1. INTRODUCTION

Reproductive function is sensitive to changes in the physical and chemical environment (Younglai et al., 2005). However, there is a paucity of data regarding the association between occupational radiation exposure and risk to human fertility (Schull, 1984; Baransi, 1993; Bonde, 1999). The results in the chapter 3, have demonstrated a significant decline in sperm functional characteristics, DNA damage and hypermethylation in radiation exposed subjects. Sperm DNA is an independent measure of sperm quality that may have better diagnostic and prognostic capabilities than standard sperm parameters like concentration, motility and morphology (Zini et al., 2001). Sperm DNA integrity analysis of radiation health workers using well established techniques such as single cell gel electrophoresis (comet assay) and flow cytometry based DNA fragmentation analysis has revealed an increased level of sperm DNA fragmentation as discussed in the chapter 3.

Cellular exposure to ionizing radiation has been known to produce reactive oxygen species (ROS) which display high reactivity towards macromolecules (Azzam et al., 2012). In order to protect against ROS mediated damage, body has evolved a variety of defense mechanisms involving antioxidant systems. Antioxidant systems include enzymes such as superoxide dismutase (SOD), catalase and non-enzymatic systems such as glutathione and ascorbic acid. The seminal plasma components of the human ejaculate also have an array of enzymatic and non-enzymatic antioxidant systems (Alvarez et al., 1987) which protect the spermatozoa from reactive oxygen species (ROS) mediated damage (Kankofer, 2005). One of the ROS mediated cellular damage is the membrane
lipid peroxidation which results in the formation of malondialdehyde (MDA). MDA has been widely used as marker to monitor the degree of peroxidative damage sustained by the spermatozoa. Previous studies have observed the enhanced antioxidant levels in the blood plasma of chronically exposed radiation health workers (Durovic et al., 2008, Russo et al., 2012). However, there is no data elucidating the association between seminal plasma antioxidants and sperm DNA integrity in occupationally exposed subjects. In light of these considerations, this study was planned to quantify the seminal plasma antioxidants and lipid peroxidation level to determine their association with the sperm chromatin integrity in the subjects who are chronically exposed to radiation at workplace.

2. MATERIALS AND METHODS

2.1 Study populations:

The details of the study populations are provided in the Chapter 3, Table 1.

2.2 Exposure monitoring

The occupational exposure levels of the subjects were routinely monitored by thermoluminescent dosimeter (TLD) device. The cumulative exposure level of each subject was collected from the radiation safety officer of the respective hospital where the subject was enrolled.
2.3 Separation of seminal plasma

Seminal plasma free from spermatozoa was obtained by centrifuging the semen sample at 2000 rpm for 10 min for antioxidants and lipid peroxidation assessment. Seminal plasma was aliquoted in cryovial and stored at -80°C till the assessment of the different parameters. All the assessment and result analysis was carried out blindly.
2.3.1 Assessment of total, reduced and oxidized glutathione (GSH) in the seminal plasma of the exposed and non-exposed subjects:

Total GSH concentration was determined according to method described by Anderson (1985) with minor modification. Briefly, 50μL of seminal plasma were added to 100μL of reaction buffer (1.5mM phosphate buffer, 2.4mM NADPH, 10mM DTNB, 0.5U GSH reductase, pH 7.5). The optical density (OD) was recorded exactly after 1 minute at 405 nm using Bio-photometer (Eppendorf, Germany). A standard graph was prepared by using various concentration of GSH standard. The total GSH levels were determined by plotting the OD values of the seminal plasma against the standard graph and dropping a perpendicular to X-axis.

