Genotoxic effects of GSTP1 gene polymorphisms with respect to respiratory problems among traffic police

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Abstract

Genetic susceptibility is likely to play a role in response to vehicular pollution of GSTP1 and NAT2 (C481T). Genotoxic effects due to respiratory problems in traffic police occupationally exposed to vehicular exhaust have particular importance in the detoxification of inhaled toxicants since it is the most abundant in the lung function and the association. 161 male traffic policemen and 170 control subjects were selected for this study. Gene polymorphism was studied using PCR and RFLP method. The results on gene polymorphism of GSTP1 and NAT2 (C481T) showed significant association of homozygous mutant genotype (GG) in GSTP1 gene and homozygous mutant genotype (CC) in NAT2 gene with traffic police having respiratory problems. However, no such association was observed in traffic police without respiratory problems and control group also. Our results suggest that long term exposure to urban air pollution (with traffic as the main contributor) polymorphisms in genetic susceptibility provides evidence to suggest a role in determining the respiratory problems in GSTP1 and NAT2 (C481T) variants associated with reduced detoxification ability, increase susceptibility to such damage and appears to be associated with occupationally exhaust vehicular exhaust in traffic policemen.

1. Introduction

Polymorphisms in genes of xenobiotic metabolizing enzymes are expected to modulate individual responses to carcinogens (Vineis 2002; Mehrotra et al., 2010; Kukkonen et al., 2011). Incorporation of such susceptibility markers in polymorphisms studies may improve the precision of carcinogen exposure and health risk estimates (Perera and Weinstein 2002; Kamel and Hoppin 2004; Ryan et al., 2007). GSTP1 variant proteins that are thought to function in xenobiotic metabolism and play a role in susceptibility of diseases (Spiteri et al., 2000; Poloninkov et al., 2009). Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles and involve the detoxification of environmental pollutants (Bi et al., 2007; Hu et al., 1999). The highly homologous human gene N-acetyltransferase (NAT2), and appear to code for the genetically invariant and variant NAT proteins, respectively. Because they catalyze the N-acetylation (usually deactivation) and O-acetylation (usually activation) of aromatic and heterocyclic amine carcinogens (Hein et al., 2000; Hong et al., 2007). Genetic polymorphisms NAT2 may modify risk associated with carcinogen exposures because of their high prevalence in the general population, genetic variants that determine
susceptibility to environmental exposures may contribute greatly to the development of occupational diseases in the setting of specific exposures occurring in the workplace (Liella et al., 2006; Rothman et al., 2001). For diseases with more complex and multifactorial aetiology such as occupational asthma and chronic airways disease, susceptibility studies for genetic polymorphisms provide additional insight into the biological mechanisms of disease. However, polymorphisms for genetic susceptibility have a clear role in identifying disease risk (Christiani et al., 2008; Russo et al., 2013).

Environmental ambient air pollutants and endotoxin associated molecular patterns are the ambient exposures studied most frequently for interactions with genetic polymorphisms in respiratory problems such as asthma (Kleeberger and Peden 2005; Delfino et al., 2006; Stephanie and Romieu 2009). The health impacts in developing world have been driven by population growth and increased use of vehicles (Han and Naheer 2006). Exposure assessment studies in the developing world are very important, seen an increasing number of traffic related problems (Millman et al., 2008). Hyderabad is one of the rapidly growing metropolitan cities of India. The growth is associated with an enormous increase in vehicular traffic emitting exhausts and polluting the atmosphere. The main city has no industries which can be blamed for air pollution in the city. It may be presumed that city pollution is entirely due to automobile exhausts (Thippanna et al., 1999; Pucher et al., 2005).

In recent years, an enormous increase in vehicles was observed in metropolitan city of Hyderabad and the traffic police are found to be most the vulnerable group for exposure to vehicular pollution. Traffic policemen who work in the busy traffic signal areas for years together are exposed to the risk of air traffic pollution genes involved in variants combination with environmental factors to modify disease progression, severity of prolong exposure. Therefore, understanding the role of genetic variability and the interaction between genetic and environmental or occupational factors provides new insights into disease susceptibility. In the long run, the pollutants may produce disease like asthma, bronchitis and emphysema in the exposed individuals with changes in normal lung functions. No systemic study has been conducted the association of gene polymorphisms in traffic policemen with respiratory problems. Hence, this study has taken to improve the traffic policemen health and respiratory impacts due to vehicular pollution.

