Oxidative Stress And Anti Oxidant Status In Male Infertility.

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ABSTRACT:
Background: one of the major causes of infertility is a defective sperm. The male factor of couple infertility is estimated to be about 25 percent to 50 percent. Sperm DNA integrity is important to successful fertilization and the normal development of embryos. Furthermore, sperm oxidative stress (OS) is associated with decreased sperm motility, decreased acrosome reaction, DNA damage and lower levels of implantation for in vitro fertilization.

Materials and Methods: The study was conducted on two groups of 40 infertile men and 40 fertile men. Blood sample and semen sample was collected.

Result: Seminal MDA was highly increased found in infertile men compared to fertile healthy men and serum level of antioxidant are low in infertile men as compare to fertile healthy men.

Conclusion: The present study concludes that oxidative stress was involved in low sperm quality and male infertility's etiological agent, they should take the antioxidant rich diet and antioxidant therapy to boost infertility, and the measurement of MDA could be a useful diagnostic tool to assess oxidative stress.

Keywords: ROS, antioxidants, OS, infertility and MDA.

INTRODUCTION:
The WHO describes infertility as a disability for couples in childbirth to attain pregnancy within 1 year of daily sexual intercourse. Infertility affects 13–20 per cent of couples worldwide, regardless of race or ethnicity[1–5]. The male factor of couple infertility is estimated to be about 25 percent to 50 percent [2,3,4, 5,6].Oligozoospermia is main cause for 90 percent infertility in men, a low sperm count and normal medical condition[7,8]. OS is an indication of high oxidants or ROS in cells against a reduced antioxidant defense network that is triggered by normal or non-
specific reactions to neighbouring cellular components such as unsaturated lipids, DNA, and proteins. Sperm cells in the respiratory chain developed a controlled level of free radicals. However, without a small amount of ROS, the sperm cells can not obtain their fertilizing capacities. For example, substantial increases in the levels of ROS, intracellular calcium, and tyrosine kinase result in increased cyclic adenosine monophosphate (cAMP) in the capacitation cycle. [9] Sperm motility is improved by cAMP, a disorder usually called hyperactivation. However, only hyperactivated motility passes through a physiological shows capable sperm exhibit acrosome reaction by acquiring the capacity to fertilize. [10] Sperm DNA uprightness is essential to the effective treatment and ordinary early stage improvement. Besides, sperm oxidative pressure was related with diminished sperm motility, overabundance DNA harm, diminished acrosome response and diminished in vitro treatment implantation rates [11–13].

MALE INFERTILITY
1. Impairment of spermatozoa in humans: one of the main causes of infertility is defective sperm. It has been reported that the nucleohistone sperm compartment containing histone-bound DNA sequences such as telomeres and gene promoters for embryonic development is frequently at high risk of oxidative stress, resulting in infertility[14,15]. The relative decrease in protamine 2 levels also results in infertility in the ratio of protamine1: protamine2, at the m-RNA and protein rates. Once, gene mutations encoding protamines induce structural changes in the composition of the sperm chromatin, leading to infertility[16,17].

2. Sperm dysfunction: The sperm dysfunction mechanism is related to oxidative stress, which actually induces sperm DNA integrity distortion by destroying the lipids and protein present in the plasma membrane of the sperm cells. This has a negative effect on fluidity and permeability of the sperm cell membrane which leads to infertility[18-19].

3. Sperm DNA Fragmentation (SDF): SDF is regulated by free-radicals, the researchers say[20,21]. Again, the underlying SDF mechanisms include single and double-strand breaks, fragmentation of DNA, abasic site entry, purine, pyrimidine modifications, and DNA crosslinking, which results in gene transcription induction, induction of signal transduction pathways; Accelerated depletion of telomeric RNA, defects in replication, genomic instability and transmission of GC to TA[22,23]. Since the causes of carcinogenesis are also known to be these mechanisms, this may explain a connection between infertility and cancer. Male infertility is also closely associated with DNA damage caused by ROS, which in turn accelerates the apoptosis of the germ cells, leading to a decrease in sperm count [24]

4. Physiologic role of ROS: ROS have detrimental effects on the sperm functions depending on the type and the concentration of ROS, and the length and location of ROS exposure [25]. Sperm acquires the capacity to travel gradually during epididymal movement. However, through a series of physiological changes, termed "capacitation," they gain the capacity to fertilize in the female tract [26,27].

