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ORIGINAL ARTICLE

Association of glutathione-S-transferase polymorphism with genetic damage in paint workers

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Abstract

Background Occupational exposure to toluene causes serious health problems ranging from drowsiness to lethal diseases such as cancer. Paint workers are exposed to toluene through inhalation or the dermal route, which can induce genetcic damage. The increased DNA damage could be linked to genetic polymorphism. Therefore, we evaluated the association of glutathione-S-transferase polymorphism with DNA damage in paint workers.

Methods First, we included skilled paint workers ($n=30$) as exposed and healthy individuals ($n=30$) as control belonging to the same socio-economic strata. The genotoxicity biomarkers, Cytokinesis-block micronucleus (CBMN), and single-cell gel electrophoresis (SCGE)/Comet assay were used to assess genotoxicity while Multiplex-PCR and PCR-RFLP were used to assess polymorphism in glutathione-s-transferase (GST) genes. Using linear curve regression analysis, we assessed the association between genetic damage and polymorphism in the glutathione-s-transferase (GST) gene in the exposed and control subjects.

Results A significantly higher frequency of CBMN (4.43 ± 1.50) and tail moment (TM) (11.23 ± 1.0) respectively in paint workers as compared to the control(1.50 ± 0.86 and (0.54 ± 0.37) underlined significantly high genetic damage in paint workers.Regression curve analysis reveals that polymorphism in the GST gene is significantly associated with higher MN and TM in paint workers.

Conclusion Overall, our study provides a strong rationale for identifying a clear association between glutathione-S-transferase polymorphism and genetic damage in paint workers.

Keywords Toluene · Paint workers · CBMN · GST polymorphism · TM value

Introduction

Aliphatic, aromatic, and chlorinated hydrocarbons are widely used as paint thinners that are potentially mutagenic and can induce oxidative stress leading to redox status imbalance [1]. Toluene and other solvents used in the paint industry are extensively applied to increase the fluid consistency of paints, thus contributing to their ease of application. Inhalation of toluene causes serious health problems in humans, including brain dysfunction and drowsiness [2] or even death in severe cases. Biological monitoring of toluene exposure in the work area is an important parameter for assessing potential health risks and occupational safety. For assessing the effects of exposure on the human population, biomarkers are available to measure genetic damage in the exposed population [3]. Biomarkers of effects such as SCE (sister chromatid exchange), chromosomal abnormalities, etc. have been used to determine the extent of damage caused by exposure to various toxins [4]. The Cytokinesis-block micronucleus assay (CBMN) has been used in the biomonitoring of human exposure to various chemicals and mixtures of benzene, toluene, and xylene [5]. Second, biomarkers of susceptibility include epidemiological analysis

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to estimate genetic and environmental toxicity induced by exposure and recurrence of risk factors.

Gene families of glutathione S-transferase (*GST*) and cytochrome P450 (*CYP*) gene families are significantly involved in toluene metabolism [6]. *CYP* family genes such as *CYP1A1* and *CYP2E1* essentially mediate toluene metabolism followed by detoxification by conjugation with glutathione, thus protecting cells from genotoxicity. The critical step is catalyzed by *GST* isoenzymes (*GSTP1, GSTT1,* and *GSTM1*) encoded by the *GST* genes which differ in specificity against genotoxic or oxidative stress [7]. This study encompasses the use of cytokinesis block micronucleus assay (CBMN) [8, 9] and single-cell gel electrophoresis (SCGE) [10–12] as biomarkers of effects to assess genetic damage and influence of *GST* gene polymorphism. CBMN frequency was also previously used to assess a correlation between the *CYP2E1* polymorphisms in car paint workers exposed to mutagenic and carcinogenic solvents [13].

There is astrong rationale for identifying a clear association between glutathione-S-transferase polymorphism and genetic damage in paint workers. The increased DNA damage could be linked to genetic polymorphism. Therefore, we evaluated the association of glutathione-S-transferase (GST) gene polymorphism with DNA damage in paint workers.