Estimation of reduced GSH was done according to Beutler et al., (1963) with minor modification. Briefly, 50μL of seminal plasma were mixed with 50μL phosphate buffer, 25μL of precipitating reagent and 25μL of fresh DTNB. The optical density of the test solution was determined at 405nm exactly after one minute. A standard graph was prepared by using various concentration of reduced GSH. The reduced GSH levels in seminal plasma were determined by plotting the optical density (OD) values of the seminal plasma against the standard graph and dropping a perpendicular to X-axis. Estimation of oxidized GSH was performed by subtracting the concentration of reduced GSH from the total GSH.
2.3.2 *Assessment of total antioxidant capacity (TAC) in seminal plasma of the exposed and non-exposed subjects:*

The ability of the seminal plasma to scavenge ABTS\(^+\) [2, 2’-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation was compared to trolox standard (Re *et al.*, 1974). Briefly, the ABTS\(^+\) radical cation were pre-generated by mixing 7mM ABTS (Cat. No. A1888; Sigma Aldrich Inc. USA) stock solution with 2.45mM potassium persulfate and incubating for 12–16h in dark condition at room temperature. The absorbance of the ABTS\(^+\) solution was equilibrated to 0.70 ± 0.02 OD value by diluting with phosphate buffered saline (PBS) at room temperature, then 1 ml was mixed with 10μL of the seminal plasma followed by measurement of absorbance at 650nm after one minute. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard and sample to determine the trolox equivalent antioxidant concentration (TEAC). To calculate the TEAC, the gradient of the plot for the sample were divided by the gradient of the plot for trolox.

2.3.3 *Assessment of superoxide dismutase (SOD) in the seminal plasma of the exposed and non-exposed subjects:*

Estimation of SOD was done according to Marklund & Marklund (1974) with minor modifications. One unit of SOD is defined as the amount of enzyme which inhibits auto-oxidation of pyrogallol by 50%. Briefly, 100μL seminal plasma was added to 2.85mL of 0.1M phosphate buffer (pH 8.4) and 50μL of 100mM pyrogallol (Cat No. RM170; HiMedia Laboratories) prepared in 20mM HCl. The absorbance of the reaction mixture
was recorded at 0 and 3 min. Similarly, the auto-oxidation is measured by adding 2.95mL phosphate buffer (pH 8.4) and 50μL pyrogallol at 0 and 3 min using a Bio-photometer at a wavelength of 405nm. SOD activity was measured using calibration curve of percentage inhibition and expressed as enzyme units/μL of seminal plasma.

2.3.4 Measurement of lipid peroxidation by malonaldehyde (MDA) assay in seminal plasma of the exposed and non-exposed subjects:

The lipid peroxidation levels in the seminal plasma was measured using thiobarbituric acid reactive substances (TBARS) assay, which monitors MDA production based on the method of Beuge & Aust (1978). Briefly, 100μL of seminal plasma were added to 1.8mL of 3% phosphoric acid (Cat. No. 215104; Sigma Aldrich Inc. USA) and 0.6 mL of 0.6% TBA (Cat. No. T5500; Sigma Aldrich Inc. USA). These mixtures were heated in boiling water for 45 min. After cooling, the intensity was measured at 550nm using Bio-photometer and the level was determined using the molar absorption coefficient at 550nm.

2.4 Statistical analysis:

The data were analyzed using Statistical Package for Social Sciences (SPSS 15.0). Data has been summarized using mean and standard error (Mean ± SEM) for continuous variables and percentages for qualitative variables like alcohol and smoking. Comparison has been done using independent ‘t’ test for continuous variables and chi-square test for categorical variables. The graphs were plotted using SPSS 15.0 and Origin 6.0 (Northampton, MA, USA). Multivariate analysis was performed using analysis of
covariance (ANCOVA) to determine the influence of confounding factors like smoking, alcohol, diet and gutkha.

3. RESULTS

3.1 Sperm DNA integrity/fragmentation in the subjects recruited for this study:

The detailed information about the DNA integrity/fragmentation of the study population has been summarized in the chapter 3 (Results 3.3; Table 5).

3.2 Glutathione (GSH) level in the seminal plasma of the exposed subjects:

Total GSH level in the exposed group was 1.294 ± 0.06 compared to 1.021 ± 0.05 mM in the non-exposed subjects and this difference was statistically significant ($P < 0.01$). Further, the level of the total GSH in the HAD group (1.358 ± 0.08), which was significantly higher than the non-exposed subjects ($P < 0.001$, Table 1; Fig 1). Though, subjects of HAD groups had elevated levels of total GSH in comparison to the subjects with LAD, the differences were not statistically significant ($P = 0.06$) (Table 1; Fig 1).

