1. INTRODUCTION

Physical and chemical hazards encountered at the workplace during the adulthood are best documented in epidemiological research. Reproductive hormones such as follicle stimulating hormone (FSH), leutinizing hormone (LH) and testosterone are considered as the most important predictors of semen quality in epidemiologic studies (Jensen et al., 1997; Uhler et al., 2003; Steven and Katherine, 2014). Several studies have reported that semen quality is associated with reproductive hormones in men (Sina et al., 1975; Subhan et al., 1995; Jensen et al., 1997; Mahmoud et al., 1998; Meekar et al., 2007) and possible disruption of hormonal regulation due to low exposure of environmental or occupational hazards on humans (Guevel and Pakdel, 2001; Welshons et al., 2003).

Spermatogenesis is a complex process which is regulated by several endocrine and paracrine signals. The release of gonadotropin releasing hormone (GnRH), from the hypothalamus is considered as master regulator of the spermatogenesis which is very important for the proliferation, differentiation and survival of the germ cells (McLachlan et al., 2002a; Ruwanpura et al., 2010). FSH supports the spermatogonial development whereas, testosterone partly supports spermatocyte maturation (McLachlan et al., 2002a; Ruwanpura et al., 2008a,b) and spermiation. Further, both testosterone and FSH is required for the release of spermatids from Sertoli cells (McLachlan et al., 2002a) while LH acts on the Leydig cells which synthesize the testosterone hormone (Wahlstrom et al., 1983).
The potential impact of chronic occupational exposure to pesticides has shown to induce damage to sperm chromatin, decrease semen quality and reproductive hormones (Yucra et al., 2006; Miranda et al., 2013). Similarly, occupational exposure to occupational hazards can alter the FSH and LH concentration (Gustafson et al., 1989; Ng et al., 1991) which reflects both primary and secondary effects on the testes and the hypothalamo-pituitary-testicular (HPT) axis. Most of this physical and chemical hazards acts on the male reproductive system by disrupting the hypothalamo-pituitary-testicular (HPT) axis. In contrast, till now influence of occupational radiation exposure on the male reproductive hormone was not elucidated so far. In light of these considerations, the study was planned to investigate the possible influence of occupational radiation exposure on FSH, LH and testosterone level in a chronically exposed population.

2. MATERIALS AND METHODS

2.1 Separation of blood plasma

Peripheral blood was obtained from each subject by venipuncture in a heparinized sterile tube. Immediately after collection, blood was centrifuged at 3000 rpm for 10 min and blood plasma was obtained. Blood plasma was aliquoted in at least four small cryovial and stored at -80°C till the assessment of the reproductive hormones after the subject coding. One cryovial containing blood plasma was taken during the analysis of one hormone. All the assessment and result analysis was carried out blindly.
2.2 Assessment of reproductive hormones in the exposed and non-exposed subjects:

Hormonal evaluation in blood plasma has been performed by means of a commercial Enzyme Linked Immuno Sorbent Assay (ELISA) kit following the manufacturer's instructions (Human GmbH Diagnostics, Germany). Assessment of FSH (Cat. No: 53020), LH (Cat No: 53010) and testosterone (Cat. No: 55010) was performed in the blood plasma of the both exposed and non-exposed subjects. All the tests were performed in duplicates and a mean value were considered for the data analysis.

2.2.1 Quantitative determination of luteinizing hormone (LH) level in blood plasma:

The ELISA for direct antigen detection uses the high affinity of biotin for streptavidin, which has been coated on the surface of microliter well. Briefly, 50µL of calibrators and
blood plasma were added in the pre-coated microliter well. Again, 100µL of enzyme conjugate were mixed to form a specific immuno complex, which was bound to the surface of the wells by the interaction of biotin with the immobilized streptavidin. This mixture were mixed gently and incubated for 60 min at room temperature. At the end of the incubation, excess enzyme conjugate and monoclonal antibodies were washed out. After this, 100µL substrate was added and the resulting color, which turns in to yellow after stopping the reaction with the stop solution (50µL) was measured with the help of ELISA reader at 450nm. The intensity of color was directly proportional to the LH concentration in the blood plasma.

2.2.2 Quantitative determination of follicle stimulating hormone (FSH) level in Blood plasma:

Briefly, 50µL of calibrators and blood plasma were added in the microliter well pre-coated with anti-follicle stimulating hormone antibodies. Again, 100µL of enzyme conjugate were mixed to form a specific immuno complex, which was bound to the surface of the microliter wells. This mixture were mixed gently and incubated for 60 min at room temperature. At the end of the incubation, excess enzyme conjugate and monoclonal antibodies were washed out. After this, 100µL substrate was added and incubated for 15 min at room temperature. The reaction was terminated by addition of 50µL of stop solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is directly proportional to the amount of FSH in the sample and the intensity is measured at 450nm with the help of ELISA reader.
2.2.3 Quantitative determination of testosterone level in blood plasma:

This test is based on competitive interaction of testosterone and the hormone enzyme conjugate for a limited number of monoclonal anti-testosterone antibodies. Briefly, 25µL of calibrators and blood plasma were added in the pre-coated microliter well. Thereafter, 200µL of enzyme conjugate were mixed to form a specific immuno complex, which was bound to the surface of the wells. This mixture were mixed gently and incubated for 60 min at room temperature. At the end of the incubation, excess enzyme conjugate and monoclonal antibodies were washed out. After this, 200µL substrate was added and the resulting color, which turns in to yellow after stopping the reaction with the stop solution (100µL), was measured with the help of ELISA reader at 450nm. The intensity of signal is inversely proportional to the amount of testosterone present in the blood plasma.