Materials and Methods

Subject selection and cohort

For this purpose, we enrolled a cohort of subjects ($n=60$) that includes exposed ($n=30$) and control subjects ($n=30$) The exposed subjects were exposed to toluene during their work shift for at least 8 h with a minimum occupational exposure of at least 2 years. In contrast, control subjects were those who had no occupational exposure to toluene but had similar socio-economicconditions. Different confounding factors such as smoking, tobacco, and alcohol consumption were also studied to assess their impact on genetic damage. Individual participation was voluntary and any individual who could not meet the inclusion criteria was excluded.

Inclusion criteria:

(1) Duration of occupational exposure should not be less than 2 years.
(2) Medical history: The person should not have received any medications, long-term treatments for critical diseases, vaccination, or diagnosis such as X-ray/chemotherapy/radiation, etc.in the last 12 months. People with other symptoms such as fever, asthma, headache, pain, skin irritation, sleep disorders, nausea, itching, and eye irritation have not been included.
(3) Individuals should not be having any exposure to other occupational genotoxic and chemical agents.

This study was approved by the Institutional Human Ethics Committee of Kurukshetra University, Haryana, India (IEC/12/240).

Sample collection

Blood samples of different individuals were taken after their work shifts at ten workplaces near the Kurukshetra, Panipat, and Sonipat districts of Haryana state in India. We collected the blood samples in 2 vials. For biomarker studies, 3 mL blood was collected in heparin-coated vials and for genotype analysis, 2 mL blood samples were collected in EDTA-coated tubes(HiMedia,India). After proper labeling, the samples were transferred to the laboratory for further processing in a 4 ℃ portable icebox.

Culture setup

The collected blood samples were processed for peripheral blood lymphocytes (PBLs) culturing at least in duplicate. All chemicals and reagents were of analytical grade and procured from HiMedia, India.

Genetic damage analysis

To determine chromosomal instability or damage due to toluene exposure, cytokinesis-block micronucleus assay (CBMN) [14] and tail moment (TM) values [12] were analyzed. For analysis of CBMN, 6 µg/mL of cytochalasin B was added after 44 h of culture incubation and incubation was continued for the next 24 h. Then the cells were harvested and fixed in freshly prepared fixatives {Methanol (CH_3OH) and Acetic acid (CH3COOH) (ratio-3:1)}. After fixing these cells, slides were prepared using the cell suspension from a height of more than 1.5–2 m (approximately 6 feet), followed by Giemsa (4%) staining for 8–10 min. The frequency of CBMN was evaluated by microscopically scoring 1000 binucleated cells per subject.

DNA damage was assessed by measuring the tail moment value (TM; arbitrary unit) using the SCGE assay using the methodology of [12] with some modifications. Briefly, cells were mixed with the 0.5% low melting agarose to prepare a second layer over the already prepared first layer of 1% normal melting agarose. Then, slides were transferred at 4˚C for 15 min,an additional layer of 0.5% low-melting agarose was applied and allowed to freeze for 15 min. Then, cells were subjected to lysis using chilled lysis solution (25 mM sodium chloride, 100 mM sodium ethylene diamine tetra acetic acid, 10 mM Tris, 1% Triton X-100, and 10% DMSO added before use) at 4 °C and next incubated in electrophoresis buffer (10

N NaCl and 200 mM EDTA) followed by electrophoresis for 30 min. In the last step, the cells were neutralized, followed by staining with ethidium bromide (10 µg/mL). Around 50 slides were scored for tail moment (TM) value by Lucia comet software (version 7.12) under a fluorescent microscope (Olympus) [15] where the tail moment is the product of tail length and the fraction of the total DNA in the tail (TM = Tail Length X % of DNA in the tail).