Similar to total GSH level, there was about 1.5 fold increase in reduced GSH level in the exposed subjects (0.979 ± 0.05) compared to non-exposed subjects (0.674 ± 0.05) which was statistically significant ($P < 0.001$, Fig 1; Table 1). Further, there was increase in the level of reduced GSH in HAD groups (1.040 ± 0.07) compared to LAD group (0.890 ± 0.07) but but this difference was statistically not significant. The level of reduced GSH in the LAD and HAD group was significantly higher ($P < 0.05$, $P < 0.001$ respectively) compared to non-exposed subjects (Fig 1; Table 1).
Though, there was a marginal decrease in the oxidized GSH level in exposed subjects (0.385 ± 0.04) in comparison to non-exposed group (0.413 ± 0.04), the differences were not significant (Fig 1; Table 1). Similarly, there was no difference in oxidized concentration in the LAD (0.360 ± 0.06) and HAD group (0.440 ± 0.05) (Fig 1; Table 1).

The ratio of reduced/oxidized GSH in the non-exposed subjects and exposed subjects was 1.629 ± 0.19, 2.119 ± 0.15 mM respectively, and this difference was significant ($P < 0.05$). However, there was no difference in the ratio of reduced/oxidized GSH in LAD (2.331 ± 0.24) and HAD group (1.942 ± 0.18) groups (Fig 2; Table 1).

**Fig 1: Glutathione (GSH) level in the exposed and non-exposed subjects:**

![Bar chart showing glutathione levels in different groups](image-url)
Table 1: Comparison of antioxidants and ROS level in the exposed and non-exposed subjects:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Non-exposed (Mean ± SEM)</th>
<th>Exposed (N= 83) (Mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All subjects</td>
<td>LAD</td>
</tr>
<tr>
<td>Glutathione (GSH; mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1.021 ± 0.05</td>
<td>1.294 ± 0.06 (^a)</td>
</tr>
<tr>
<td>Reduced</td>
<td>0.674 ± 0.05</td>
<td>0.979 ± 0.05 (^c)</td>
</tr>
<tr>
<td>Oxidized</td>
<td>0.413 ± 0.04</td>
<td>0.385 ± 0.04</td>
</tr>
<tr>
<td>Ratio (Reduced/Oxidized)</td>
<td>1.629 ± 0.15</td>
<td>2.119 ± 0.15 (^f)</td>
</tr>
<tr>
<td>Total Antioxidant capacity (TAC; mM)</td>
<td>3.39 ± 0.14</td>
<td>4.49 ± 0.13 (^g)</td>
</tr>
<tr>
<td>Superoxide Dismutase (SOD; Units/µL)</td>
<td>0.0356 ± 0.0009</td>
<td>0.0364 ± 0.0007</td>
</tr>
<tr>
<td>Malonaldehyde (MDA; nmol/L)</td>
<td>2054.70 ± 170.65</td>
<td>2058.24 ± 106.31</td>
</tr>
</tbody>
</table>

\(^a\)Vs Non-exposed Total GSH; \(^b\)Vs Non-exposed Total GSH; \(^c\)Vs Non-exposed reduced GSH; \(^d\)Vs Non-exposed reduced GSH; \(^e\)Vs Non-exposed reduced GSH; \(^f\)Vs Non-exposed ratio; \(^g\)Vs Non-exposed TAC; \(^h\)Vs Non-exposed TAC; \(^i\)Vs LAD TAC.
3.3 Total antioxidant capacity (TAC) in seminal plasma of exposed subjects:

