



Glutathione S-Transferase gene polymorphisms (*GSTT1*, *GSTM1*, *GSTP1*) as increased risk factors for asthma and COPD among Isocyanate exposed population of Bhopal, India

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Abstract

The release of methyl isocyanate (MIC) in Bhopal, India, caused the worst industrial accident in history. Isocyanates are the most common low molecular weight chemicals to cause asthma and Chronic Obstructive Pulmonary Disorder (COPD), whereas their metabolites may be conjugated with glutathione by glutathione S-transferases (GSTs). Glutathione S-transferases (GSTs) are enzymes involved in the detoxification of hazardous agents. We examined whether polymorphisms in the *GSTM1*, *GSTP1* and *GSTT1* genes modify allergic responses to isocyanate exposure, thus increasing risks for the development of isocyanate related- pulmonary disorders in a cohort of Bhopal. We compared clinical data - including gender, age and smoking habits - between the 2 groups. The study population consisted of 54 methyl isocyanate exposed subjects. Genotyping the polymorphisms in the *GSTT1* and *GSTM1* genes was performed using the multiplex PCR. The *GSTP1* ILe105Val polymorphism was determined using PCR-RFLP. *GSTP1* genotype was significantly associated with the increased risk of asthma and COPD (RR =1.66, 95%CI, 0.412-3.29 and RR=1.586 ; 95%CI, 0.75-3.33 respectively). Asthmatic had a higher prevalence of the *GSTP1* ILe105val allele than the COPD group (53.48% and 44.1%, respectively; $p =0.002$). Also, the presence of the *GSTP1* homozygote Val/Val was less common in subjects with asthma (39.53%) than in COPD group (62.79%). Polymorphisms within genes of the GST superfamily were associated with risk of asthma and COPD in Bhopal population. No differences in genotype frequencies were perceived in patients stratified by age and gender. *GSTM1* and *GSTT1* had almost no association. The results suggest, for the first time, that the polymorphic GSTs, especially *GST(P1)*, play an important role in inception of ill effects related to exposure to methyl isocyanate. Although about half of the subjects with lower FEV1 had never smoked, smoking was the main risk factor for the decreased FEV1 in COPD and asthmatic subjects. Our series of studies identified GST variants as a potential susceptibility locus to asthma and to lower lung function in COPD, asthmatic subjects, which may support the contention that genetic determinants of lung function influence susceptibility to asthma.

Key words: Isocyanate, asthma, chronic obstructive pulmonary disease, airflow obstruction, Bhopal, smoking.

Introduction

Isocyanates are reactive organic chemicals widely used in the industrial production of polyurethane polymers, pesticides, fungicides, and other materials. Methyl isocyanate (MIC), a precursor in pesticide production, was the major causative agent of the environmental disaster in Bhopal, India, responsible for more than 3000 immediate deaths and several thousand additional casualties in the years after the accident. Dependent on exposure levels and duration, MIC-exposed individuals present with airway hyperresponsiveness, inflammation, reactive airway dysfunction syndrome (RADS) and airway edema and injuries¹. To minimise the potential for oxidative injury, the human lung has an integrated system of antioxidant enzymes and expendable soluble molecules. This system includes several mechanisms by which ROS are converted to products that are further detoxified by other enzymes. If the oxidant burden is sufficiently great, ROS may overwhelm the antioxidant

system leading to a state of “oxidative stress”, which is thought to contribute to the pathogenesis of a number of respiratory diseases. Although antioxidant defences are available to decrease oxidative stress in the airways, individuals differ in their ability to deal with an oxidant burden; such differences are, in part, determined genetically².