Assessment of GST gene polymorphism

To assess genotype-based susceptibility to oxidative stress in exposed individuals, a spin column kit was used to isolate DNA from blood samples (Bangalore Genei, India). Specific primers for the *GSTP1, GSTT1,* and *GSTM1* genes and the internal control (*CYP1A1*) were custom synthesized according to their sequences described in Table 1. Multiplex PCR was used to assess the presence and absence of *GSTT1*, and *GSTM1* genotypes [16] while PCR-RFLP was used for genotyping of the *GSTP1*rs1695 GG variant [17].

Statistical analysis

All experiments were carried out at least in duplicate, the statistical evaluation was carried out with SPSS and the results were presented as mean ± SD. A student t-test and chi-square test were applied for multivariate comparison and distribution analysis of the genotypes (*GSTM1* and *GSTT1*), respectively. Next, the statistical significance of *GSTP1* genotype frequency was evaluated using the Hardy–Weinberg equilibrium equation. A One-way ANOVA test with post hoc analysis (Tukey's test) was used to assess any significant impact of different confounding factors on DNA instability and genotoxicity biomarkers (CBMN and TM). Finally, regression analysis was used to identify any significant impact of *GST* polymorphisms on biomarkers while considering all other confounding factors in the study.

Table 1 Primer sequences of candidate genes used in this study

Gene	Primer Sequence	Product Size (base pair)
GSTT1	F5′-TCTCCTTACTGGTCCTCACAT CTC-3′ R 5′-TCACCGGATCATGGCCAGCA-3′	480
GSTM1	F 5′-GAACTCCCTGAAAAGCTA AAGC-3′ R 5′-GTTGGGCTCAAATATACG GTGG-3′	215
CYP1A1	F,5′-GAACTGCCACTTCAGCTG TCT-3′; R,5′-CAGCTGCATTTGGAAGTG CTC-3′	312
GSTP1 rs1695 GG variant	F5′- ACCCCAGGGCTCTATGGGAA-3′ R5′-TGAGGGCACAAGAAGCCCCT-3′	176

Results

Subjects selection

As previously mentioned, we have selected 60 individuals including 30 toluene exposed and 30 control of similar socio-economic strata except for occupational exposure to toluene.Various confounding factors such as age, diet, tobacco use, and alcohol use were also taken into consideration. The exposed and control subjects had a median age of 28 years and 29.5 years, with an age range of 18–43 years and 18–46 years, respectively.

Analysis of CBMN frequency and TM value

CBMN frequency and TM value (arbitrary unit) were analyzed to assess genetic damage. We observed a significantly higher CBMN frequency (4.43 ± 1.50) and TM value (11.23 ± 1.0) in exposed individuals as compared to control i.e. (1.50 ± 0.86) and (0.54 ± 0.37) respectively (Tables 2,

Table 2 Impact of different confounding factors in exposed and control individuals as analyzed byCBMN frequency

Variables	Control (30) MN/1000 (Mean ± SD)	Exposed (30) MN/1000 (Mean ± SD)
All	1.50 ± 0.86	4.43 ± 1.50*
Age groups (years)		
Less than 30	1.25 ± 0.93	3.82 ± 1.51
30–40	1.79 ± 0.70	5.23 ± 1.09*
More than40	2.23 ± 0.67¥	5.45 ± 1.89*¥
Smoking		
Non-smokers	1.44 ± 0.87	4.42 ± 1.61
Smokers	1.80 ± 0.84¥	4.50 ± 1.05¥
Alcohol use		
Alcohol non -takers	1.50 ± 0.88	4.32 ± 1.38
Alcohol takers	1.50 ± 0.84	5.00 ± 2.12¥
Tobacco use		
Tobacco non- users	1.58 ± 0.86¥	4.43 ± 1.55
Tobacco users	1.00 ± 0.82	4.50 ± 0.71¥
Duration of exposure (years)		
< 6		3.53 ± 0.94
> 6		5.62 ± 1.26*¥

*Significant at $p < 0.05$, a multivariate ANOVA with post-hoc analysis was applied for MN frequency comparison between multiple sub-groups.Whereas, the highest mean rank (¥) was evaluated by Kruskal–Wallis H-test

3). Therefore, our data showed that paint workers exposed to toluene have higher geneticdamage as compared to the control group.

