POLYMORPHISMS OF GSTM1 AND GSTT1 GENES CONTRIBUTE TO THE RISK OF AVERSE REPRODUCTIVE OUTCOME IN STEEL INDUSTRY WORKERS

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ABSTRACT

Context: The root causes of many adverse pregnancy outcomes are not well understood, but there is growing evidence that both the environmental and genetic factors play an important role. The present study aims to investigate the association of polymorphisms of GSTM1 and GSTT1 genes with adverse reproductive outcome in steel industry workers.

Methods: The study populations consisted of 150 male steel industry workers in the age group of 18-55 years and 146 males in the same age group and socio economic status and not occupationally exposed to any chemical agents were studied for the reproductive outcome in their spouses. The information on reproductive outcome including the number of pregnancies, fertility, infertility, live births, spontaneous abortions, premature births, neonatal deaths, still births etc. was collected. Blood samples were collected, DNA extraction and genotyping was done for GSTM1 and GSTT1 using multiplex PCR. The study was approved by the Institutional Ethics Committee of the Centre and written informed consent was obtained from all the participants of the study. The results were analyzed statistically using the appropriate chi square test and logistic regression analysis to find the significance of the association of GSTM1 and GSTT1 polymorphisms with reproductive outcome in steel industry workers and control subjects.

Results: The results showed an increase in the frequency of abortions, still births, premature births and neonatal deaths in the workers with homozygous deletions of GSTM1 and GSTT1 but the increase was not statistically significant compared to that of active GSTM1 and GSTT1 variants.

Conclusion: The results did not provide any evidence for the influence of polymorphisms of GSTM1 and GSTT1 genes on the reproductive outcome. Neither GSTM1 nor GSTT1 null variants were associated with adverse reproductive outcome.

INTRODUCTION

Recent epidemiological studies indicated involvement of genetic and environmental factors for the risk of adverse reproductive outcome in males (Fisch et al., 2000; Oliva et al., 2001; Sharpe, 2001; Damgaard et al., 2002; Fisher, 2004) and females (Kamrin et al., 1994; Sharara et al., 1998; Nicolopouloustamati and Pitsos, 2001).

During the last few decades deterioration of male reproductive capacity in industrialized countries as a result of exposure to environmental contaminants (toxic and heavy metals) has been reported (Sengupta et al., 2013). A drastic decline in the sperm count associated with sperm quality over the years was shown by Waissmann et al., (2002). Exposure to heavy metals showed adverse effects on male reproductive system that include size of testis, semen abnormality, semen quality, sperm motility, seminal vesicle, impotency, altered genetic material of sperm, altered spermatogenesis and genetic diseases in offspring (Astrid Sigel et al., 2011; Sengupta et al., 2013). Elbetieha, et al., (1997) observed increased risk of infertility and reduced semen quality among male welders. Xenobiotic compounds are associated with oxidative stress in male reproductive organs which may contribute to adverse reproductive outcome (Aitken and Krausz, 2001; Agarwal and Sushil, 2005; Tremellen, 2008; Turner and Lysiak, 2008). Gerhard et al., (1998) and Kumar,
GSTM and GSTT are the cytosolic enzymes that play a key role in the Phase II detoxification pathways in humans against various physiological and xenobiotic substances and also act as important antioxidants in testis tissues (Listowsky et al., 1998; Strange et al., 2001). They are extensively present in the testis and seminal tubule fluid as well as in the sperm (Hemachand et al., 2002; Mukherjee et al., 1999) and protect germ cells against the damage caused by oxidative stress. Some studies showed that GSTs might be involved in spermatogenesis impairment (Castellon, 1999). The homozygous deletion (null genotype) of the GSTM1 or GSTT1 gene results in the total absence of the enzyme activity and increases the level of oxidative stress resulting in male infertility (Seidegard et al., 1988). Recently we have studied reproductive outcome in steel industry workers and reported infertility and an increase in the frequency of abortions, premature births, still births and neonatal deaths which might be due to undue exposure of workers to steel dust at work place. (Indira Priyadarshini et al., 2017). Thus in this study, we investigated the influence of polymorphisms of GSTM1 and GSTT1 genes on adverse reproductive outcome in steel industry workers.

MATERIALS AND METHODS

150 male steel industry workers in the age group of 18-55 years and 146 males belonging to the same age group and socio economic status and not occupationally exposed to any chemical agent were studied for the reproductive outcome in their spouses. Subjects for the present study were selected among the male workers of the steel industry situated at Patancheru, Hyderabad, India. The information on reproductive outcome including the number of pregnancies, fertility, infertility, live births, spontaneous abortions, premature births, neonatal deaths, still births, etc. was collected.