Total antioxidant capacity was determined to find out the effect of chronic workplace radiation exposure on the total enzymatic and non-enzymatic antioxidant activity in the seminal plasma. Similar to seminal GSH level, TAC level in the exposed subjects were significantly (\(P<0.0001\)) increased to 4.49 ± 0.13 compared to non-exposed subjects (3.39 ± 0.14). Further, the TAC level in the HAD group was increased to 4.75 ± 0.17 compared to LAD (4.14 ± 0.20), and this difference was statistically significant (\(P<0.05\)). In addition, there was a significantly higher level of TAC level in the LAD (\(P<0.01\)) and HAD (\(P<0.0001\)) groups compared to non-exposed subjects (Fig 3; Table 1).
Fig 3: Total antioxidants capacity (TAC) in the exposed and non-exposed subjects:

![Bar chart showing TAC levels for Non-exposed, Exposed, LAD, and HAD groups.](image)

Note: *p < 0.0001 vs Non-exposed; *p < 0.01 vs Non-exposed; *p < 0.0001 vs Non-exposed; *p < 0.05 vs LAD

Fig 4: Level of superoxide dismutase (SOD) concentration in the exposed and non-exposed subjects

![Bar chart showing SOD levels for Non-exposed, Exposed, LAD, and HAD groups.](image)
3.4 Superoxide dismutase (SOD) level in the seminal plasma of exposed subjects:

The level of SOD in the exposed and non-exposed group was 0.0364 ± 0.0007 and 0.0356 ± 0.0009 Units/µL respectively and the difference was not significant. Similarly, SOD level in the LAD group was 0.0363 ± 0.0012 whereas HAD group had 0.0365 ± 0.001 nmol/L and this difference was not significantly different (Fig 4; Table 1).

3.5 Lipid peroxidation level in the seminal plasma of the exposed subjects:

The extent of lipid peroxidation in both non-exposed and exposed subjects was evaluated by the assessment of malonaldehyde (MDA) level in the seminal plasma. The level of MDA in the exposed group was 2058.24 ± 106.31 nmol/L, which was not significantly different from the non-exposed group (2054.70 ± 170.65). Further, the concentration of the MDA in the LAD and HAD group was 2019.25 ± 171.22, 2086.67 ± 136.34 nmol/L respectively and this was not significant (Fig 5; Table 1).

Fig 5: Lipid peroxidation level in the exposed and non-exposed subjects
3.6 Association between radiation exposure level and antioxidants & lipid peroxidation in the radiation exposed subjects:

An attempt was made to find out the association between the radiation exposure level and the antioxidants and lipid peroxidation level in the exposed subjects. The radiation exposure level in the exposed subjects failed to exhibit any association with total GSH (R= 0.08), reduced GSH (R= 0.05), oxidized GSH (R= 0.01) and reduced/oxidized GSH ratio (R= 0.05) (Fig 6A-D). Further, radiation exposure level also failed to show any association with SOD (R= 0.04) and lipid peroxidation (R= 0.01) (Fig 6F, 6G). However, TAC level in the exposed subjects showed positive correlation with radiation exposure level in the health workers (R= 0.20) (Fig 6E).
Fig 6: Association between antioxidants, lipid peroxidation and radiation exposure level in the exposed subjects (A-G)
3.7 Association between total glutathione (GSH) level and sperm DNA damage in the exposed and non-exposed subjects:

An attempt was made to determine the association between the sperm DNA damage and the seminal plasma GSH level. The total GSH of the exposed subjects showed a weak positive correlation with OTM ($R = 0.15$) and tail DNA ($R = 0.23$). However, total GSH showed a good positive correlation with alpha T value ($R = 0.48$) but failed to demonstrate any correlation with the TUNEL positive spermatozoa ($R = 0.11$) (Fig 7). Total GSH of the non-exposed subjects showed weak negative correlation with OTM ($R = 0.15$), tail DNA ($R = 0.21$) and TUNEL positive spermatozoa ($R = 0.16$) but showed a weak positive correlation with alpha T value ($R = 0.22$) (Fig 7).

