Section: Miscellaneous

Original Article

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Oxidative Stress and its Impact on Semen Quality

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Abstract

Background: Male Infertility is on an inclining trend and there has been alarming rise of deteriorated semen quality in the past few decades. Recent evidences have suggested the role of elevated levels of oxidative stress and in impairing spermatogenesis. Therefore, in the present study effect of oxidative stress on semen quality has been explored. **Objective:** This study aimed to determine the relationship between oxidative stress (OS) and the results of semen analysis among men from infertile couples. **Subjects and Methods:** Hundred Semen samples from the males of infertile couple attending the infertility clinic JNMCH AMU were collected in the year 2016-2018 for detailed Semen analysis after proper consent. Simultaneously Total Antioxidant Capacity and Oxidative stress status of semen samples was evaluated by colorimetric method. The effect of Oxidative stress on semen parameters was assessed using appropriate tests for both Quantitative and Qualitative variables. **Results and Conclusion:** There was significant reduction in Total sperm Concentration and sperm motility in semen samples with high Oxidative stress Index and low Total Antioxidant capacity, however semen volume and Morphology was unaltered. The result suggests that Oxidative stress has deleterious effect on semen parameters.

Keywords: Oxidative stress, Male Infertility, Total Antioxidant capacity, Semen, motility, Total sperm concentration.

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Introduction

Infertility is usually defined as the inability to conceive after 12 months of regular unprotected intercourse.[1] Infertility is a major public health problem affecting around 5-15% of couple worldwide.[2] Male factors are responsible for overall infertility in 40%–50% of cases, and approximately 7% of men worldwide are affected by infertility.[3]

Male reproductive potential is largely defined by semen parameters which is suggestive of optimum spermatogenesis with aligned hormonal milieu. However abnormal semen analysis not always result in infertility still Male infertility diagnosis is commonly based on standard semen parameters analysis 4 Due to alarming trend of declining semen quality in the past decade5 there has been surge in efforts to identify the causes for the same.

Multiple causes are responsible for male infertility. Anatomical, neurological environmental, behavioral factors influence spermatogenesis,[6] and eventually affects the semen parameters.

Recent evidences have pointed to the presence of elevated oxidative stress in abnormal semen samples.[7] Oxidative stress is defined as an imbalance between the generation of reactive oxygen species (ROS) and the protective action of antioxidant systems responsible for their neutralization and removal8 OS has become a growing concern for researchers and clinicians because of association within infertility and reproductive outcomes. Declining semen quality over the years have prompted the research in areas which are still shady like oxidative stress in the semen samples which may impair semen quality.

Oxidative damage to sperm, with a reduction in fertility, occurs when the production of reactive oxygen species (ROS) by sperm and seminal leukocytes exceeds the neutralization capacity of protective antioxidants system of male reproductive organs.[8] Hydroxyl radicals, hydrogen peroxides and superoxide anion are the major reactive oxygen species (ROS) in the seminal plasma. ROS help in Acrosomal reaction, sperm capacitation and sperm oocyte fusion when it is present in physiological amount. However, when ROS is present in excessive amount it leads to infertility.[9] Sperm parameters are ultimately affected as ROS leads to loss of motility of sperm and its ability to fuse with oocyte by damaging sperm plasma membrane. It also damages sperm DNA which results in passage of defective paternal DNA to the conceptus which may results in implantation failure.[10]

Oxidative stress may be an outcome of various exogenous and endogenous factors like alcohol consumption, tobacco chewing and smoking etc. Several studies affirm the hypothesis that oxidative stress has a role to play in male infertility and therefore the present study was planned to evaluate the impact of oxidative stress on semen

parameters. This facilitates identification of causes, formulation of preventive and therapeutic measures to optimize sperm health and improve reproductive outcomes

Subjects and Methods

We conducted a Prospective cohort study between June 2016 to June 2018. All the males of infertile couple attending infertility clinic outpatient department (OPD) of Jawaharlal Nehru Medical College and Hospital, Aligarh, Uttar Pradesh, India were taken as subjects. Ethical clearance from the Institutional Ethics Committee (IEC) of Jawaharlal Nehru Medical College and hospital was taken in order to conduct this study. Patients were enrolled after taking written informed consent and satisfying inclusion and exclusion criteria.