This genetic variability may account for the considerable between-subject variability seen in both the lung function and airway inflammatory responses to MIC. Humans vary in their ability to metabolize exogenous and endogenous compounds, and individuals with a genetically determined reduced capacity to detoxify hazardous compounds may be at increased risk of adverse health effects compared with those with unaltered metabolic capacity. In this respect, conjugation of electrophilic compounds to glutathione, mediated by glutathione S transferases (GSTs), may be of great importance. The glutathione S-transferases M1 (*GSTM1*), T1 (*GSTT1*) and (*GSTP1*) P1 genes are

polymorphic in humans, and a phenotypic absence of enzyme activity results from the homozygous deletion of the respective genes (i.e., the null genotypes). The GSTM1 and GSTT1 genes exhibit null (i.e., deletion) polymorphisms; in specific individuals, homozygous deletion (i.e., both copies lost) of these genes can be detected³.

The current authors investigated the relationships between common polymorphisms in these three genes involved in response to oxidative stress, and decline in respiratory symptoms and lung function in response to isocyanate among asthmatics and Chronic Obstructive Pulmonary Disorder (COPD). The full spectrum of exposures and susceptibility genes involved in the pathogenesis of asthma and wheezing have yet to be established. Tobacco smoke is an exposure of interest, especially among groups with high prevalence of asthma and increased sensitivity to air pollutants. An extensive body of evidence indicates that involuntary tobacco smoke exposure increases the prevalence of wheezing, cough, and phlegm, and that household ETS (Environmental tobacco smoke) exposures cause exacerbations in asthma and COPD⁴.

Pulmonary function is among the most important health indicators, being a strong predictor of long-term morbidity and mortality. Impaired pulmonary function is a strong risk factor for the development of asthma and COPD and a marker of disease severity. Chronic stimulation of the innate immune system by microbes, inhaled allergens or components of tobacco smoke is involved in the inflammation and remodeling of airways that underlies asthma and COPD, and certain genetic variants may play a role in the common pathogenesis of both asthma and COPD⁵. Our group has recently identified the *GST* gene as an asthma susceptibility gene among the methyl isocyanate exposed cohort in Bhopal. Since it has been suggested that genetic determinants of lung function influence susceptibility to asthma as well as COPD, our finding may indicate a role of *GST* in the development of airflow obstruction that originates from oxidative stress.

Material and Methods

Study Subjects: This was a cross-sectional analysis of 54 Indian men and women resident of Bhopal city since past 3 decades aged 35 to 67 years who visited the Bhopal Memorial Hospital and Research Centre for annual medical checkup since past years. Detailed information on respiratory health, lifestyle, and exposure to MIC, other environmental irritants such as tobacco smoking, allergens, and air pollutions was collected. Based on a detailed questionnaire on pulmonary symptoms we carefully excluded the presence of pulmonary diseases such as chronic bronchitis, old tuberculosis, and pulmonary fibrosis.

Our diagnosis of asthma basically relied on the physicians diagnosis. Clinical diagnosis of COPD was defined as

reporting of any respiratory symptom (exertional breathlessness, chronic cough, regular sputum, frequent winter bronchitis, or wheeze) and evidence of airway obstruction on spirometry (both $FEV_1/FVC < 0.7$ and $FEV_1 < 80\%$ predicted).

Classification of the subjects: Of the 54 subjects, 20 subjects with a history of asthma, 23 subjects with COPD participated in the study. Of the total subjects 14 subjects had $FEV_1/FVC < 70\%$.

Pulmonary function test (PFT): Data for FEV_1 , FVC, and FEV_1/FVC ratio were available for all participants. Lung function was measured in the standing position using an electronic spirometer (Autospiro series; Minato Medical Science Co., Ltd, Osaka, Japan). Participants performed up to 3 forced expiratory maneuvers to obtain acceptable maneuvers; FEV_1 and FVC values taken to characterize each participant were the maximum results obtained from acceptable maneuvers. FEV_1/FVC ratio was calculated using actual values.

Determination of Genetic Polymorphisms: DNA was isolated from clot with a Qiamp Blood DNA Maxi kit (Qiagen Inc., Santa Clarita, CA, USA) in accordance with the manufacturer's instructions and stored at -80°C until use. Genotyping for the *GST*(s) polymorphism was carried out following a previously reported protocol⁶.