**Fig 7: Association between total GSH level and sperm DNA damage in the exposed and non-exposed subjects**
Reduced GSH level of the exposed subjects showed a weak negative correlation with OTM (R= 0.16), tail DNA (R= 0.15), TUNEL positive spermatozoa (R= 0.14), however, it showed a positive correlation with alpha T value (R= 0.23). In addition, reduced GSH in the non-exposed subjects showed a positive correlation with tail DNA (R= 0.28) but failed to demonstrate any correlation with OTM (R= 0.11), TUNEL positive spermatozoa (R= 0.06) and alpha T value (R= 0.1) (Fig 8).
Oxidized GSH of the exposed subjects failed to show any association with OTM (R=0.02), tail DNA (R=0.01) but showed a weak negative correlation with the TUNEL positive spermatozoa (R=0.11). However, oxidized GSH in the exposed subjects showed a strong positive correlation with αT value (R=0.46), suggesting that as the level of radiation exposure increases oxidized GSH increased in the health workers. Further, oxidized GSH in the non-exposed subjects showed a weak negative correlation with TUNEL positive spermatozoa but failed to show any association with the OTM (R=0.04), tail DNA (0.03) and αT value (R=0.09) (Fig 9).
3.8 Association between total antioxidant capacity (TAC) and sperm DNA damage in the exposed and non-exposed subjects:

The TAC of the exposed subjects showed a negative correlation with OTM (R= 0.24), tail DNA (R= 0.27) and TUNEL positive spermatozoa (R= 0.21). However, it showed a strong positive correlation with the alpha T value (R= 0.57), which means as the level of DNA damage in the spermatozoa increases, TAC level in the seminal plasma also increased (Fig 10). Further, TAC in the non-exposed subjects showed weak positive correlation with tail DNA (R= 0.24), TUNEL positive spermatozoa (R= 0.21) and alpha T value (R= 0.32) but failed to demonstrate any association with OTM (R= 0.02) (Fig 10).

Fig 10: Association between total antioxidant capacity (TAC) and sperm DNA damage in the exposed and non-exposed subjects
3.9 Association between superoxide dismutase (SOD) and sperm DNA damage in the exposed and non-exposed subjects:

The SOD level in the exposed subjects failed to demonstrate any correlation with OTM (R= 0.09), tail DNA (R= 0.01) and TUNEL positive spermatozoa (R= 0.08) but showed a weak negative correlation with alpha T value (R= 0.17) (Fig 11). Similarly, non-exposed subjects also failed to demonstrate any correlation with tail DNA (R= 0.01), TUNEL positive spermatozoa (0.11) and alpha T value (0.1) but showed a good positive correlation with OTM (R= 0.27) (Fig 11).

Fig 11: Association between superoxide dismutase (SOD) and sperm DNA damage in the exposed and non-exposed subjects
3.10 Association between lipid peroxidation and sperm DNA damage in the exposed and non-exposed subjects:

The lipid peroxidation was assessed by estimation of the MDA level in the seminal plasma. The MDA level in the exposed subjects showed a weak negative correlation with OTM (R= 0.15), tail DNA (R= 0.08) and αT value (R= 0.1). However, it showed a weak positive correlation with TUNEL positive spermatozoa (R= 0.19). Further, MDA level in the non-exposed subjects failed to demonstrate association with OTM (R= 0.05), tail DNA (R= 0.01), TUNEL positive spermatozoa (R= 0.05) and αT value (R= 0.08) (Fig 12).

Fig 12: Association between lipid peroxidation and sperm DNA damage in the exposed and non-exposed subjects
3.11 Influence of confounding factors on study parameters:

To rule out the effect of confounding factors such as alcohol consumption, smoking and diet on the present outcome, the cross tabulation of the smokers, alcoholics and diet in both the exposed and non-exposed groups was performed (Table 1). The percentage of subjects who was consuming alcohol in the non-exposed subjects was 72.5% whereas 67.5% subjects from exposed group was consuming alcohol and the difference was not significant. In addition, the percentage of alcoholics subjects in the LAD (71.43%) and HAD (64.58%) group was also not significantly different. Further, the percentage of smokers in the non-exposed and exposed subjects was 68.6%, 67.8% respectively and the difference was not significant (Table 1). Similarly, the percentage of smokers in the LAD (34.29%) and HAD (27.08%) groups was not significant. The effect of diet pattern on the present outcome was also studied. The percentage of mixed diet subjects in the exposed group was 91.57% whereas an 86.28% subject in the non-exposed group was mixed diets and this difference was not statistically different. Similarly, the percentage of mixed diet subjects in the LAD and HAD group was 94.29%, 89.58% respectively which was not significant (Table 1).