Peripheral blood samples were collected from all the participants. DNA was extracted and genotyping of GSTM1 and GSTT1 was carried out using multiplex PCR. The study was approved by the Institutional Ethics Committee of the Centre and written informed consent was obtained from all the participants of the study. The results were analyzed statistically using the appropriate chi square test and odds ratio (OR) and 95% confidence intervals (95%CI) were calculated to assess the relative risk conferred by null genotype. In addition, logistic regression analysis was carried out to find the significance of the association of GSTM1 and GSTT1 polymorphisms with reproductive outcome in steel industry workers and control subjects.

Genetic analysis of GSTM1 and GSTT1 gene polymorphisms by multiplex PCR

GSTM1 and GSTT1 genotyping: 5ml blood samples were collected from the male steel industry workers and control subjects and genomic DNA was extracted by Spin column kit (Bangalore Genei, India). Multiplex PCR assay was used for analyzing the GSTM1 and GSTT1 gene deletions. To detect the GSTM1 deletion, the following primers was used: Forward primer 5’ GAA CTC CCT GAA AAG CTA AAGC 3’ and Reverse primer 5’ GTT GGG CTC AAA T 3’. For GSTT1, Forward primer 5’ TTC CCT CTG CCT CAC ATCTC- 3’ and Reverse primer 5’ TCACCGGATCATGGCCAGCA-3’ were used .The PCR amplified products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and the results were documented using a gel documentation system. The presence of GSTM1 and that of GSTT1 genes were indicated by the resulting 215 and 480 bp PCR amplicons, respectively. As an internal control, HAB was amplified (350bp) using the primers, HAB F (5’-CAACTTCATCCAGTTCACC-3’) and HAB R (5’-GAAGAGCCAAGGACAGTAC-3’) for the authentication of multiplex PCR.

### Table 1

<table>
<thead>
<tr>
<th>Genotype (Case/Control)</th>
<th>Steel Industry Workers (n=150)</th>
<th>Controls Subjects (n=146)</th>
<th>OR(95% Confidence interval) p value</th>
<th>Steel Industry Workers (n=150)</th>
<th>Controls Subjects (n=146)</th>
<th>OR(95% Confidence interval) p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1 Active (94/95)</td>
<td>3(3.1)</td>
<td>2(2.1)</td>
<td>Reference 0.58&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1(1.0)</td>
<td>0</td>
<td>Reference 1.0&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Null(56/51)</td>
<td>6(10.7)</td>
<td>3(5.8)</td>
<td>1.9(0.39-10.3)</td>
<td>2(3.5)</td>
<td>1(1.9)</td>
<td>1.8(0.12-53.3)</td>
</tr>
<tr>
<td>GSTT1 Active (92/91)</td>
<td>4(4.3)</td>
<td>1(1.0)</td>
<td>Reference 1.0&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1(1.0)</td>
<td>0</td>
<td>Reference 1.0&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Null(58/55)</td>
<td>5(8.6)</td>
<td>4(7.2)</td>
<td>1.2(0.26-5.72)</td>
<td>2(3.4)</td>
<td>1(1.8)</td>
<td>1.9(0.13-55.4)</td>
</tr>
</tbody>
</table>

Note: Differences in frequencies between the subjects and control groups were analyzed for statistical significance using logistic regression analysis. Odds ratios(OR) are reported with 95% confidence limits, NS = not statistically significant (P > 0.05).

### Table 2

<table>
<thead>
<tr>
<th>Genotype (Case/Control)</th>
<th>Steel Industry Workers (n=150)</th>
<th>Controls Subjects (n=146)</th>
<th>OR(95% Confidence interval) p value</th>
<th>Steel Industry Workers (n=150)</th>
<th>Controls Subjects (n=146)</th>
<th>OR(95% Confidence interval) p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSTM1 Active (94/95)</td>
<td>1(1.0)</td>
<td>1(1.0)</td>
<td>Reference 0.81&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>2(2.1)</td>
<td>1(1.0)</td>
<td>Reference 1.0&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Null(56/51)</td>
<td>5(8.9)</td>
<td>3(5.8)</td>
<td>1.5(0.30-8.84)</td>
<td>3(5.3)</td>
<td>2(3.9)</td>
<td>1.4(0.17-12.48)</td>
</tr>
<tr>
<td>GSTT1 Active (92/91)</td>
<td>2(2.1)</td>
<td>2(2.1)</td>
<td>Reference 0.72&lt;sup&gt;NS&lt;/sup&gt;</td>
<td>1(1.0)</td>
<td>0</td>
<td>Reference 1.0&lt;sup&gt;NS&lt;/sup&gt;</td>
</tr>
<tr>
<td>Null(58/55)</td>
<td>4(6.8)</td>
<td>2(3.6)</td>
<td>2(0.29-16.2)</td>
<td>4(6.8)</td>
<td>3(5.4)</td>
<td>1.2(0.22-7.67)</td>
</tr>
</tbody>
</table>

