiology as well as by lowering gonadotropin level. So OS and serum gonadotropin levels can be emphasized in case of treatment of female infertility.

KEY WORDS: Infertility; Oxidative Stress, Reactive Oxygen Species, Gonadotropin

INTRODUCTION

The infertility has become a common problem amongst women nowadays. The infertile couples suffer from anxiety and shame.[1] Infertility is defined as “a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.”[2] Primary infertility refers those patients who have never conceived and secondary infertility refers those patient who conceived previously but unable to conceive subsequently.[3]

Approximately 85–90% of healthy young couples conceive within 1 year and approximately 10–15% of couples suffer from infertility.[4] The rate has continuously increased over the past 30 years.[5]

As per the survey of the World Health Organization, in developed countries, female infertility accounts for 37% of total infertile couples. The most common causes of female
infertility are ovulatory disorders (25%), endometriosis (15%), pelvic adhesions (11%), tubal blockage (11%), tubal abnormalities (11%), and hyperprolactinemia (7%).[6] Miller et al. also have shown that ovulatory dysfunction (15%), tubal and peritoneal pathology (30–40%), and uterine pathology are responsible for female infertility.[7]

In the human body physiological levels of Reactive Oxygen Species (ROS) are necessary for various functions. Low levels of free radicals play role in female reproductive physiology, like maturation of oocytes, ovulation, fertilization of the ovum, atresia of the follicle, regression of corpus luteum, and formation of corpus luteum in pregnancy.[5]

ROS are treated as a double-edged sword. Though they play role in reproductive physiology but also may be responsible for different pathology in the female reproductive system. The balance of ROS is maintained by the antioxidants system present in the human body. Imbalance between ROS and the antioxidant system may results development of oxidative stress (OS).

Different studies show that OS may influence the entire physiology of reproduction like oocyte maturation, fertilization, and embryo development and thus results infertility.[9-12] Cellular membrane damage, retardation of embryo development may be inevitable due high level of ROS. Adverse effects of ROS are lipid peroxidation, alteration of the primary, secondary and tertiary structures of proteins, and formation of carbonylated protein derivatives via various mechanism such as fragmentation and amino acid oxidation.

ROS can oxidize amino acids side chain and protein backbone. ROS also can result protein-protein cross-linkage. Cross-linking and aggregation of the proteins that occur due to the formation of free radical induce carbonyl adducts have been observed in several studies.[13] ROS can results in modification of base, oxidation of deoxyribose. Other impacts of ROS in DNA are single strand or double-strand breaks, cross-linking of strands, and adducts formation.

Gonadotropins, the follicle-stimulating hormone (FSH), and the luteinizing hormone (LH) both are secreted from the anterior pituitary gland. FSH and LH act synergistically in the physiology of reproduction. In females, FSH initiates follicular growth, especially affecting the granulosa cells and the decline in serum levels in the late follicular phase. FSH is essential for the advanced follicle to proceed to ovulation. LH surge induces ovulation. The FSH levels of 42.9% of infertile women go toward the lower end of the normal range. The LH level of 42.9% of infertile women was below normal. The decreased level of LH in the mid-cycle may be responsible for anovulation and thereby infertility.[14] Hence, it can be hypothesized that there might be some correlation between OS and gonadotropins level in infertile women.

Primary Objectives
To assess the role of OS and serum gonadotropins level and their correlation in infertile women.

Secondary Objectives
1. To study the OS in infertile women and compare it with control
2. To study serum level of gonadotropin hormones in infertile women and compare it with control
3. To study whether the OS has any effect on the serum levels of gonadotropins in infertile women.

MATERIALS AND METHODS
Approval has been taken from the Institutional Ethical Committee and written consents were taken from the participants.

This hospital-based cross-sectional study was undertaken in the Department of Biochemistry and Department of Obstetrics and Gynecology of Bankura Sammilani Medical College and Hospital, Bankura, West Bengal, India which is a tertiary care teaching hospital encompassing the whole Bankura district with some adjacent areas. Fifty infertile women, in the age group of 20–45, attending the Obstetrics and Gynaecology outpatient department, satisfying the inclusion criteria were selected as cases. Age-matched 50 women without a history of infertility were included in the study as control subjects. The study has been conducted for a period of 1 year.

Inclusion Criteria
1. Infertile women in age group of 20–45 years age
2. The infertile women having normal genitalia, uterus, and adnexa by clinical examination and ultrasonography
3. Husband’s semen report showing no abnormality
4. Women with no previous history or documented report of deranged serum gonadotropin levels.

Exclusion Criteria
1. Chronic medical ailment (hypertension, diabetes mellitus, etc.)
2. Abnormal semen analysis report of husband
3. With genital ambiguity, lack of sex differentiation.