Descriptive statistics were expressed as mean \pm standard deviation (SD). We compared clinical data – gender, age, body mass index (BMI), smoking habits (never smoker, ex-smoker, and current smoker), using a 2-tailed Student's *t*-test or Pearson's chi-square test as appropriate. Smoking index was calculated by multiplying smoking dose (cigarettes per day) and duration (years smoked).

Statistical Methods The odds ratio (OR) and risk ratio (RR) is an estimate of the relative risk of disease associated with specific genotypes and is defined as the odds of a case patient having the at-risk genotype divided by the odds of a control subject having the same genotype. ORs and 95% confidence intervals (CIs) were calculated from 2 x 2 tables with the Fisher's exact model, and Pearson coefficients of correlation were calculated (Statistical Package for the social sciences software-SPSS 16 for windows, SPSS Inc., Chicago, III, USA).

Ethics This study was undertaken after ethical clearance from the Institutional Review Board and informed written consent was taken from each patient after briefing them regarding the study.

Results and Discussion

The association between *GST* genotype and susceptibility was studied in 20 unrelated asthmatic and 23 COPD patients

residing at varying radial distance from Union Carbide India Limited (UCIL), using a control group of 50 healthy individuals. Table-1 summarizes the characteristics of subjects conducted in this study. In total, 34% of asthmatic children and 21% of COPD patients were active smokers or subjects with a past smoking history of 2 to 5 years. With an average age of 54 (ranging between 35 and 67 years). Low Body mass index (BMI) among COPD patients were obvious as compared to asthmatics (20.1 and 23.6 respectively). The odds of Asthma and COPD occurring in the isocyanate exposed population of Bhopal was 1.8 and 4.43 times greater than the not exposed population (OR_{Asthma}, 1.8, 95%CI,0.67-4.81 ; OR_{COPD},6.67, 95%CI, 2.06-21.56). The control group had significantly higher lung function parameters than the population under study. Smoking was found to be highest among the gas affected population viz. 32% as compared to the control population being 16%. Our data exhibited very significant association of smoking resulting in asthma and COPD (OD_{Asthma},1.6829,95%CI, 0.5417 – 5.2288 ; OR_{COPD}, 6.67, 95%CI, 2.06-21.56 respectively). Table-2 summarizes the data found regarding the genotype frequencies for the RFLP in the GSTP1 gene, as well as the homozygous deletions of the GSTM1 and GSTT1 genes. Genotype frequencies (GSTP1, GSTM1, and GSTT1) were found that GSTP1 genotype was significantly associated with increased risk of asthma (p = .002). Indeed, the GSTM1 null genotype was present among 30% of the asthmatics and among 8.6% of the COPD group. On the other hand, GSTT1 null genotype was present in 40% asthmatics and 17.39% COPD subjects. As for the GSTP1, the homozygote GSTP1 Val/Val genotype and heterozygote Ile/Val genotype was significantly higher and common among the COPD patients than in the asthmatic group .

Table- 1
Demographic description of studied cohort from Bhopal

Parameters	Asthma (n=20)	COPD (n=23)	Control (n=50)
Age range (years)	39-62	47-67	35-66
Av.Body Mass Index (BMI)	23.6	20.1	25.8
Gender (% Male)	76	84	63
Income below Rs.1000/- per month (%)	59	96	55
Smoking history (%)	34	21	16
Av. FEV1/FVC ratio	0.65	0.51	0.83
Within 5 kms. radial distance of residence from UCIL plant (Kms.)(%)	62	88	59
Note , FEV1, Forced Expiratory volume in one second, FVC, Forced Vital Capacity , BMI , Body Mass Index			

Subjects with the GSTP1Val/GSTP1Val genotype registered a 1.22 fold (OR) lower risk of asthma and 2.01(OR) in case

of COPD patients. This was in comparison with the GSTP1Ile/Ile genotype (95% CI 0.31–4.74 and 95% CI 0.65-6.19 respectively). Between both study samples, there was a significant difference in the frequency of the GSTP1 alleles (p = .02).