Impact of different confounding factors on MN frequency and TM value

Our results showed significant DNA damage due to toluene exposure as assessed by CBMN score and TM value in paint workers. Furthermore, we observed that the age factor is directly associated with an increase in micronuclei and tail moment value in exposed individuals compared to control subjects (Table 2, 3). Another confounding factor, the exposure duration also showed a positive association with a remarkably high CBMN frequency (5.62 ± 1.26) and a TM value (11.98 ± 0.86) in subjects occupationally exposed for more than 6 years compared to subjects who had occupational exposure less than 6 years. On the other hand, other confounding factors (smoking, alcohol, tobacco) showed no significant association with CBMN frequency and TM value in exposed and control subjects. Based on this analysis, we concluded that age and exposure duration are associated with micronuclei frequency and TM value with an increase of 42% and 59%, respectively, in exposed subjects.

Table 3 Impact of different confounding factors in exposed and control individuals as analyzed by tail moment (TM)

Variables	Control TM value (µM)	Exposed TM value (µM)
All	0.54 ± 0.37	11.23 ± 1.0*
Age (years)		
< 30	0.61 ± 0.38¥	10.69 ± 0.95
> 30	0.48 ± 0.37	11.95 ± **0**.86*¥
Smoking		
Non-smokers	0.56 ± 0.39¥	10.84 ± 1.16
Smokers	0.49 ± 0.31	11.33 ± 1.08¥
Alcohol Use		
Alcohol non- takers	0.52 ± 0.37	11.20 ± 1.12
Alcohol takers	0.65 ± **0**.39¥	11.39 ± 1.05¥
Tobacco Use		
Tobacco non-users	0.51 ± 0.37	11.46 ± **0**.77¥
Tobacco users	0.77 ± 0.41¥	11.22 ± 1.13
Duration of exposure (years)		
< 6		10.66 ± 0.91
> 6		11.98 ± 0.86*¥

*Significant at $p < 0.05$, a multivariate ANOVA with post-hoc analysis was applied for MN frequency comparison between multiple sub-groups.Whereas, the highest mean rank (¥) was evaluated by Kruskal–Wallis *H*-test

Distribution of *GSTP1, GSTM1,* and *GSTT1* genotypes

The distribution of GST genotypes (*GSTM1, GSTT1,*and *GSTP1*) was evaluated to establish an association with genetic damage in exposed and control subjects. Table 4 shows the frequency distribution of *GSTM1, GSTT1,*and *GSTP*1 rs1695 GG variant genes. The frequency of *GSTM1* null genotype in exposed group is found to be significantly higher (p value- 0.02, OR-0.286, 95%CI- 0.098–0.832), however, in the case of *GSTT1* no significant difference in frequency was observed between exposed and control subjects (p value- 0.05, OR-0.327, 95% CI: 0.104–1.032). Considering the frequencies in the North Indian population, 33% and 18.4% of the population showed null *GSTM1* and *GSTT1* respectively. In the case of *GSTP1*, 44.3%, 50.3%, and 5.4% of the population showed Ile/Ile, Ile/Val, and Val/Val genotypes, respectively [18]. While in the South Indian population, the frequency distribution of *GSTM1*and GSTT1 null genotypes was found to be 22.4% and 17.6% respectively. Different *GSTP1*genotypes- Ile/Ile, Ile/Val, and Val/Val were observed in 58.4%, 38.4%, and 3.1% of the population, respectively [19].