Inclusion Criteria

All the males of infertile couple attending infertility clinic for couple infertility in reproductive age group

Exclusion Criteria

Males having systemic diseases (such as diabetes, hypertension, cancer), Endocrinal abnormalities which may affect male reproductive function. Subjects diagnosed with any sexually transmitted diseases, Males undergoing therapies such as radiotherapy and chemotherapy, urogenital problems or any other serious chronic diseases that might have an impact on reproductive system were not included.

Methodology

100 men who attended infertility clinic were recruited after fulfilling the inclusion criteria. Detailed history was taken and physical examination performed. Baseline characteristics such as age, BMI (WHO classification 2014), alcohol addiction,[11] smoking Index,[12] educational qualification, and religious status were documented.

Semen samples and blood investigations were collected. Semen samples of male subjects were self-collected after 3-5 days of abstinence and following the prerequisites of WHO criteria 2010. Detailed evaluation of each sample was done in accordance with WHO recommendations 2010.

WHO 2010 Semen parameters

Semen characteristic	Lower reference limit
Volume, mL	1.5
Sperm concentration, 10^6/mL	39
Total sperm number, 10^6	15
Total motility (PR + NP), %	40
Progressive motility (PR), %	32
Vitality (live spermatozoa), %	58
Sperm morphology (normal forms), %	4
pH	≥7.2
Seminal fructose, µmol/ejaculate	≥13

PR, progressive motility; NP, non-progressive motility.

Figure 1:

Oxidative Stress Index (OSI) and Total antioxidant capacity (TAC)

The semen samples were collected from the subjects, centrifuged for separation of plasma, and stored at -80°C until further analysis was done for oxidative stress index (OSI) and total antioxidant capacity (TAC).

From this sample, semen plasma OSI and TAC was measured by colorimetric method and expressed as millimole (mM).

Total Oxidative Status (TOS)

Oxidants present in the semen sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules, which were abundantly present in the reaction medium used in the test. The ferric ion made a colored complex with xylenol orange in an acidic medium. The color intensity was measured spectrophotometrically, and was proportional to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per litre (µmol H202 Equiv./L)

Total Antioxidant Capacity (TAC)

The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical cation-based assays are among the most abundant antioxidant capacity assays. Measurement methods were based on the decolorization of ABTS radical by the presence of antioxidants in the sample in acetate buffer. (ABTS) is incubated with methmyoglobin and hydrogen peroxide to produce ABTS+. These species were blue-green. Antioxidants present in the sample cause a reduction in

absorption proportional to their concentration.

This reaction was monitored spectrophotometrically and the bleaching rate was inversely related to the TAC of the sample. The reaction was calibrated with Trolox, which is widely used as a traditional standard for TAC measurement assays, and the assay results are expressed in mmol Trolox equivalent/L.

Automated Calibration-Afte manual spectrophotometric optimization processes, the assay kits were applied to an automated analyzer (Aeroset) and final TOS and TAC is estimated. Followed by calculation of Oxidative Stress Index through Formula for each semen sample.