2.3 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 Follicle stimulating hormone (FSH) level in the exposed subjects:

FSH level was determined to find out the effect of chronic workplace radiation exposure on the FSH level of the recruited subjects. A direct comparison was performed between level of FSH in non-exposed and exposed subjects in blood plasma. The FSH level in the non-exposed subjects was $4.119 \pm 0.139$ which was decreased to $3.886 \pm 0.163$ U/L, but this difference was not significant. Further, there was no difference in FSH level in the LAD ($3.829 \pm 0.159$) and HAD ($3.927 \pm 0.259$) groups (Table 1; Fig 1).

Table 1: Reproductive hormones level in the blood plasma of the exposed and non-exposed subjects

<table>
<thead>
<tr>
<th></th>
<th>Non-exposed (Mean ± SEM)</th>
<th>Exposed (N= 83)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>All subjects</td>
</tr>
<tr>
<td>FSH (U/L)</td>
<td>$4.119 \pm 0.139$</td>
<td>$3.886 \pm 0.163$</td>
</tr>
<tr>
<td>LH (U/L)</td>
<td>$6.695 \pm 0.689$</td>
<td>$5.967 \pm 0.376$</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>$5.854 \pm 0.212$</td>
<td>$5.773 \pm 0.264$</td>
</tr>
</tbody>
</table>
Fig 1: Follicle stimulating hormone (FSH) level in the exposed and non-exposed subjects

Fig 2: Luteinizing hormone (LH) level in the exposed and non-exposed subjects
3.2 Luteinizing hormone (LH) level in the exposed subjects:

The level of LH in the exposed and non-exposed group was 6.695 ± 0.689 and 5.967 ± 0.376 U/L respectively. Although, the LH level was decreased in the exposed subjects compared to non-exposed, it was not significant (Table 1; Fig 2). Similarly, level of LH in the LAD group was 5.938 ± 0.55, whereas HAD group had 5.989 ± 0.515 U/L, and the difference was not significant (Table 1; Fig 2).

3.3 Testosterone level in the exposed subjects:

The level of testosterone in the non-exposed and exposed group was 5.854 ± 0.212 and 5.773 ± 0.267 ng/mL respectively which was not significant. Although, a decline in the 12% in the level of testosterone was observed in the HAD group (5.404 ± 0.352) compared to LAD (6.280 ± 0.382), it was not significant (Table 1; Fig 3).

Fig 3: Testosterone level in the exposed and non-exposed subjects
3.4 Association between the reproductive hormones and radiation exposure level in the exposed subjects:

The association between the FSH, LH, and testosterone with the radiation exposure level was determined in the radiation exposed subjects. FSH showed weak positive correlation with the radiation exposure level ($R = 0.21$; Fig 4A). However, LH ($R = 0.17$) and testosterone ($R = 0.18$) showed weak negative correlation with radiation exposure dose of the radiation exposed subjects (Fig 4B, 4C).
Fig 4: Association between reproductive hormones and radiation exposure level in the exposed subjects
3.5 Association between follicle stimulating hormone (FSH) and sperm concentration in the recruited subjects:

Effect of FSH on the sperm concentration in the exposed and non-exposed subjects was studied. FSH exhibited a weak negative correlation (R= 0.14; Fig 5A) with sperm concentration in the exposed subjects, whereas non-exposed subjects failed to show any meaningful correlation (R= 0.08; Fig 5A). Similarly, total sperm number (TSN) also failed to show any association in both non-exposed (R= 0.06) and exposed subjects (R= 0.05) (Fig 5B).

Fig 5: Association between follicle stimulating hormone (FSH) level and sperm concentration in the exposed and non-exposed subjects

3.6 Association between luteinizing hormone (LH) level and sperm concentration in the exposed and non-exposed subjects:

Effect of LH on the sperm concentration in the exposed and non-exposed subjects was also studied. LH exhibited a weak negative correlation with sperm concentration (R= 0.21) and total sperm number (R= 0.17) in the exposed subjects, whereas non-exposed
subjects showed weak correlation with sperm concentration (R= 0.18) (Fig 6A). However, total sperm number (TSN) did not show any association in non-exposed subjects (R= 0.001; Fig 6B).