Antioxidants and Fertility
ROS has both physiological and pathological functions, antioxidants in the seminal plasma maintains a stable state of ROS. Antioxidants serve as free radical scavengers, in order to shield sperm from ROS. These antioxidants are superoxide dismutase (SOD), catalase, and peroxidase glutathione (GPX). However, a semen includes a variety of non-enzymatic antioxidants[28] such as vitamin Cand E, pyruvate, glutathione, and carnitine, which in effect decreases endogenous repair mechanisms and enzymatic defenses. [29] [28]

Seminal Malondialdehyde
The primary component of ROS-initiated sperm harm which prompts barrenness is lipid peroxidation of the sperm film. Malondialdehyde (MDA), a lipid peroxide side-effect, mirrors the lipid peroxidation level [30].

Role of Glutathione
Glutathione is one of the most bottomless non-thiol protein in the mammalian cells.[31] Glutathione insufficiency may prompt mid-piece insecurity, coming about in the imperfect
motility.[32,33] It shields plasma layer from lipid peroxidation, superoxide forestalls the development of O2. Glutathione peroxidase (GPx) assumes a significant job in lessening hydrogen peroxide and natural peroxides that include phospholipid peroxides. Selenium happens in selenocysteine type in the dynamic site of GPx[32]. It is contained in the mitochondrial sperm matrix but a nuclear portion of GPx is associated with sperm DNA protection from oxidative disruption. It has also been documented that the nuclear process plays a part in condensing chromatin. The appearance of GPx in the seminal plasma indicates the prostate may be the source of it[35].

**Superoxide Dismutase**

Superoxide dismutase (SOD) scavengers both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane.[36] SOD also prevents superoxide radicals from premature hyperactivation and pre-ejaculation capacitation.[37] Superoxide dismutase or superoxide oxide reductase catalyzes the conversion of superoxide anions to dismutation. For both extra- and intracellular forms, otherwise known as metaloenzymes, these also occur. The two intracellular forms in their active centers and the organelle where they are located are separated by the metal(s). The first intracellular component in the active center contain copper and zinc (SOD-1) and is mainly contained in the cytoplasm, while the second component present in the active center of mitochondria with manganese is called SOD-2. In extracellular space (SOD-3), the extracellular shape of the SOD functions. This is connected to the polysaccharide surface although it can be found in a free form.[38,39]

**Catalase**

Catalase catalyses the split of H₂O₂ into H₂O and O₂. H₂O₂ is one of the reactive oxygen species involved in oxidative stress, catalase helps in detoxifying both intracellular and extracellular H₂O₂ to water and oxygen.[40] However, it triggers sperm capacitation caused by nitrous oxide (NO), which is a complex process involving H₂O₂.[41]

**Vitamin E**

Vit-E is an intense cancer prevention agent operator found in the cells membrane and it inhibits lipid peroxidation, and rummages free radicals created during univalent decrease of sub-atomic oxygen and during ordinary oxidative chemical movement. The arrangement of these radicals brings about phospholipid peroxidation in the mitochondrial sperm bringing about low motility [42] Suleiman et al. (1996) found that Vit-E supplementation could fundamentally decrease lipid peroxidation in original plasma, improve sperm motility and improve pregnancy frequency [43,44].

**Vitamin C**

Vit-C is a keto-lactone containing six carbons that is biosynthesized in liver. Nevertheless, human inability to synthesize vitamin necessitates the inclusion in the diet or as the supplement[45]. Vit-C used asacofactor, which helps in the folic acid, tyrosine, and tryptophan metabolism processes[46].

**AIM AND OBJECTIVE:**

**AIM:**
To study the levels of seminal MDA, CAT, GPx, SOD, Vit-C, Vit-E in male infertility.

**OBJECTIVE:**
To correlate the levels of seminal MDA, CAT, GPx, SOD, Vit-C, Vit-E between infertile Male patients and healthy fertile male as controls (age matched) attending AVBRH Wardha and SMHRC Nagpur, Maharashtra.

