Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6506

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (3):** 214-221 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Association of Polymorphisms of *GSTM1* and *GSTT1* Genes with Male Infertility in Steel Industry Workers

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Received: 22.03.2018 | Revised: 28.04.2018 | Accepted: 5.05.2018

ABSTRACT

Many factors such as exposure to physical and chemical agents, life style, smoking, and alcoholism habit may affect our reproductive health and ability to produce healthy children. The present study aims to investigate the association of polymorphisms of GSTM1 and GSTT1 genes with infertility in male steel industry workers.

Key words: *Epidemiology, infertility, occupational exposure, genetic polymorphism, glutathione-S transferase.*

INTRODUCTION

Over the past two decades, the quality and quantity of human semen has declined in the global population, which has now raised concerns about male fertility[15]. Men are forced to work anywhere under the influence of economic gain, to earn and meet the financial needs of the family. Therefore male reproductive health that faces various risks during his working time should be considered as a pain factor. The emitted material coming from steel industry contains heavy metals such as nickel, chromium, iron, manganese, cobalt, tungsten, molybdenum and vanadium etc. and hence workers exposed to steel dust are at high risk for health and reproductive problems.

Astrid Sigel *et al.*[6] , showed adverse effects of metals on male fertility that include altered genetic material of sperm, altered spermatogenesis, pregnancy loss, genetic diseases in offspring. Nordberg *et al.*[23], reported adverse male reproductive functions that include size of testis, semen quality, seminal vesicles, reproductive endocrine function, impotency, and fertility. Welding fumes could an increased risk of infertility and reduced semen quality among male welders. There are the few studies that explained semen abnormalities correlated with the number of years of exposure to chromium Cr (VI) Elbetieh. Hong lif *et al.*[18], observed occupational exposure to Cr (VI) reduced sperm counts and sperm motility in electroplating workers.

Cite this article: Priyadarshini, U.I., Baluka, V., Prashanth, Ch., Krishna, P. P. and Reddy, P.P., Association of Polymorphisms of *GSTM1* and *GSTT1* Genes with Male Infertility in Steel Industry Workers, *Int. J. Pure App. Biosci.* **6(3):** 214-221 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6506

These metals exposure could cause oxidative stress in steel industry male workers *et al.*[29], Irvine[28] and Krausz[33] showed that genetic problems, health problems, and environmental factors affect sperm quality and its function.

Xenobiotic compounds are associated with oxidative stress in male reproductive organs, which may contribute to adverse reproductive outcome Aitken and Krausz[4]; Agarwal and Sushil[1]; Tremellen[30, 53]; Turner and Lysiak[54].

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[33, 49]. They are extensively present in the testis and seminiferous tubule fluid as well as in the sperm[24, 34], and can protect germ cells against the damage caused by oxidative stress. Some studies showed that GSTs might be associated with male reproduction and might be involved in spermatogenesis impairment[13]. 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, which might be related to male infertility[47]. In this study, we illustrate the association of polymorphisms of GSTM1 and GSTT1 genes with infertility in male steel industry workers.

Heavy metals exposure and its use have risen drastically in the last 10-40 years in steel industries. In this aspect many international and national studies, related to reproductive performance have been carried out in industrial workers. Vani *et al.*[52], identified an association between the GSTM1 null genotype with male infertility in India. Olshan *et al.*[38], from the United States of America reported that reduced sperm concentration and count in semen were associated with the GSTT1 non-null genotype. Safarinejad *et al.*[45], revealed an increased risk of the GSTM1 and/or GSTT1 null genotypes for developing infertility. Da-Ke Xiong *et al.*[57], showed individuals with the combination of GSTM1-present and GSTT1-null were also at higher risk of infertility in Sichuan population, China. Wu *et al.*[55], found that the GSTT1 null genotype was a risk factor for idiopathic infertility in China. Tang *et al.*[50] revealed that GSTM1 and GSTT1 null genotypes increase oxidative damage in infertile men with varicocele. Wu *et al.*[50], conducted meta-analysis of 6934 individuals and showed that the genotype GSTM1 null type was significantly associated with the risk for male infertility in both Asian and Caucasian populations.