**Fig 6: Association between luteinizing hormone (LH) level and sperm concentration in the exposed and non-exposed subjects**

![Fig 6: Association between luteinizing hormone (LH) level and sperm concentration in the exposed and non-exposed subjects](image)

**Fig 7: Association between testosterone and sperm concentration in the exposed and non-exposed subjects**

![Fig 7: Association between testosterone and sperm concentration in the exposed and non-exposed subjects](image)
3.7 Association between testosterone level and sperm concentration in the exposed and non-exposed subjects:

Testosterone exhibited a weak negative correlation with sperm concentration (R= 0.20) and failed to demonstrate any association with total sperm number (R= 0.08) in the radiation exposed subjects, whereas non-exposed subjects showed weak positive correlation with sperm concentration (R= 0.18) and total sperm number (R= 0.12) (Fig 7).

3.8 Influence of potential confounding factors on FSH, LH and testosterone:

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 alcoholic 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% and 67.8% respectively and, the difference was statistically not significant (Table 1). Similarly, there was no difference in the percentage of smokers in the LAD (34.29%) and HAD (27.08%) groups. 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 7, 8, 9). 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 to find out the effect of confounding factors on FSH, LH and testosterone:

<table>
<thead>
<tr>
<th></th>
<th>FSH (Mean ± SEM)</th>
<th>LH (Mean ± SEM)</th>
<th>Testosterone (Mean ± SEM)</th>
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</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>4.060 ± 0.295</td>
<td>6.746 ± 0.913</td>
<td>5.594 ± 0.477</td>
</tr>
<tr>
<td>Yes</td>
<td>3.997 ± 0.380</td>
<td>6.841 ± 1.176</td>
<td>6.726 ± 0.615</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3.964 ± 0.232</td>
<td>6.916 ± 0.719</td>
<td>6.280 ± 0.376</td>
</tr>
<tr>
<td>Yes</td>
<td>4.093 ± 0.421</td>
<td>6.671 ± 1.304</td>
<td>6.040 ± 0.981</td>
</tr>
<tr>
<td>Diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetarian</td>
<td>3.955 ± 0.453</td>
<td>6.578 ± 1.400</td>
<td>6.482 ± 0.732</td>
</tr>
<tr>
<td>Mixed</td>
<td>4.101 ± 0.163</td>
<td>7.009 ± 0.505</td>
<td>5.838 ± 0.264</td>
</tr>
</tbody>
</table>
4. DISCUSSION

The data from the present study indicates that occupational radiation exposure is not associated with the alteration in the level of FSH, LH and testosterone in the health workers. Earlier studies have clearly demonstrated the detrimental effect of occupational hazards like pesticides (Padungtod et al., 1998; Yucra et al., 2006; Miranda et al., 2013) and heavy metals (Gustafson et al., 1989; Ng et al., 1991) on serum reproductive hormones.

Several studies have shown the importance of reproductive hormone such as FSH, LH and testosterone in spermatogenesis (Wahlstrom et al., 1983; McLachlan et al., 2002a; Ruwanpura et al., 2008a,b; 2010) and considered as most important predictor of semen quality in epidemiologic studies function (Jensen et al., 1997; Uhler et al., 2003; Steven and Katherine, 2014). Studies have demonstrated that that semen quality can be associated with changes in reproductive hormones in men (Sina et al., 1975; Subhan et al., 1995; Jensen et al., 1997; Mahmoud et al., 1998; Meekar et al., 2007). Disruption of hormonal regulation due to low exposure of environmental or occupational hazards on humans have been demonstrated by earlier studies (Guevel and Pakdel, 2001; Welshons et al., 2003). Further, these occupational hazards reflects both primary and secondary effect on the testes and HPT axis as an altered sperm functional characteristics in lead (Gustafson et al., 1989; Ng et al., 1991) and pesticides exposed subjects (Yucra et al., 2006; Miranda et al., 2013). However in the present study, FSH, LH and testosterone level was found to be similar in both exposed and non-exposed subjects. This finding is in agreement with observed result in chapter 3, where concentration of spermatozoa was
not different in the exposed and non-exposed subjects. In addition, regression analysis performed in this study did not find any association of occupational radiation exposure to FSH, LH and testosterone in health workers.

In the previous chapters, detrimental effects of occupational radiation exposure on functional, genetic and epigenetic integrity of spermatozoa in health workers were clearly observed. Further, alteration in the plasma antioxidants and chromosomal abnormalities in these subjects was also observed. However, there was no sperm concentration in both exposed and non-exposed subjects in chapter 3, which confirms the normal spermatogenesis in both exposed and non-exposed subjects, suggesting that chronic radiation exposure is not disrupting the endocrine function of the exposed subjects like other occupational hazards (Sina et al., 1975; Subhan et al., 1995; Jensen et al., 1997; Mahmoud et al., 1998; Meekar et al., 2007).

In conclusion, this study distinctly shows that there is no alteration in the FSH, LH and testosterone concentration in radiation health workers and occupational radiation exposure may not act as an endocrine disrupter. However, the exact mechanism of the decline in the sperm functional characteristics in the radiation health workers reported in chapter 3, needs to be studied.