**MATERIAL AND METHOD:**
This Study was conducted in the Department of Biochemistry and Dept. of OBGY at DattaMeghe Medical College, ShalinitaiMeghe Hospital & Research Centre, Nagpur in collaboration with
Jawaharlal Nehru Medical College DattaMeghe Institute of Medical Sciences, Sawangi (Meghe) Wardha Maharashtra.
Total 80 subjects were selected for study. Out of which 40 infertile male patients and 40 age-matched healthy control.

**Sample Collection:**
Semen samples were obtained after at least 72 hours (3-4 days) of sexual abstinence through masturbation in a sterile and broad mouthed tub. Samples were allowed to liquefy for a minimum of 45 minutes at room temperature (25°C).
Blood sample was collected for oxidative stress and antioxidant measurement.

**Estimation of MDA:**
MDA levels were estimated by using “thiobarbituric acid (TBARS) assay (by Rao et al method)”. [47]

**GSH-Px Measurement:**
“GSH-Px activity was measured by the method of Paglia and Valentine”. [48]

**SOD Measurement:**
SOD behavior was determined by the "Sun and Colleagues" test. [49]

**Estimation of vitamin C:**
Vitamin C was estimated by high performance liquid chromatography (HPLC) with electrochemical or ultraviolet light detection. [50]

**Estimation of vitamin E:**
Vitamin E was estimated by Modified simple method by “baker and frank method”. [51].

**Estimation of Catalase:**
Estimation of catalase done by spectrophotometric method. [52]

**INCLUSION CRITERIA:**
- Infertile patients in reproductive age group persisting infertility for more than one year.

**EXCLUSION CRITERIA:**
- Patients with smoking history
- Drinking alcohol and chewing tobacco.
  - Patients with mellitus diabetes, COPD, asthma, obesity and AIDS.
  - Patients who take supplements of vitamin E, vitamin C or glutathione.
  - Anomalous cases such as cryptorchidism (undescended checks) -atrophic or varicocele checks.

**STATISTICAL ANALYSIS**
All results were expressed as mean ±SD. Mean values will be assessed for significance by unpaired student –t test.
A statistical analysis will be performed using the Statistical Package for the Social Science program (SPSS, 24.0). Frequencies and percentages will be used for the categorical measures. Probability values p < 0.05 will be considered statistically significant.

**OBSERVATION AND RESULTS**

**Table 1: Levels of seminal MDA, CAT, GPx, SOD, Vit-C, Vit-E in infertile male and Healthy control.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Infertile Male Mean±SD (n=40)</th>
<th>Fertile male Mean±SD (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit-C</td>
<td>0.30±0.127</td>
<td>0.98±1.53</td>
</tr>
<tr>
<td>Vit-E</td>
<td>4.38±1.6</td>
<td>7.455±11.49</td>
</tr>
<tr>
<td>SOD</td>
<td>130.67±26.79</td>
<td>180.975±35.40</td>
</tr>
<tr>
<td>Cat</td>
<td>13.36±1.87</td>
<td>18.55±5.148</td>
</tr>
<tr>
<td>Seminal MDA</td>
<td>0.85±0.38</td>
<td>0.53±0.35</td>
</tr>
<tr>
<td>GPx</td>
<td>1.91±0.930</td>
<td>3.98±2.14</td>
</tr>
</tbody>
</table>
Above table no 2 shows the concentration of seminal MDA, CAT, GPx, SOD, Vit-C and Vit-E were decreased found in infertile male compare to fertile male.

Table 2: Correlation of seminal MDA, CAT, GPx, SOD, Vit-C, Vit-E in infertile male and Healthy control.