The presence of the *GSTT1* null polymorphism was compared in both groups. The difference showed to be nonsignificant ($P > .05$) between COPD and asthmatics, 17.39% and 40%, respectively. The characteristics of the population studied are shown in table-1. We studied lung function results from a these subjects with pulmonary disease. This study showed that the group of individuals who have normal FEV₁/FVC ratio and decreased FEV₁ mainly consists of smokers with higher cumulative tobacco consumption. About 20 subjects who had a reduced FEV₁ (<80% of predicted) could not be classified as having COPD because they had a FEV₁/FVC ratio $\geq 70\%$.

In cross-sectional analyses, the percentage of males in the lower FEV₁ group was significantly higher than the percentage of males in the higher FEV₁ group. No significant differences in the following factors were observed between the 2 groups – lower FEV₁ and higher FEV₁, age (range), Among subjects who had a normal FEV₁/FVC ratio and a decreased FEV₁% (the lower FEV₁ group), 48.8% had never smoked. Nevertheless, smokers were more common in the lower FEV₁ group than in the higher FEV₁ group ($P < 0.0001$). The smoking index was also significantly higher in the lower FEV₁ group than in the higher FEV₁ group ($P < 0.0001$).

In contrast, there was no association between the genotype *GSTP1* and the FEV₁% predicted or the FEV₁/FVC phenotype. We did not observe any significant association between any one of these SNPs and the decline in FEV₁. Furthermore, this study provided preliminary evidence that functional SNPs in the *GST* gene influence FEV₁ and FEV₁/FVC in healthy subjects independently of the impact of tobacco smoke, emphasizing a distinct role for *GST* in airway obstruction that is a potentially important shared risk factor for asthma and COPD.

Although smoking is a major, modifiable risk factor, nonsmokers are similarly at risk for lung function decline. Therefore, our findings indicate that the group of healthy subjects with lower FEV₁ is a mixture of individuals who are susceptible to 1 or more irritants including microbial infections, allergens, or tobacco smoking⁷. As seen in figure-1, *GSTT1* and *GSTM1* genotypes displayed weak correlation (0.001 and 0.28 respectively, p,0.05).

On the other hand, *GSTP1* showed significant correlation 0.5, p, 0.05. High smoking index otherwise COPD patients may be a marker for an increased FEV₁ decline and an increased risk of deteriorating condition chronic

inflammatory lung diseases such as asthma and COPD, indicating the importance of recognizing decreased FEV₁ in targeting intervention efforts among smokers .

Our study has several limitations that require discussion. Reversible obstructions, however, are mostly undiagnosed asthma, and in this study, we carefully excluded asthma by a detailed questionnaire on pulmonary symptoms (cough, sputum, exertional dyspnea, wheeze), and physical examinations including pulmonary auscultation⁸. In addition, prebronchodilator lung function has been used in many epidemiological studies and has been shown to have value in predicting health outcomes on a population level.

Another limitation of our study is the retrospective nature of the FEV₁ follow-up. The follow-up periods and numbers of spirometry measurements varied among subjects. This variation may have contributed to a biased estimation of the annual decline in FEV₁ among subjects; however, we used a linear mixed-effects model to control for correlations among repeated measures within each subject, and we limited the analysis to include only subjects who had at least 4 separate FEV₁ measurements during 2 years follow-up. Nonetheless, our results will need to be replicated in other, ideally prospective, studies with large cohorts.

Table – 2
Genotype profile of Asthmatics, COPD and control population in the studied cohort

Gene	Chromosomal location	Polymorphisms	Genotypes	Asthma	COPD	Control
GSTP1	11q13	Ile105Val	Ile/Ile	9 (20.93)	4 (9.3)	22 (44)
			Ile/Val	5 (11.63)	11 (25.5)	15 (30)
			Val/Val	6 (13.95)	8 (18.6)	13(26)
			A(Ile)	23 (57.5)	19 (41.3)	59
			G(Val)	17 (42.5)	27 (55.1)	41
GSTM1	1p13	null allele	null	6	2	7
			WT	14	21	43
GSTT1	22q11	null allele	null	8	4	5
			WT	12	19	45