2. Materials and methods

2.1. Study Subjects

The study consisted of 161 male traffic policemen discharging their duties in heavy traffic areas of Hyderabad city, India. During their duties, they worked upto 8 h/day and 7 days a week. 170 volunteers participated as control subjects who had similar group, sex and habits with no exposure to traffic pollution. Information on personal identity, life style, smoking status, alcohol consumption, employment history, prior occupational exposure, health status and reproductive history was recorded on a proforma prepared for the purpose. The subjects had health problems such as eye irritation, hearing problem, backache, joint pain, skin allergy, acidity, hypertension, diabetes, renal problems and major and minor respiratory problems such as sore throat, stuffy nose, coughing, asthma, pneumonia, bronchitis and emphysema were recorded in both control and exposed groups. Both study subjects were informed about the nature of study and their acceptance to participate in the study was obtained in the regional language. Two ml of venous blood sample was collected from each subject with permission from the administrative authority, Joint Commissioner of Police Office, Hyderabad. Genomic DNA was isolated by Triton-X method (Lahari et al., 1992).

2.2. Genotyping of GSTP1 polymorphism

GSTP1 gene was analyzed according to the method of Harries et al. (1997) and Burim et al. (2004) using the primers to amplify 176-bp fragment specific primer sequences: P105 5‘- ACCCAGGGCTCTATGGGAA 3’ and P261 5’- TGAAGGGCAAGAAAGCCCT. The 25 μL PCR reaction mixture consisted of approximately 100-150 ng of genomic DNA, 15 pmol/L of each primer, 200 μmol/L of dNTPs, 20 mmol/L of Tris HCl, 50 mM of KCl, 2.5 mmol/L of MgCl2, 0.5 U of Taq DNA polymerase. The PCR cycling conditions include initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 5 min. After amplification, PCR products were subjected to restriction digestion using of Alw 261 (fast digest enzyme). The samples were genotyped on 3% agarose gel electrophoresis. The PCR product of 176 bp compared with smaller fragments of 85 and 91 bp. Polymorphisms were divided into three groups, wild type (AA) heterozygous (AG) and mutant (GG).

2.3. Statistical Analysis

All the statistical tests were carried out used by openepi.com version 3.01 statistical software package include the odds ratios (OR) and corresponding 95% confidence intervals (CI) and the associated variables, compared to traffic policemen with and without respiratory problems against control group with and without respiratory problems. p value less than 5% was considered statistically significant.

3. Results

3.1. Molecular analysis of GSTP1 gene polymorphism

In the present investigation, molecular analysis of GSTP1 and NAT2 genes were carried out in 161 traffic police (95 traffic police with respiratory problems and 66 traffic police without respiratory problems) and 170 control subjects (123 control subjects without respiratory problems and 47 control subjects with respiratory problems). The traffic police were categorized into two groups i.e. traffic police without respiratory problems (exposed group I) and traffic police
with respiratory problems (exposed group-II). In a similar way the control subjects were also categorized into two groups i.e. control subjects without respiratory problems (control group-I) and control subjects with respiratory problems (control group-II).

3.2. GSTP1 gene polymorphism

3.2.1. GSTP1 genotype distribution and allelic frequencies in traffic police without respiratory problems (exposed group-I) and control subjects without respiratory problems (control group-I)

The genotypic distribution of GSTP1 gene was studied in traffic police and control subjects without respiratory problems and the results are presented in Table 1. The results showed that the distribution of AA, AG and GG genotypes was 58.53%, 32.52% and 8.94% in control subjects without respiratory problems, while the genotypes were 56.06%, 34.84% and 9.09% in traffic police without respiratory problems respectively. The frequencies of alleles A and G were 0.75 and 0.25 in controls as against 0.73 and 0.27 in traffic police respectively. Distribution of genotypic and allelic frequencies of the GSTP1 gene polymorphism was in agreement with Hardy-Weinberg law of equilibrium. Statistical analysis of genotype distribution of GSTP1 gene in the traffic police and control subjects with no respiratory problems was carried out using chi-square test ($\chi^2$), odds ratio (OR), 95% CI (confidence interval) and the p values are shown in Table 2. Statistical analysis of genotype distribution of GSTP1 gene showed no statistically significant differences between traffic police and control group.