<table>
<thead>
<tr>
<th></th>
<th>Cases Mean±SD(n=40)</th>
<th>Controls Mean±SD(n=40)</th>
<th>t-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit-C</td>
<td>0.30±0.127</td>
<td>0.98±1.53</td>
<td>2.801</td>
<td>0.0064</td>
</tr>
<tr>
<td>Vit-E</td>
<td>4.38±1.6</td>
<td>7.455±11.49</td>
<td>1.676</td>
<td>0.0977</td>
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<tr>
<td>SOD</td>
<td>130.67±26.79</td>
<td>180.975±35.40</td>
<td>7.167</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cat</td>
<td>13.36±1.87</td>
<td>18.55±5.148</td>
<td>5.993</td>
<td>0.0001</td>
</tr>
<tr>
<td>SeminalMDA</td>
<td>0.85±0.38</td>
<td>0.53±0.35</td>
<td>-</td>
<td>0.0002</td>
</tr>
<tr>
<td>GPx</td>
<td>1.91±0.930</td>
<td>3.98±2.14</td>
<td>5.611</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Above table no 2 shows the levels of seminal Malondialdehyde was increased in infertile men as compared to fertile men. The levels of Catalase(CAT), Glutathione Peroxidase, Superoxide dismutase, Vitamin C, Vitamin E was decreased in infertile Male patients as compared to healthy fertile male.

DISCUSSION:
It is understood that reactive oxygen species play an important role in the etiology of defective sperm function through mechanisms that include inducing peroxidative damage to the plasma membrane[53]; ROS is linked to the spermatozoa hyperactivation, capacitation and acrosome reaction[54]. The development of ROS is negatively related to human spermatozoa's ability to fuse oocytes[55]. ROS at low concentrations will inhibit sperm-egg fusion with SHproteins via sperm membrane oxidation[56]. High lipid peroxidation would reduce the ability of the sperm to undergo acrosomal reaction and fertilize[55]. Addition of hydrogen peroxide has weakened the oocyte fusion ability of spermatozoa during epidididymal maturation and storage. Human sperm are especially susceptible to oxidative harm, but seminal plasma's antioxidant properties are naturally shielded from such harm. ROS plays a vital role in sperm biology, such as sperm maturation and sperm capacitation. Abnormal ROS production has to do with abnormal sperm function [58,59]. A fine balance between the synthesis of ROS and its reusing is essential for spermatogenesis. High seminal ROS development, mainly through neutrophils but also through immobile sperm, abnormal sperm, or morphologically normal but functionally abnormal sperm, may be a major trigger for males infertility [60]. Nonetheless, sperm is rich in unsaturated fatty acid making it more susceptible to oxidation, and sperm cells with oxidatively damaged DNA are subjected to apoptotic removal through a p53-dependent and independent mechanism that can contribute to infertility. Recent research have revealed that disorders such as insufficient fertilization, pregnancy failure, birth defects, insufficient growth in embryos[61,62]. Humans have evolved a highly regulated, and complex antioxidant defense mechanism to protect the body's cells and organ systems from ROS[63,64]. The endogenous antioxidants are primarily enzymatic antioxidants including superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and the levels of these antioxidants is low in infertile males in our study as compared to healthy controls [65,66,67].

Vitamin E and Vitamin C are also low in infertile males as compared to healthy control as they are another important chain-breaking antioxidant that contributes up to 65% of the antioxidant capacity of the seminal plasma. Seminal levels of malondialdehyde are elevated in infertile males relative to healthy controls; high levels of MDA in infertile men's seminal plasma is a sign of increased oxidative stress associated with reduced sperm quality and a major risk of idiopathic male infertility [68,69].
CONCLUSION:
The present study indicated that low sperm quality and male infertility etiology included oxidative stress, a diet rich in antioxidant and antioxidant therapy to boost infertility, and MDA calculation could be a quite useful diagnostic method to estimate oxidative stress.

REFERENCES:
14. Tay, P.Y.; Tan, C.P.; Abas, F.; Yim, H.S.; Ho, C.W. Assessment of extraction parameters on antioxidant capacity, polyphenol content, epigallocatechin gallate (EGCG), epicatechingallate (ECG) and iriflophenone 3-C-β-glucoside of agarwood (Aquilariacrassna) young leaves. Molecules 2014, 19, 12304–12319.
15. Dr.Anubha Singh, Dr.PreetaishKaul (2018) Effectiveness of ManSure, a herbal reproductive health supplement, on semen quality (sperm count, sperm quality, sperm motility) in infertile men International Journal Of Scientific Research And Education.06,01 (Jan-18) 7788-91