The multivariate analysis using analysis of covariance (ANCOVA) was also performed to confirm the effect of these potential factors on present outcome (Table 2). However, these factors did not show any effect on all the parameters studied which suggest that the confounding factors did not influence the study results.
Table 2: Multivariate analysis of the confounding factors on antioxidants and lipid peroxidation in the recruited subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Alcohol</th>
<th>Smoking</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>No</td>
<td>Vegetarian</td>
</tr>
<tr>
<td>αT value (Mean ± SEM)</td>
<td>0.2853±0.0026</td>
<td>0.2822±0.0011</td>
<td>0.2838±0.0042</td>
</tr>
<tr>
<td>Total GSH (Mean ± SEM)</td>
<td>1.25±0.09</td>
<td>1.27±0.06</td>
<td>1.04±0.1</td>
</tr>
<tr>
<td>Reduced GSH (Mean ± SEM)</td>
<td>0.86±0.06</td>
<td>0.94±0.06</td>
<td>0.87±0.183</td>
</tr>
<tr>
<td>Oxidized GSH (Mean ± SEM)</td>
<td>0.45±0.07</td>
<td>0.43±0.04</td>
<td>0.34±0.08</td>
</tr>
<tr>
<td>TAC (Mean ± SEM)</td>
<td>4.284±0.21</td>
<td>4.22±0.13</td>
<td>4.257±0.5</td>
</tr>
<tr>
<td>SOD (Mean ± SEM)</td>
<td>0.0344±0.001</td>
<td>0.0355±0.0007</td>
<td>0.03614±0.0019</td>
</tr>
<tr>
<td>MDA (Mean ± SEM)</td>
<td>2227.52±181.35</td>
<td>2173.91±118.56</td>
<td>1830.21±241.57</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>Yes</th>
<th>Mixed</th>
</tr>
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<tbody>
<tr>
<td>αT value (Mean ± SEM)</td>
<td>0.2797±0.0010</td>
<td>0.2819±0.0025</td>
<td>0.2812±0.0011</td>
</tr>
<tr>
<td>Total GSH (Mean ± SEM)</td>
<td>1.16±0.05</td>
<td>1.03±0.05</td>
<td>1.21±0.04</td>
</tr>
<tr>
<td>Reduced GSH (Mean ± SEM)</td>
<td>0.88±0.54</td>
<td>0.7±0.05</td>
<td>0.86±0.042</td>
</tr>
<tr>
<td>Oxidized GSH (Mean ± SEM)</td>
<td>0.37±0.03</td>
<td>0.34±0.03</td>
<td>0.41±0.03</td>
</tr>
<tr>
<td>TAC (Mean ± SEM)</td>
<td>3.99±0.13</td>
<td>3.73±0.18</td>
<td>4.049±0.11</td>
</tr>
<tr>
<td>SOD (Mean ± SEM)</td>
<td>0.0368±0.0007</td>
<td>0.0372±0.0009</td>
<td>0.03607±0.0006</td>
</tr>
<tr>
<td>MDA (Mean ± SEM)</td>
<td>1981.66±102.78</td>
<td>1800.50±118.88</td>
<td>2080.3±97.09</td>
</tr>
</tbody>
</table>

*aP< 0.05; bP< 0.05
4. DISCUSSION

In chapter 3, results have demonstrated the detrimental effects of occupational radiation exposure on semen characteristics and genetic/epigenetic integrity of the spermatozoa. The key findings in this study were: 1) an adaptive response to chronic occupational exposure as the level of antioxidants are higher and no significant correlation with MDA level and 2) direct correlations between seminal plasma antioxidant status and sperm chromatin integrity in the radiation health workers. These findings indicated the functioning of antioxidant defense mechanism in the seminal plasma to protect the functional and genetic integrity of the spermatozoa.