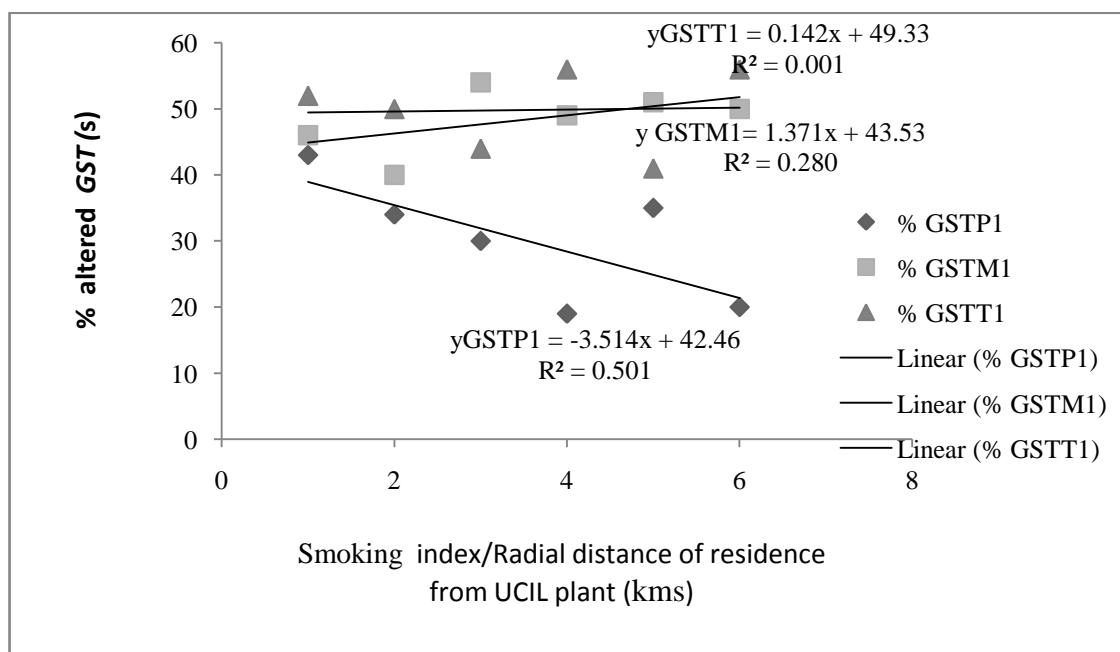


Figure- 1
Linear Regression analysis of Percent GST (P1, M1 and T1) gene polymorphism versus smoking index and Radial distance of residence from UCIL plant (giving an estimate of MIC exposure) among studied cohort in Bhopal

Conclusion

Finally, although the *GSTP1* genotype, smoking index and FEV₁/FVC ratio is a relatively sensitive index of mild airflow obstruction, it seems that lower FEV₁ in COPD and asthmatic subjects may be of some help in identifying subjects at risk for these chronic inflammatory pulmonary diseases. Given the increasing societal burden of airflow obstruction, early detection of an altered lung function in asymptomatic adults is important⁹. The demonstration of increased decline of FEV₁, even in healthy adults with lower FEV₁, should alert clinicians to the importance of case detection in subjects at risk, irrespective of their smoking status. Increased oxidative stress in COPD patients derive from the increased burden of inhaled oxidants such as cigarette smoke and other forms of particulate or gaseous air pollution and from the increase in reactive oxygen species (ROS) generated by several inflammatory, immune, and structural airways cells¹⁰. There is increasing evidence that genetic factors may also contribute to the pathogenesis of COPD, particularly antioxidant genes, which may confer a susceptibility to environmental insults such as cigarette smoke and thereafter development of COPD. In addition, we identified *GSTP1* as associated with natural variation in lung function, bringing new insight into our understanding of the genetic basis of lung development and related airway disorders, such as asthma and COPD.

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