Oxidative Stress Index (OSI) = TOS/TAC x 100

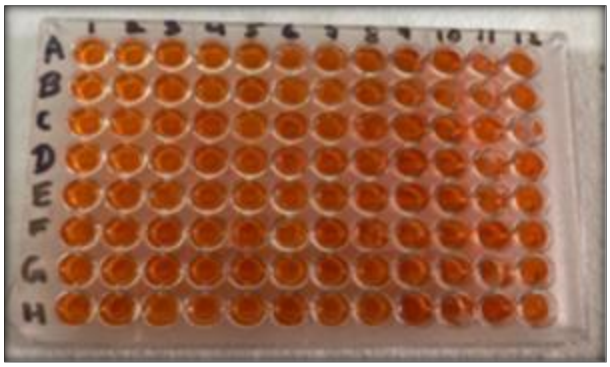

Figure 2: TOS assay kit

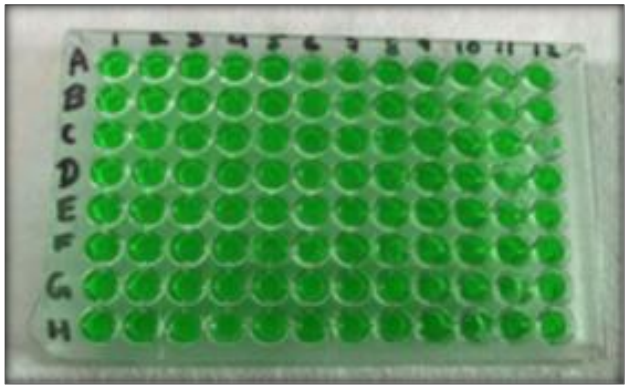

Figure 3: TAC assay kit

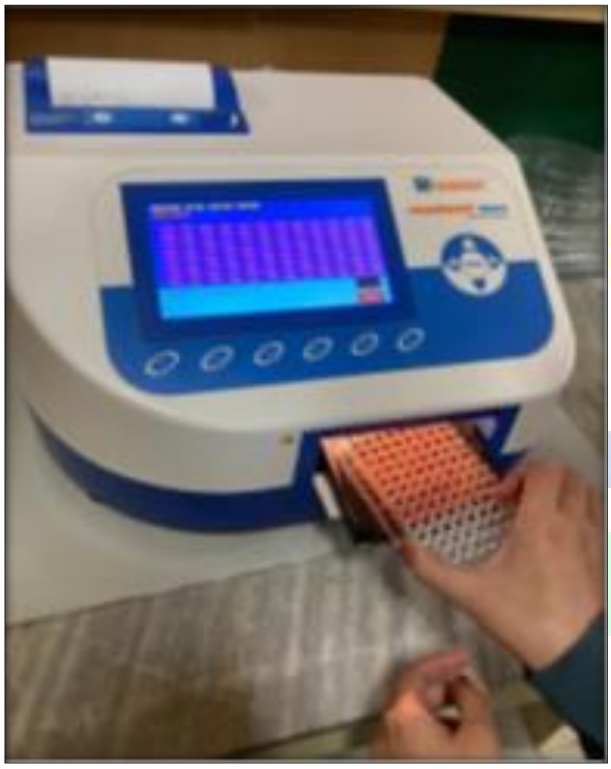

Figure 4: Automated ELIZA Kit Analyzer

Results

Study group characteristics are depicted in Table 1.

In present study, mean age distribution of the study subjects(N=100) was 34.27+5.3 yrs. Mean BMI was 25.99+2.97. Majority of the population followed religion Islam. Primary infertility was the major concern of the study population.

Of 100 subjects, 22(22.00%) were smokers12 where 15 were moderate,6 light and 1heavy smoker and 8(8.00%) were alcoholics11 where 7 were mild and 1 moderate drinker respectively. Gutka was consumed by 32 % of the population.

Detailed semen analysis showed normal range of Total Sperm concentration (10^6/mL) in 67.00% of cases while 33(33.00%) subjects had any one value below the reference range and thus were categorized as abnormal or Low. Mean value of Total sperm concentration(10^6/mL) was 34.67 ± 26.54 while median (25th-75th percentile) was 35(12.8-46.5).

Percentage total progressive motility in 55(55.00%) cases was normal, whereas 45(45.00%) of them had motility below the 5th percentile of reference ranges. Mean value of progressive motility (%) of study subjects was 35.51 ± 24.65 with median (25th-75th percentile) of 30(15-58.5).