Oxidative stress refers to an imbalance between excessive production and accumulation of reactive oxygen species (ROS) and an impaired antioxidant mechanism (Sikka, 2001; Agarwal et al., 2003). A high level of ROS in human seminal plasma is related to poor sperm morphology, poor motility and a low sperm concentration (Aitken, 1989). Similarly, an excessive accumulation of ROS such as superoxide radicals (O$_2^-$) or hydrogen peroxide (H$_2$O$_2$) can cause human sperm plasma membrane damage and DNA damage in both mitochondrial and nuclear genomes (Aitken and Krausz, 2001). Moreover, it can freely cross cell membranes and cause DNA damage (Halliwell, 1997; Baumber et al., 2003). It has been shown that seminal oxidative stress can be induced by over-production of ROS through blood cells, dead and immature spermatozoa which is one of the important causes of male infertility. In contrast, seminal plasma is well equipped with an array of antioxidant defense mechanisms to protect the spermatozoa.
against oxidants. Antioxidant rich seminal plasma probably compensates the deficiency in cytoplasmic antioxidant enzymes and molecules in the spermatozoa (Agrawal et al., 2003). Specifically, free radical scavengers in seminal plasma such as SOD and GSH have found to be very crucial in reducing the oxidative stress to the spermatozoa (Potts et al., 2000). These considerations represent the rationale for the investigation of antioxidant defenses in the seminal plasma of occupationally exposed subjects presented in this study. Our data suggest an efficient antioxidant response as shown by increased production of GSH, the major antioxidant, in the exposed population which is probably helping to protect the spermatozoa from the deleterious effects of long-term radiation exposure.

Prolonged exposure to high levels of testicular toxicant such as ionizing radiation may produce testicular atrophy thereby shutting off spermatogenesis. However, at lower levels, the adverse effect may be limited to only changes in the motility pattern without affecting fertility significantly (Perreault & Cancel, 2001). The International Commission on Radiological Protection (ICRP), in its 2007 recommendations based on occupational classification, limits artificial irradiation of the public to an average of <1 mSv of effective dose per year, and ≤ 6 or ≤ 20 mSv/year in the case of occupationally exposed subjects (ICRP, 2007). Since occupational radiation exposure levels now strictly fall well within the accepted limits (Maffei et al., 2002), the long-term consequence of low level exposure is possibly observed as altered seminal plasma antioxidants as an adaptive response in the exposed subjects.
A number of compensatory and preparatory mechanisms are activated in response to the damage caused by ionizing radiation. In the present study, we have observed an increase in the seminal plasma GSH and TAC concentrations. Our results are in agreement with the previous finding by Durović et al., (2008) who observed an increase in antioxidant enzymes as a protection against the increased production of ROS during occupational exposure. Recently, enhanced cellular antioxidant defense to chronic radiation exposure in the blood plasma of the interventional cardiologists has been considered as an adaptive response (Russo et al., 2012).

GSH is an abundant tripeptide antioxidant molecule present in the cytoplasm and body fluids. It plays an important role in many biological processes including the protection against damage produced by oxidants, electrophiles, and free radicals due to its ability to react directly with hydrogen peroxide and superoxide-anion, hydroxyl, and alkoxyl radicals by its free sulphhydryl groups (Meister and Anderson, 1983). The scavenging properties of GSH are mainly due to its function as cofactor for antioxidant enzyme glutathione peroxidase (GPX) and also its ability to react directly with ROS by donating hydrogen ion (H+) from its free sulfhydryl groups. It neutralizes the cytotoxic aldehydes produced during lipid peroxidation and thus protects the sperm plasma membrane (Sorenson et al., 1999). Catalyzed by glutathione peroxidase, GSH reduces H₂O₂ to H₂O. Glutathione disulfide, thus formed, is converted back to glutathione by glutathione reductase. This “glutathione cycle” has been demonstrated to be an effective, intracellular defence mechanism against a variety of oxidant stresses (Kosower et al., 1971). Higher levels of total GSH and reduced GSH levels in seminal plasma indicate that GSH plays a
significant role in protection of spermatozoa against oxidative damage and also improves the sperm motility and morphology (Atig et al., 2012). Whenever the tissues are challenged with oxidative stress, the body tries to overcome the deleterious effect by elevating the expression of antioxidant enzymes and antioxidant molecules as an adaptive response (Limón-Pacheco and Gonsebatt, 2009). Similar response has been observed in the occupationally exposed group in the present study. Radiation is known to induce oxidative stress, mainly through hydrolysis of water. Further, the level of total GSH and reduced GSH were significantly higher in HAD group which further justifies the argument that elevated oxidative stress induced by chronic low dose radiation exposure induces adaptive response in biological fluid such as seminal plasma. Similar findings are reported in the literature which has demonstrated high antioxidant level in blood of radiation health workers (Durovic et al., 2008, Russo et al., 2012).