Note: Differences in frequencies between the subjects and control groups were analyzed for statistical significance using logistic regression analysis. Odds ratios(OR) are reported with 95% confidence limits, NS = not statistically significant (P > 0.05).
The PCR protocol included an initial denaturation temperature of 94 °C (5 min) followed by 35 cycles of amplification (denaturation at 94 °C for 1 min, annealing at 59 °C for 1 min and extension at 72 °C for 1 min). A final 10 min extension step (72 °C) terminated the process. The final PCR products were visualized in ethidium bromide stained gel. Individuals with active (+) genotype of GSTM1 will have 215 bp band while the individuals with null (-) genotype of GSTM1 will not have this band. Similarly individuals with active (+) genotype of GSTT1 will have 480 bp band while the individuals with null (-) genotype of GSTT1 will not have this band.

Statistical Analysis

The results were analyzed statistically using the appropriate chi squared test and odds ratio (OR) and 95% confidence intervals (95%CI) were calculated to assess the relative risk conferred by a null genotype and also to assess the relationship between GSTM1 and GSTT1 gene polymorphisms with adverse reproductive outcome in steel industry workers. In addition, logistic regression analysis was done to find the significance of the association of GSTM1 and GSTT1 polymorphisms with reproductive outcome in steel industry workers and control subjects. The results were considered to be significant at p values of less than 0.05 (indicated by *). Genotype frequencies were checked for deviation from Hardy-Weinberg equilibrium and were not significantly different from those predicted.

RESULTS

The results on the frequency of abortions, premature births, neonatal deaths and still births of steel industry workers with GSTM1 and GSTT1 gene polymorphisms are presented in Tables 1-2.

The results showed an increase in the frequency of abortions, premature births, neonatal deaths and still births in the spouses of steel industry workers with null genotypes of GSTM1 and GSTT1 when compared to controls. However, the logistic regressions analysis showed no significant increase in all the parameters.

DISCUSSION

The aim and purpose of reproductive epidemiology in the industrial workers is to promote, protect, and restore good health and reduce incidence of reproductive problems by understanding the risk factors in industry workers. In the early 1980s, Levin (1983) and Baird et al., (1986) carried out epidemiological research related to adverse reproductive outcomes. Recent epidemiologic studies have shown that both genetic and environmental factors are responsible for adverse reproductive outcome (Edward et al., 2005; Ramos, 2008; Edwards, 2007).

We have shown an increased frequency of abortions, stillbirths, neonatal deaths and a significant decrease in live births in spouses of steel industrial workers as a result of occupational exposure to steel dust at work place (IndiraPriyadarshini et al., 2017).

The steel dust contains nickel, chromium, iron, manganese, cobalt, tungsten, molybdenum and vanadium which are carcinogenic and mutagenic (Cornelia 2002). Thus the adverse effects might be due to exposure to complex mixtures of these heavy metals whose combined effect may be greater than the sum of their individual effects on reproductive health. Earlier studies carried out in the workers exposed to nickel, chromium, iron, manganese and lead showed adverse effects in both male and female reproductive systems at the workplace (Baranski et al., 1993, Bonde et al., 1999; Danadevi et al., 2003, Kumar et al., 2005, Sengupta, 2012, Agrawal et al., 2012, IndiraPriyadarshini et al., 2017). Although pregnancy loss is a common occurrence, its environmental determinants are largely unknown. Heavy metals are considered as environmental teratogens, and exposure could contribute to pregnancy loss (Gardella and Hill, 2000). It has been reported that the both null genotypes of GSTM1 and GSTT1 are associated with a reduced survival rate in women with epithelial ovarian cancer (Howells et al., 1998). Tina et al., (2000) have shown a reduced quantity and quality of semen in men exposed to welding metals. Further, genetic polymorphism in xenobiotic metabolizing genes may influence the effect of environmental contaminants causing adverse reproductive outcomes such as preterm delivery (Mustafa et al., 2013). Mustafa et al., (2010) showed that GSTM1/GSTT1 (null) genotype may be one of the associated genetic factors for the increased risk of PTL. In this context, the present study was taken up to understand the influence of GSTM1 and GSTT1 gene polymorphisms on the adverse reproductive outcome in male steel industry workers. The GST system includes one of the most important detoxifying genes in protecting cells from oxidative damage (Chen et al., 2002; Quinones et al., 2006). Among the GST’s, GSTM1 preferentially detoxifies carcinogens derived from tobacco, whereas GSTT1 causes the biotransformation of many toxins. Any alterations due to genetic polymorphisms affect the activities of these genes, thereby increasing the genotoxic risk in humans (Peddireddy et al., 2016). It has been demonstrated that GST has a protective role during spermatogenesis in males (Castellon, 1999). Oxidative stress could lead to biological effects in males and females. Studies that have shown the acceleration of spermatozoa apoptosis (Aitken et al., 2012), abnormality of sperm parameters (Badade et al., 2011), decrease of sperm and oocyte fusion capacity (Griveau and Le Lannou, 1997), and damage of DNA integrity in sperm mitochondrial (Aitken et al., 1998) due to oxidative stress in males. These detoxifying genes inactivate xenobiotic compounds especially the heavy metals when the males are occupationally exposed and if this gene is inactive, it results in the male infertility (Sharma, et al., 2004, Axelsson et al., 2010).