3.2.2. GSTP1 genotype distribution and allelic frequencies in traffic police with respiratory problems (exposed group-II) and control subjects with respiratory problems (control group-II)

The genotypic distribution of GSTP1 gene was studied in traffic police and control subjects with respiratory problems and the results are presented in Table 1. The results showed that the genotypic distribution of AA, AG and GG genotypes was 57.44%, 31.91% and 10.63% in the control group with respiratory problems, while the genotypes were 37.89%, 40.00% and 22.10% in the traffic police respectively. The frequencies of alleles A and G were 0.73 and 0.27 in controls as against 0.58 and 0.42 in traffic police respectively. GG Vs AA genotype showed statistically significant difference between control group and traffic police.

Table 1. Genomic distribution and allelic frequencies of GSTP1 gene in control subjects and Traffic Police without/with respiratory problems.

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Genotypic Distribution</th>
<th>Allelic Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>Controls without respiratory problems- (C-I)</td>
<td>72 (58.53)</td>
<td>40 (32.52)</td>
</tr>
<tr>
<td>Traffic Police without respiratory problems- (E-I)</td>
<td>37 (56.06)</td>
<td>23 (34.84)</td>
</tr>
<tr>
<td>Controls with respiratory problems- (C-II)</td>
<td>27 (57.44)</td>
<td>15 (31.91)</td>
</tr>
<tr>
<td>Traffic Police with respiratory problems- (E-II)</td>
<td>36 (37.89)</td>
<td>38 (40.00)</td>
</tr>
</tbody>
</table>

Table 2. Comparison of genotypic distribution and allelic frequencies of GSTP1 gene between control subjects and Traffic Police without/with respiratory problems.

<table>
<thead>
<tr>
<th>Control subjects and traffic police without respiratory problems</th>
<th>Control subjects and traffic police with respiratory problems</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes/ Alleles</td>
<td>$\chi^2$</td>
</tr>
<tr>
<td>GG Vs AA</td>
<td>0.01</td>
</tr>
<tr>
<td>GG Vs AG</td>
<td>0.008</td>
</tr>
<tr>
<td>GG Vs AA+AG</td>
<td>0.001</td>
</tr>
<tr>
<td>G Vs A</td>
<td>0.07</td>
</tr>
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</table>
GG Vs GA genotype and GG Vs AA+AG genotype showed no significant difference between controls and traffic police. However, G Vs A allele showed statistically significant difference between the exposed group-II and the control group-II shown in Table 2.

4. Discussion

The present study was carried out in GSTP1 and NAT2 (C481T) gene polymorphisms association with respiratory problems among traffic police due to occupational exposure of vehicular exhaust. With an ever increasing vehicle density of the city, there was a need to evaluate the genotoxic risk on traffic policemen exposed to automobile exhausts. Previous studies in GSTP1 and NAT2 gene polymorphisms and the association with various workers in different type of occupational exposure, among them some of the studies associated, however others did not find any association.

Gilliland et al. (2004) found that GSTP1 ile105 wild type genotypes modify the adjuvant effect of diesel exhaust particles on allergic inflammation. Liu et al. (2006) showed that GSTP1 polymorphism is associated with increased risk of disease susceptibility. GSTP1 has particular importance in the detoxification of inhaled toxicants since it is the most abundant GST isoform in the lungs (Castella et al., 2005; Silva et al., 2008). Broberg et al. (2010) studied the GSTP1 Ile105 Val polymorphism and the metabolism of toluene di-isocyanate (TDI) in workers exposed to TDI which is widely used in the production of polyurethane foams and paints. Polymorphisms in TDI metabolizing enzymes may affect elimination kinetics, resulting in differences in body retention and in its turn differences in adverse effects. The results showed that genetic modification on the human TDI metabolism and the results support earlier findings of GSTP1 105 Val as protective against TDI related asthma.