Sample Collection
About 10 ml venous blood was collected in clotted vials without any anticoagulant from both cases and controls on
the 3rd day of menstrual cycle accordingly. Then, the collected samples were centrifuged at 3000 rpm speed for 5 min in order to separate serum. All the serum samples were tested within 24 h.

**Assay of Parameters**

Carbonylation of serum protein as a marker of OS was assayed by Levine’s method. Serum FSH and LH were measured by the Chemiluminescence Immunoassay method in ADVIA, Centaur CP (Siemens) autoanalyzer, which is a competitive immunoassay using direct chemiluminescence technology.

**Levine’s Method for Estimation of Carbonylated Serum Protein**[15]

About 1 ml of serum was treated with 0.5 ml of 10% Tri-Chloro Acetic Acid (TCA) to precipitate the protein present in the serum. Then, the mixture was centrifuged at 5000 rpm for 5 min and the supernatant was discarded. Then, 0.5 ml 10 mmol/L 2,4-DNPH in 2 mol/L HCL was added to the precipitate and incubated at room temperature for 30 min. Sample was mixed vigorously at every 10 min. After that 0.5 ml, 10% TCA was added again to the precipitate and centrifuged at 5000 rpm for 5 min. Supernatant was discarded and 0.5 ml 10 mmol/L 2,4-DNPH in 2 mol/L HCL was added to the precipitate and incubated at room temperature for 30 min. Sample was mixed vigorously at every 10 min. After that 0.5 ml, 10% TCA was added again to the precipitate and centrifuged at 5000 rpm for 5 min. Supernatant was discarded and the precipitate was washed with 1 ml protein washing solution (ethanol:ethylacetate = 1:1) three times. Then the precipitate was dissolved in 1.5 ml protein dissolving solution (2 g SDS and 50 mg EDTA in 100 ml of 80 mmol/L phosphate buffer, pH-8) and incubated in water bath at 37°C for 10 min. Now the supernatant was collected and measured spectrophotometrically at 370 nm using 2 mol/L HCL as blank.

The concentration of carbonylated protein can be calculated using Molar Extinction Coefficient of carbonylated serum protein- 21 × 10^3 L/mol. The concentration of carbonylated protein has been expressed in n mol/mg of serum protein.

**RESULTS**

Table 1 shows mean and SD values of age in years of control and infertile group. This table also reflects mean age in years in primary infertility and secondary infertility group. Table 2 shows the result of the Independent sample t-test of age in years between infertile and control group and it is evident that there was no significant difference between mean age of the subjects in infertile and control group (P > 0.05). Moreover, the Levene’s test for equality of variances was not significant (P = 0.301) which indicates that equality of variances were present in both infertility and control groups. Table 3 shows mean and SD values of measured parameters of control group, infertile group, primary infertility and secondary infertility group. Table 4 shows Independent samples t-test of parameters of infertility and control group and it is evident that there are significant differences in means of carbonylation of serum protein, serum FSH, serum LH levels between infertile group and control group (P < 0.001). Table 5 shows that there are no significant differences in means of Carbonylation of serum protein, serum FSH, serum LH levels between primary and secondary infertility group (P > 0.05). Table 6 shows Pearson’s bivariate correlation analysis of the parameters in the infertile group which reveals significant negative correlation between serum level of carbonylation of serum protein and serum FSH (r = –0.381, P < 0.01). Significant negative correlation between serum level of carbonylation of serum protein and serum LH is also evident (r = –0.463, P < 0.01). All the significant negative correlations observed have been displayed by Scatter Diagrams [Figures 1 and 2].

**DISCUSSION**

In our study carbonylation of serum protein levels was significantly higher among the infertile women than the control group (P < 0.001). On the other hand serum level of OS marker do not differ significantly between primary and secondary infertility group (P > 0.05). These findings of our study suggest that OS has an equal impact in both primary and secondary infertility group.

![Figure 1: Scattered diagram showing distribution of Carbonylation of serum protein and serum Follicle stimulating hormone level in infertility group](image-url)

Table 1: Mean and SD values of age in years of control and infertility group. (Infertility group is divided into primary infertility and secondary infertility group)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 50)</th>
<th>Infertility (n = 50)</th>
<th>Infertility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Primary infertility (n = 25)</td>
</tr>
<tr>
<td>Age in years (Mean±SD)</td>
<td>30.16±5.09</td>
<td>28.64±4.63</td>
<td>27.48±4.68</td>
</tr>
</tbody>
</table>

and secondary infertility. In the present study serum levels of FSH and LH are found significantly lower in the infertility group than the control group ($P < 0.001$). However, there are no significant differences of serum FSH and LH levels between primary and secondary infertile group ($P > 0.05$). In our study, we also noticed significant negative correlations between carbonylation of serum protein level with both serum FSH level ($P < 0.01$) and serum LH level ($P < 0.01$) in infertile women. These results indicate a pathological correlation between OS and lower gonadotropin level in female infertility.