Influence of *GST* genotypes CBMN frequency

The effect of *GST* genotypes on micronuclei frequency is presented in Table 5. In the case of *GSTT1*, null genotypes (5.46 ± 1.33; 1.67 ± 0.52) showed a significantly higher frequency of CBMN than non-null genotypes (3.65 ± 1.11; 1.46 ± 0.93) in both groups. Null *GSTM1* genotypes showed a significantly ($p < 0.05$) higher frequency of CBMN in both exposed (5.17 ± 1.42) and control (2.22 ± 0.67) subjects. In the case of *GSTP1*, the variant *GSTP1* rs1695 GG also showed significantly higher

Table 4 Frequency Distribution of *GSTM1*, *GSTT1*and *GSTP1* genotypes in control and exposed subjects

Genotype Frequency N(%)	*GSTM1*		*GSTT1*		*GSTT1 /GSTM1*	*GSTT1 /GSTM1*	*GSTP1* rs1695 GG variant		
	Null	Non null	Null	Non null	Null	Non-null	AA	AG	GG
Control	9 (30)	21 (70)	6(20)	24(80)	15(30)	45(64.3)	22 (73.4)	7 (23.3)	1 (3.3)
Exposed	18 (60)	12 (40)	17(56.7)	13(43.3)	35(70)	25(35.7)	13 (43.3)	14 (46.7)	3 (10)

χ^2 test along with confidence interval (95% CI) was used for frequency distribution of *GSTM1* and *GSTT1* genotypes whereas the Hardy–Weinberg equation was used for frequency distribution of *GSTP1*genotypes

Table 5 Influence of *GST* genotypes on CBMN frequency and TM value

Genotype	CBMN/1000 (Mean ± SD)		TM (Mean ± SD)	
	Control	Exposed	Control	Exposed
GSTT1				
Null	1.67 ± 0.52	5.46 ± 1.33*	0.63 ± 0.39	11.89 ± 0.97*
Non-null	1.46 ± 0.93	3.65 ± 1.11	0.53 ± 0.38	10.73 ± 0.92
β[a]	− 0.208	− 1.814	− 0.105	− 1.161
R^2	− 1.814	0.285	0.013	0.285
P[b] value	0.605	< 0.05	0.55	< 0.05
GSTM1				
Null	2.22 ± 0.67*	5.17 ± 1.42*	0.36 ± 0.31	11.67 ± 0.95*
Non-null	1.19 ± 0.75	3.33 ± 0.78	0.63 ± 0.38	10.58 ± 1.00
β[a]	0.268	− 1.092	0.268	− 1.092
R^2	0.111	0.246	0.111	0.246
P[b] value	0.072	< 0.05	0.072	< 0.05
GSTP1 rs1695 GG variant				
wt/wt	1.23 ± 0.75	3.38 ± 0.96	0.58 ± 0 .38	10.51 ± 0.91
wt/mt	2.14 ± 0.69*	4.79 ± 0.97*	0.49 ± 0.37	11.58 ± 0.84*
mt/mt	3.00 ± 0.00*	7.33 ± 0.58*	0.22 ± 0.00	12.78 ± 0.27*
β[a]	0.904	1.763	− 0.129	1.111
R^2	0.315	0.602	0.034	0.448
P[b] value	< 0.05	< 0.05	0.332	< 0.05

wt/wt- wild type, wt/mt-heterozygous, mt/mt-homozygous mutant, Significant at $p < 0.05$**,** Multivariate ANOVA test was used for the comparison in CBMN frequency and TM value in multiple subgroups among the studied cohort. Here, "a" represents the coefficient that was unstandardized and "b" is the model p-value. The differences in CBMN and TM values (adjusted with respect to age, consumption habits, and duration of exposure) were identified through regression analysis

*Significant at $p < 0.05$

CBMN frequency in both exposed (7.33 ± 0.58) and control (3.00 ± 0.00) subjects. The results of linear regression analysis adjusted for models of various confounding factors are presented in Table 5.