Jonsson et al. (2008) studied impaired lung function among rubber industry workers and control group. The comparison levels of 2 thiothiazolidine-4 carboxylic acid exposed group showed that association of increased levels 2-thiothiazolidine-4 carboxylic acid when compared to control group. The association was modified by glutathione S transferase P1. Heuser et al. (2007) carried out the analysis of GSTP1 gene polymorphism footwear employees in manufacturing industry. Employees in the footwear manufacturing industry are routinely exposed to complex mixtures of solvents used in cleaning and as diluents in glues, primers and degreasers. The result on genetic polymorphism, GSTP1 Ile/Val or Val/Val individuals from the exposed group relative to those with GSTP1 Ile/Ile, especially in younger subjects in footwear worker can be associated with genetic polymorphism. Schultz et al. (2010) studied the role of GSTP1 polymorphisms and smoke exposure increased association in children with acute asthma.

Knudsen et al. (1999) studied the genotoxic effects of urban air pollution in nonsmoking bus drivers and postal workers. The analysis results showed that long term exposure to urban air pollution NAT2 genotype effect, which affected all subjects, may influence the individual response exposure of air pollution. Wikman et al. (2002) results showed that NAT2 gene polymorphism play an important role in inception of asthmatic reactions related to occupational exposure to diisocyanates. Dai et al. (2000) analyzed the effects of genetic polymorphisms of N-acetyltransferase on trichloroethylene (TCE) induced hypersensitivity dermatitis among exposed workers. The results revealed that subjects with intermediate or slow acetylators of NAT2 have 2 fold higher risk for the disease than subjects with the fast acetylators. Zhang et al. (2009) investigated the effect of multiple factors of exposure to polycyclic aromatic hydrocarbons (PAHs), lifestyle and genetic polymorphism N-acetyltransferase wild type had significantly increased DNA adduct levels in coke oven workers.

Nielsen et al. (2000) carried out a study on PAH/DNA adducts in bus drivers, occupationally exposed to air pollution and the effect of NAT2 metabolizing enzymes on adduct levels and the results showed that no influence of NAT2 on adduct levels. Fanlo et al. (2004) also studied the urinary mutagenicity, NAT2 activity in textile industry workers and assess the association between occupational derived exposure to mutagens and NAT2 activity. The results showed that no significant differences observed when the urinary mutagenicity of slow and fast acetylators was compared, and the urinary mutagenicity was not significantly associated with the NAT2 activity.

5. Conclusion

Our study conclude that polymorphisms in GSTP1 and NAT2 genes in the traffic police indicated lack of functional protein causing either increased or reduced metabolic activity. These polymorphisms may alter the ability of enzymes to metabolize the chemical carcinogens and mutagens suggesting that polymorphic variations of GSTP1 and NAT2 genes are associated with disease susceptibility. Long term exposure to chemicals, gases and fumes present in the environment near heavy traffic are harmful for the lungs and traffic policemen are highly vulnerable for respiratory impairment due to vehicular exhaust at workplace environment, suggested to the traffic police personnel should use masks during the duty hours in busy traffic area and should undergo regular health check up to identify respiratory symptoms if any, and follow suitable management procedures and awareness should be created in the public regarding the harmful effects of traffic air pollution. The results are an important contribution to understanding the interactions between genetic and environmental factors that may modify risk of human health is important considering these issues; necessary steps need to be taken to improve the quality of the environment as well as the health of the traffic policemen.
Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgements
The authors are also thankful to the Director, Scientists and Technical Assistants of Regional Occupational Health Centre, Indian Council of Medical Research (ICMR), Bangalore & Institute of Genetics, Hyderabad for providing necessary grant and help to conduct the present study. As well as thank the traffic policemen Hyderabad city for participating in the study.

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