Although studies have been carried out on semen quality in welding workers[5, 9, 11, 17, 34, 39], no studies have been carried out on the association of GSTT1 and GSTM1 gene polymorphisms with infertility in male steel industry workers. The aim of our study is to assess the association of GSTT1 and GSTM1 gene polymorphisms as genetic biomarker with infertility in male steel industry workers occupationally exposed to the steel dust.

MATERIAL AND METHODS

150 male steel industry workers and for comparison 146 normal subjects who belonged to the same age group and not occupationally exposed to chemical compounds (control group) were studied for infertility. Subjects for the present study were selected among the male workers of the steel industry situated at Patancheru, Hyderabad, India. After informed consent was obtained, information on reproductive history including the age, occupation, nature of job, duration of service, socio economic status, life style, income, occupational history, tobacco and alcohol use, type of marriage, health problems, family history, spouse health, hormonal, medical, or surgical history of spouse, duration of married life, adoption of family planning procedures were collected for the study. The male subjects with varicocele, infection, other diseases and the spouses with problems related to their

reproductive system including gynec problems are excluded from study. 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.

Genetic analysis of GSTM1 and GSTT1 gene polymorphisms by multiplex PCR:

GSTM1 and GSTT1 genotyping: Genomic DNA was extracted from 200 µL of whole blood 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 primers used were Forward primer (5′ GAA CTC CCT GAA AAG CTA AAGC 3′) and Reverse primer (5′ GTT GGG CTC AAA TAT ACG GTG G-3′). For GSTT1, the primers used were Forward primer (5′-TTC CTT ACT GGT CCT CAC ATCTC- 3′) and Reverse primer R (5′-TCACCGGATCATGGCCA GCA-3′). The PCR amplified products were electrophoreses 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. A DNA sample with GSTM1 and GSTT1 alleles present was run as a positive control in each run. As an internal control, HAB gene was amplified (350bp) using the primers HAB F (5′-CAACTTCATCCACGTTCACC-3′) and 5′-GAAGAGCCAAGGACAGGTAC-3′ for the authentication of multiplex PCR. Individuals with null (–) genotype of GSTM1 did not have 215 bp band while they did have the 350bp band. Similarly the individuals with null (–) genotype of GSTT1 did not have 480 bp band.

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. The size of the GSTM1 was visualized as 215bps, GSTT1 as 480 bps and the HAB internal control as 350 bps fragment.

Statistical Analysis

The results were analyzed statistically using appropriate chi squared test with infertility risk in steel industry workers. In addition, logistic regression analysis was done to find the significance of the association of GSTM1 and GSTT1 polymorphisms with infertility risk 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. Odds ratios and 95% confidence interval (95% CI) were calculated to assess the relationship between GSTM1 and GSTT1 gene polymorphisms with infertility in male steel industry workers.

RESULTS

The comparative analysis of distribution of wild and null genotypes of GSTM1 and GSTT1 is presented in **Table 1.** The results showed an increased frequency of infertility among the workers and control group with null genotypes when compare with the males with wild genotype of GSTM1 and GSTT1 in both the groups. Further analysis showed that the percentage of infertility among null genotypes was higher compared to the controls. While the percentage of infertility was 5.8 among the control group with null genotype, it has increased to 19.6 among the steel industry workers with GSTM1 as shown in **Table 1**.A similar phenomenon was noted among the workers with GSTT1.The statistically analysis of the data by using chi square test and logistic regression analysis showed that the frequency of infertility was significantly high among the workers with null genotypes.

Table 1: Distribution of GSTM1 and GSTT1 genotypes among steel industry workers and control subjects with infertility

Genotype	Steel industry workers(n=150)		Controls Subjects (n=146)		OR (95% CI)	*p* value
	Fertility	Infertility	Fertility	Infertility		
GSTM1	85 (90.4%)	9 (9.57%)	90(94.7%)	5(5.3%)	1.90(0.61- 5.91)	0.26
Present	45 (80.4%)	11 (19.6%)	48(94.1%)	3(5.8%)	3.91(1.02-14.9)	0.04*
Null	85 (92.3%)	7 (7.6%)	87(95.6%)	4(4.4%)	1.79(0.50-6.34)	0.36
GSTT1	45 (77.6%)	13 (22.4%)	51(92.7%)	4(7.3%)	3.68(1.12-12.11)	0.03*
Present						
Null						

P- Value was calculated by $\chi 2$ test with 2 x 2 contingency table and considered <0.05 as significant.*

DISCUSSION

Despite concern about the harmful effects of industrial and agricultural chemicals very little attention has been paid to generate data on reproductive outcome and the association of GSTM1 and GSTT1 gene polymorphisms with the risk to infertility in male steel industry workers. Recently we reported an adverse reproductive outcome in the spouses of steel industry workers[27].