Total antioxidant capacity (TAC) is the total capacity of antioxidants to scavenge harmful free radicals in the biological sample and used as specific biomarker for assessing the oxidative stress in spermatozoa (Pahune et al., 2013). The detection of TAC in the seminal plasma is considered as one of the most reliable and simple tests for the diagnosis of male infertility (Mahfouz et al., 2009) as lower TAC levels were observed in seminal plasma of subfertile men as compared with fertile men (Lewis et al., 1995, 1997; Smith et al., 1996). Interestingly, this study showed a significant increase in TAC in the seminal plasma of the exposed subjects especially between HAD and LAD group which suggests an association between antioxidant defense and high radiation absorbed dose in the health workers.
Studies have demonstrated inverse relation between seminal plasma TAC or GSH levels and sperm morphology (Smith et al., 1996; Raijmakers et al., 2003). In contrast, our study has demonstrated a direct correlation between seminal plasma total GSH, TAC and sperm chromatin integrity especially with flow based SCSA method as this is one of the most common and sensitive test employed in assessing sperm DNA integrity in epidemiological studies. Since sperm chromatin integrity is known to be affected by ROS, it is possible that as a survival strategy, antioxidant defense functions effectively activated to counteract ROS mediated detrimental effects to sperm functions in occupationally exposed subjects to radiation. Superoxide dismutase enzymes are present in both spermatozoa (Alvarez et al., 1987) as well as seminal plasma (Mennella and Jones, 1980). In the present investigation the assessment of SOD activity and MDA level in seminal plasma, did not show strong correlation with chromatin integrity. It is possible that since spermatozoa come in contact with the seminal plasma only after ejaculation and the oxidative stress induced by occupational radiation exposure for prolonged period are expressed in male germ cells, estimation of SOD and lipid peroxidation in spermatozoa itself might have given a better correlation with DNA integrity of spermatozoa.

It has been shown that confounding factors such as smoking, alcohol, and diet can influence the plasma oxidants and genetic damage induced by ionizing radiation (ICRP, 2007) in humans possibly by increasing the radio sensitivity of the cells (Wang et al., 2000). However, cross-tabulation analysis of smokers and alcoholics did not reveal any significant difference between the two groups which excludes the possible influence of
confounding factors on our results. In contrast, multivariate analysis showed further decrease in the TAC concentration of the alcoholics subjects compared to non-alcoholics. Alcohol consumption may reduce the concentration of total antioxidant activity in the human blood serum. Further, alcohol consumption is also shown to diminish both serum and brain defense mechanisms against free radical attack, which might result in many diseases, which may be by decreasing the total antioxidant activity (Sevil and Idris, 2008). In addition to this, the level of lipid peroxidation was further increased in the non-vegetarian subjects.

In conclusion, this study distinctly shows that there is altered sperm chromatin integrity in radiation health workers which is also associated with elevated level of seminal plasma antioxidants, especially GSH and TAC. Further, the increased seminal plasma GSH and TAC could be an adaptive measure to tackle the oxidative stress to protect genetic and functional sperm deformities in radiation health workers.