The objective of our study was to assess OS and serum gonadotropin level both in infertile women and control group and to find out significant correlations between them in infertile women if any. Our study was based on assumption that OS may be responsible for female infertility and worsen the condition by lowering gonadotropin hormones levels. Gonadotropins are required for normal physiology of reproduction. Measurement of carbonylation of serum protein is a good marker of OS.$^{[16,17]}$ OS has been reported as an etiological event in female infertility by various studies.$^{[12,18]}$ OS may results infertility in women through different mechanisms. ROS can directly damage oocyte and spermatozoa leading to impaired fertilization. Even after fertilization apoptosis may result embryo fragmentation, implantation failure, abortion. ROS can directly affect the embryo in the endometrium.$^{[19]}$ Oxidant-antioxidant imbalance may result luteal regression and insufficient luteal hormonal support to continue pregnancy.$^{[20]}$ Normal levels of FSH and LH are necessary for the physiology of reproduction in female. FSH is required for folliculogenesis in female reproduction physiology. FSH play role in follicle development and with the help of LH, it stimulates follicular growth in the pre-ovulatory phase. LH surge induces ovulation and formation of corpus luteum in female reproductive physiology. Decrease level of gonadotropin may impede follicle development, ovulation, steroid hormone synthesis, corpus luteum formation, resulting in infertility. The findings of our study are in concordance with the findings of some previous studies. In those studies, lower level of FSH and LH were noticed in infertile women in comparison to the control group.$^{[14,21,22]}$ The findings of our study which indicate pathological correlations between OS and lower gonadotropins levels in female infertility are in concordance with some other studies.$^{[23,24]}$ Accumulated lipid peroxides may impede membrane fluidity and integrity, and receptors activities.$^{[24,25]}$ Increase lipid peroxides in OS may disrupt the physiological feedback mechanism in Hypothalamus-Pituitary-Gonadal Axis.$^{[23]}$

The Strength of our study is that the participants were interested in the infertility issue, so they showed interest in the study. However, there are few limitations in our study. Due to time restraint, this study could not be extended to a larger population. We only measure FSH and LH as endocrine parameters. Measurement of FSH, LH, Prolactin, Estrogen, Progesterone as endocrine parameters is required.

Prospective study with antioxidant therapy in infertile women could be conducted to obtain stronger evidences. Moreover, assessment of thyroid hormones should be included, because thyroid dysfunction is known associated with female infertility. Magnetic resonance imaging should also be done for complete diagnosis of the cause of infertility.

![Figure 2: Scattered diagram showing distribution of Carbonylation of serum protein and serum luteinizing hormone level in infertility group](image)

**Table 2: Independent sample t-test between age (in years) of infertility and control group**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>$t$-value</th>
<th>Sig. (2-tailed)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>−1.562</td>
<td>0.122</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Table 3: Mean and SD values of parameters of control and infertility group (Infertility group is divided in to primary infertility and secondary infertility group)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control ($n = 50$)</th>
<th>Infertility ($n = 50$)</th>
<th>Infertility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Primary infertility ($n = 25$)</td>
<td>Secondary infertility ($n = 25$)</td>
</tr>
<tr>
<td>Carbonylation of serum protein (nmol/mg of serum protein) ($\text{Mean} \pm \text{SD}$)</td>
<td>0.90±0.15</td>
<td>1.15±0.14</td>
<td>1.17±0.13</td>
</tr>
<tr>
<td>Serum FSH (mIU/ml) ($\text{Mean} \pm \text{SD}$)</td>
<td>7.20±2.22</td>
<td>5.66±1.92</td>
<td>5.48±1.86</td>
</tr>
<tr>
<td>Serum LH (mIU/ml) ($\text{Mean} \pm \text{SD}$)</td>
<td>5.23±1.26</td>
<td>3.83±1.62</td>
<td>3.62±1.51</td>
</tr>
</tbody>
</table>

FSH: Follicle stimulating hormone, LH: Luteinizing hormone
as this can detect pituitary tumors which may be responsible for endocrine abnormality. A more planned study with greater control over confounding variables is needed for generating stronger evidence.

CONCLUSION

Our study shows that both OS and lower serum gonadotropin level may be etiological factors for infertility in women. OS may be a direct cause for infertility in women or can worsen the condition by lowering gonadotropin level. It is also evident from our study that OS and gonadotropin have similar impact both in primary and secondary infertility. Hence, OS and serum gonadotropin level can be emphasized in the management of both primary and secondary female infertility.

REFERENCES

2. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O. The international committee for monitoring assisted reproductive technology (ICMART) and the World Health Organization (WHO) revised glossary on ART terminology. Hum Reprod 2009;24:2683-87.
21. Olooto WE, Adeleye AO, Amballia A, Mosufo A. Pattern of reproductive hormones (follicle stimulating hormone, luteinizing hormone, estradiol, progesterone, and prolactin) levels in infertile women in Sagamu South Western Nigeria.


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