In the present study the influence of polymorphisms of GSTM1 and GSTT1 genes on the reproductive outcome was investigated in the steel industry workers. This is first novel study to investigate the association of GSTM1 and GSTT1 gene polymorphisms with reproductive outcome in steel industry workers. The results of the study showed that the differences in the reproductive outcome between null and active genotypes are not statistically significant thus, indicating the absence of an association with polymorphisms of GSTM1 and GSTT1 genes.

Our results are in agreement with that of Suryanarayana et al., (2004) who observed no significant association between GSTM1 and GSTT1 and recurrent pregnancy loss in the South Indian population. However they suggested the occurrence of...
the CYP1A1*2A allele as a probable risk factor in idiopathic recurrent miscarriages.

Our results are in agreement with that of Renato Polimanti et al., (2012) who observed no significant differences in the frequencies of GSTM1 and GSTT1 variants between recurrent miscarriages in Italian women. Zusterzeel et al., (2000) reported no influence of GSTT1 and GSTM1 variants with recurrent early pregnancy loss in Caucasian populations. Nonaka et al., (2011) also observed no difference in the distribution of GSTM1 and GSTT1 genotypes in recurrent pregnancy loss in Japanese populations in relation to smoking or consumption of coffee or alcohol.

Sena et al., (2009) and Aydemir et al., (2007) have studied GSTM1 and GSTT1 genotypes association with infertility in males. Aydemir et al., (2007) observed significant association with GSTT1 with idiopathic infertility in males. They did not find significant association of GSTM1 variant with idiopathic infertility. Sena et al., (2009) studied the association of GSTM1 and GSTT1 variants with infertility in Turkish males and observed significant association only with GSTT1 gene. Olshan et al., (2010) from the United States of America reported that reduced sperm concentration and semen count in fertile men were associated with the GSTT1 non-null genotype.

Contrary to our findings, Tang et al., (2012) conducted a study and reported both GSTM1 and GSTT1 null genotypes may predispose sperm to increased oxidative damage in infertile males with varicocele in Northwestern China. Li et al., (2013) carried out meta-analysis of the studies on the association of GSTs with male infertility and showed that GSTM1 null genotype contributed to increased risk of male idiopathic infertility in Caucasians while males with dual null genotype of GSTM1/GSTT1 were particularly susceptible to developing idiopathic infertility. Vani et al., (2010) observed an association of GSTM1 null genotype with male infertility in South Indian population whereas Wu et al., (2008) reported the association of GSTT1 null genotypes with infertility in males in both Asian and Caucasian groups. Finotti et al., (2009) indicated significant association of GSTM1 and GSTT1 null genotypes with idiopathic male infertility and suggested that individuals polymorphic for GSTM1 and GSTT1 genes are susceptible to reduction in sperm quality and infertility. GSTM1 null genotypes were found to be associated with RPL in Japanese (Hirvonen et al., 1996) and North Carolina (Sata et al., 2003) populations. Parveen et al., (2010) revealed an association between the GSTT1 null genotype and the risk of RPL in North Indian subjects. Rohini et al., (2013) studied the association of GSTM1 and GSTT1 with early pregnancy loss (EPL) and showed significant association of GSTT1 null genotype with EPL. Bustamante, et al., (2012) reported increased risk for preterm delivery in Spanish women with GSTM1 deletion.

The overall study revealed that the associations of detoxification genes vary greatly in different studies. This might be due to region selected, ethnicity, life style, habits and the gene-environment interactions. Further studies in more populations from different regions in larger sample size and different environmental settings are worthwhile.

CONCLUSIONS

The study did not provide any evidence for the influence of polymorphisms of GSTM1 and GSTT1 on the reproductive outcome in steel industry workers. Further studies are warranted to generate more information on the association of genetic variability of detoxification genes on reproductive outcome.

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Conflict of Interest

None of the authors of this paper had any personal or financial conflicts of interest.

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