Mean semen volume was 2.71+1.46, however none of the semen samples showed an abnormal value. Similarly, Sperm morphology was normal in 96% of the samples and the mean value was 42-60%.

Oxidative stress status was documented for each sample, using standard methods as described and similarly Total Antioxidant Capacity was obtained for each sample as shown in Table 2. Mean TAC was calibrated to be 4.253+1.297 and Mean Oxidative Stress Index was estimated to be 0.1688+0.067.

Pearson Correlation test was used to obtain correlation between TAC and Semen parameters. TAC and Total sperm Concentration showed positive and significant correlation (r-value 0.329

p-value-0.001), with Motility the results were likewise (r-value 0.466 and p-value <0.001). However no correlation was obtained with semen volume and Morphology (Volume; r-value -0.003 and p-value 0.976: Morphology; r-value-0.461 , P-value-0.554).The results showed that higher values of TAC of semen samples improves the TSC and motility , and semen volume and morphology remains unaffected.

OSI of the semen samples was calculated and correlation was obtained with semen parameters using Pearson correlation test as shown in Table 2.

OSI and TSC showed negative and significant correlation (r-value = -0.338 and p-value0.009). Similar results were obtained with motility (R-value -0.427 and p-value <0.001). However with semen volume and Morphology no correlation was obtained (Volume ;r-value 0.01 and p-value 0.923 :Morphology; r value-0.324,p-value 0.739).Thus the result showed elevated Oxidative Stress cause adverse effect on TSC and Motility.

Table 1: Patients' Characteristic distribution (N=100)

Patient characteristics	Frequency/Mean value	Percentage	
Age	34.27+5.3 yrs		
BMI	25.99+2.97		
Alcohol	8	8%	
Gutka consumption	32	32%	
Smoking	22	22%	
Total Sperm concentration (10^6/mL)			
Abnormal	33	33%	
Normal	67	67%	
Mean± SD	34.67 ± 26.54		
Median	35(12.8-46.5)		
Total Progressive motility			
Normal	55	55%	
Abnormal	45	45%	
Mean± SD	35.51 ± 24.65		
Median	30(15-58.5)		
Semen Volume			
Normal	100	100%	
Abnormal	0		
Mean± SD	2.71+1.46.		
Morphology			
Normal	96	96%	
Abnormal	4	4%	
Mean	42-60%		

Table 2: Correlation of TAC an OSI with semen Parameters

Patient characteristics	TSC Mean	Motility Mean	Semen Volume Mean	Morphology Mean
Oxidative Stress Index	34.67 ± 26.54	35.51 ± 24.65	2.71+1.46.	42-60%
Mean±SD = 0.1688+0.067	r-value - -0.338 and p-value-.009	*r*-value -0.427 and p-value <0.001).	(r-value 0.01 and p-value 0.923).	r value-0.324,p-value 0.739
Total Antioxidant Capacity	r-value 0.329	*r*-value 0.466 and	*r*-value -0.003 and p-value 0.976	r-value-0.461 , P-value-0.554
Mean±SD = 4.253+1.297	**p-value-0.001**	**p-value <0.001).**		

Discussion

In the study population of infertile couple, the prevalence of Male factor presumably responsible for couple infertility was 33%. Reported prevalence of male infertility in global data is between 9-15%.[13] Male infertility is diagnosed by one or more factors that include abnormal semen

quantitative, qualitative, and functional parameters or immunological abnormalities of reproductive system (chronic illness is also included) or conditions incompatible with sexual intercourse.

In 2018, the global prevalence of male infertility was estimated to be 56,530.4 thousand (95% UI: 31,861.5–90,211.7).[14] The higher prevalence in our study may be attributed to the targeted population of infertile couple where fertility is expectedly diminished.