The present study showed evidence for the association of detoxifying gene polymorphisms with infertility in male steel industry workers. The results of this study sound an alarm on the modulating effects of GSTM1 and GSTT1 gene polymorphisms with the risk for infertility among the workers. No studies have been carried out on the association of gene polymorphisms with risk for male infertility in steel industry workers. In this aspect this is the first novel study on the association of GSTM1 and GSTT1 gene polymorphisms with infertility in male steel industry workers. The results of the study showed a significant association of infertility with null genotypes of both GSTM1 and GSTT1 among the workers.

Our results are in agreement with that of Safarinejad *et al.*[45], who showed an increased risk of the GSTM1 and GSTT1 null genotypes for developing infertility in males. Vani *et al.*[52], observed an association of GSTM1 null genotypes with infertility whereas Wu *et al.*[55], reported the association of GSTT1 null genotypes with infertility in males Finotti *et al.*[20], 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.

The GST system is one of the most important detoxifying genes in protecting cells from oxidative damage[14, 41]. It works to 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[7, 48]. It has been demonstrated that GST has a protective role during spermatogenesis in males[13]. Oxidative stress could lead to biological effects in males and females. Studies have shown the acceleration of spermatozoa apoptosis in males[2,] abnormality of sperm quality, parameters[8] and damage of DNA integrity in sperm mitochondria of males due to oxidative stress[3.] Oxidative stress is also regarded as a common etiology of male infertility[30, 53].

Studies that have shown that oxidative stress as the potential risk factor for recurrent miscarriage *et al.*[42], decrease of sperm and oocyte fusion capacity[22] Earlier studies have shown possible association between recurrent miscarriage risk and genetic polymorphisms related to detoxifying genes GSTM1 and GSTT1 *et al.*[40], Nonaka, *et al.*[37],. Nair and Sata[46] showed GSTM1 null polymorphism had been associated with early pregnancy loss risk in women. Bustamante, *et al.*[12], reported increased risk for adverse reproductive outcome (Preterm delivery) in women with GSTM1 deletion.

The steel dust contains nickel, chromium, iron, manganese, cobalt, tungsten, molybdenum and vanadium which are carcinogenic and mutagenic Cornelia[16]. 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. The earlier studies carried out in the workers exposed to heavy metals showed adverse reproductive effects in both male and female[5, 9, 11, 17, 34, 39, 27] Heavy metals are also considered as environmental teratogens, and exposure could contribute to pregnancy loss Gardella and Hill[21] .Tina *et al.*[51] have shown a reduced quantity and quality of semen in man exposed to welding metals. It has been reported that both null genotypes of GSTM1 and GSTT1 are associated with a reduced survival rate in women with epithelial ovarian cancer *et al.*[26],. Further, genetic polymorphism in xenobiotic metabolizing genes may modify the effect of environmental health hazards causing adverse reproductive outcomes such as preterm delivery. Mustafa *et al.*[35], showed that GSTM1/GSTT1 null genotype may be one of the associated genetic factors for the increased risk of preterm labor. The problem of infertility has increased over the past two decades in industrialized countries; nearly 72.4 million couples globally experience fertility problems *et al.*[10], *et al.*[44], .The present study suggests that the absence of active genotypes of the detoxifying genes might result in the adverse reproductive outcomes both in males and females and will continue to increase unless the exposure of man to industrial compounds is minimized.

CONCLUSIONS

The results of study suggest the association of null genotypes of GSTM1 and GSTT1 with infertility in male steel industry workers. The increase in the infertility among the male steel industry workers might be due to the undue exposure to steel dust at work place.

Acknowledgments

The authors wish to thank all the workers who participated in the study. We are also indebted to the management of the steel industries. Our thanks are also due to Shri Motilal Jain, Chairman, Shri Mahendra Ranka, Vice-chairman, Shri Sushil Kapadia, Managing Trustee and Shri Ashok Kothari, Trustee Treasurer of our Research centre for their support and for providing necessary infrastructure facilities to carry out this project.

CONFLICT OF INTEREST

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

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