Influence of *GST* genotypes on TM value

The effect of *GSTM1* and *GSTT1* genotypes on Tail Moment value (arbitrary unit) has been summarized in Table 5. The null genotypes of *GSTM1* (11.67 ± 0.95) and *GSTT1* (11.89 ± 0.97) in exposed workers showed significantly high TM values as compared to the non-null genotype of *GSTM1* (10.58 ± 1.00) and *GSTT1* (10.73 ± 0.92). For *GSTP1,* we found that exposed workers with *GSTP1* rs1695 GG variants showed significantly (p < 0.05) high TM value (12.78 ± 0.27). The results of linear regression analysis adjusted for models of various confounding factors are presented in Table 5.

Discussion

The availability of biomarkers and their relationship in molecular biology and medical diagnostics even in developing and low-income countries offer unique opportunities to study occupational exposure and its health consequences to identify, differentiate and classify discrete subsets to predict the course of infection, disease outcome, and response to therapy. The relationship between key genes and genotoxicity will aid in the development of diagnostics and improved therapeutics for effectively managing the toxicological effects of such exposures. In our study, we assessed genotoxicity in skilled paint workers exposed to toluene using CBMN frequency and TM values as biomarkers. This is the first study in Indiato establish the link between genetic damage in paint workers and GST gene polymorphism. We performeda cohort-based assessment of genetic damage and GST gene polymorphism among the exposed and healthy subjects.

Regarding the genotoxic measures, our study showed that CBMN frequency and TM values were significantly higher in the exposed compared to control subjects. In support of our data, there are studies where researchers from Brazil and Greece found a significant difference in CBMN frequency in exposed paint workers compared to control subjects [20, 21]. Our results with high TM value in the exposed population were consistent with previous studies in which the occupationally exposed population had a high TM value compared to control subjects [22]. In addition, we evaluated the distribution of GST genotypes (*GSTM1, GSTT1,* and *GSTP1*) using subjects of the cohort to establish an association with genetic damage. Our results showed a significant increase in the frequency of CBMN and TM value in *GSTT1* and *GSTM1* null subjects while in the case of *GSTP1*, the *GSTP1* rs1695 GG variant has a higher frequency of micronuclei and TM value compared to their respective wild genotypes. A study of workers in the footwear industry workers also supported our results, in which workers primarily exposed to multiple organic solvents were analyzed for DNA damage and GST gene polymorphism.The results showed a low TM frequency of the *GSTT1* null genotype, while the *GSTM1* genotype showed no possible effect [3]. Similar to our results, Jiang et al. [23] in 2009 measured DNA damage in 151 workers exposed to formaldehyde and compared it with 112 non-exposed individuals. The study showed a higher TM value and CBMN frequency in the null *GSTM1* genotype and *GSTP1* Val allele of exposed individuals. These results confirm our findings and suggest that volatile organic solvents pose significantly greater genetic damage in exposed individuals compared to healthy individuals. Furthermore, our results on GST gene polymorphism showed that the

workers who have null genotypes of both *GSTM1* and *GSTT1* and the mutant *GSTP1* rs1695 GG aredevoid of detoxication of xenobiotics including organic solvents that may further change disease outcomes to chronic conditions leading to cancer.Further studies along with different permutation-combination are essentially required to find the potential cancer risk of toluene exposure.

Our results established a relationship between biomarkers of exposure and polymorphism of GST genes, where polymorphism in the GST genesis associated with extensive damage in DNA.

Author contributions Concept, design, and overall supervision: AY; Sample collection, processing, and data acquisition: KP; Analysis or interpretation of data: KP, AY, SK, NA, and RG; Drafting of the manuscript: KP and SK; Editing of the manuscript: KP, SK, AY, RG, and NA; All authors provided comments on the manuscript and evaluated critically. All authors gave their consent for publication.

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Data availability Data will be available from 1s[t] author only at reasonable request.

Declarations

Conflict of interest The authors declare no potential conflict of interest.

Ethical approval All research procedures utilized in this study involving human participants were as per the ethical standards of the Institutional Human Ethics Committee of Kurukshetra University (IEC/12/240) and informed consent was obtained from all participants involved in the study.

Consent to participate Informed consent was obtained from all individual participants included in the study.

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