The mean Age of the study population was 34.27+5.3 years and similarly multiple studies quoted the same agegroup,[15,16,17] as this age group is keen for conception and reports promptly when they are unable to conceive. In this study Age did not affect the semen quality, as suggested by Pearson correlation (Semen volume r-value 0.153 and p-value 0.129, TSC- r-value 0.058 and p-value 0.566, Motility r-value -0.04 and p-value 0.693). Other studies have reported age to be affecting the semen parameters adversely and significantly however few have reported no change in aforementioned parameters with age.[18,19,20,21,22,23]

This disparity may be due to heterogeneity of the data, racial and geographical differences.

Mean BMI of the group was 25.99+2.97, most were in overweight category and correlation with semen quality using Pearson Correlation was obtained which showed negative but insignificant correlation with TSC and Motility (TSC-r--0.104 and p- 0.305 Motility r-value-0.111 and p-value 0.273). Several studies reported BMI affecting semen quality, [24,25,26] unlike ours. The difference was presumably due lack of subjects in obese grp which was reported in majority of the studies.

Further in the study we found that 22% were smokers, we compared semen parameters in smoker vs Nonsmoker. In our study, using unpaired t-test the means of semen volume(p1), TSC(p2), Motility(p3) was compared and we found that there were no significant differences in the two group (p1=p-value 0.156 p2=p-value 0.239 p3=p-value 0.429). showing thereby that smoking didn't affect semen parameters. An association between semen quality and smoking has been reported in a number of studies, but the results are inconsistent. [27,28,29] Detrimental effect on semen quality was more pronounced where the heavy smoking was reported, unlike ours where majority of men were moderate to light smokers.

Gutka was consumed by 32% of the subjects and we compared the semen quality in Gutka consumer vs non-consumer group. We found that means of (semen volume, TSC and Motility) were comparable p value was no significant (p1=p-value 0.573, p2=0.352, p3=p-value 0.199). Studies on Gutka consumption suggest inconsistent results.[30,31] A study suggested impaired semen parameters in Gutka consumers,[31] unlike ours, the difference could be due to large number of study subjects in the study quoted.[31]

Oxidative stress status and Total antioxidant capacity was evaluated for each semen sample and was correlated with semen parameters. TAC correlated positively and significantly with TSC and Motility suggesting lower TAC may impair motility and TSC. The findings were in concordance with other studies which suggested detrimental effect of lower TAC on motility and morphology.[32,33] It is responsible for preventing membrane and DNA damage in spermatozoa by excessive Reactive Oxygen species (ROS).[34]

OSI was calculated, derived indirectly from Oxidative status and TAC. The combined index from the ROS generation and the total antioxidant status score have been reported to be better markers of the oxidative stress.[35] In our study OSI was higher in semen samples with low motility and low TSC meaning thereby that higher oxidative stress adversely affect motility and sperm number. Other studies also had similar findings, Barati et al. concluded that OS in the testes and sperm was negatively associated with sperm motility and acrosome activity.[36] OS causes damage to nuclear and mitochondrial DNA, with shorter telomeres, affects the motility and repairing capacity of spermatozoa,[37] A high incidence of genetic aberrations in embryos have been attributed to ROS-induced OS in the male germ line.[38]

Conclusion

In conclusion we propose that abnormal sperm motility and number may be an outcome of elevated Oxidative stress and decreased TAC of the semen sample which could be due to any exogenous or endogenous factors. The factors should be exhaustively searched for and dealt appropriately. OSI gives us an insight about the prevailing biochemical milieu in the semen sample.

Assessment of sperm OSI levels among infertile men can aid in determining which individuals may benefit from antioxidant therapy. Currently, there are no infertility guidelines that recommend routine OSI measurement and there is still an ongoing debate regarding the need for this test and identification of patients who are likely to be tested for the oxidative burden.

In the light of present study and many more it would be judicial to evaluate OSI in the patients with impaired semen parameters specially motility. Treatment with antioxidants and benefits in the form of improved semen parameters need further research on large scale.

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