



Polymorphism of Glutathione S-transferase Genes and the Risk of Toxic Liver Damage in Petrochemical Workers

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Abstract

Background: Exposure to numerous chemicals, including industrial ones, may result in liver damage. The body susceptibility to the environmental hazards largely depends on the activity of the enzymes in the xenobiotic detoxification system. Function abnormalities of such enzymes due to genetic variations would increase the risk of developing various diseases.

Objective: To elucidate the relationship between polymorphism in *glutathione S-transferase* genes (*GSTM1*, *GSTT1* and *GSTP1*) and the risk of toxic liver damage in a group of petrochemical workers.

Methods: This study was conducted on 72 workers with toxic liver injury, 156 healthy workers, and 322 healthy individuals without history of occupational exposure to chemicals. Genotyping of the *GSTP1* rs1695 gene polymorphism was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Polymerase chain reaction (PCR) was used to perform genotyping of the *GSTM1* and *GSTT1* genes polymorphism.

Results: There was a significant difference in genotype frequencies of the *GSTP1* rs1695 gene polymorphism among the groups studied. The distribution of Val/Val genotype of the *GSTP1* rs1695 gene polymorphism had a higher incidence in healthy workers compared with patients with toxic liver damage ($p=0.036$). No significant association was found between the *GSTM1* and *GSTT1* polymorphisms and toxic liver damage.

Conclusion: The *GSTP1* rs1695 gene polymorphism can play a protective role in the development of toxic liver damage in petrochemical workers.

Keywords: Glutathione S-transferase; Liver diseases; Polymorphism, genetic

Introduction

Toxic liver damage comprises a broad range of diseases caused by hepatotoxic effects of diverse chemicals, mainly after occupational exposure. In petrochemical industries, numerous hazardous substances with hepatotropic prop-

erties that cause both acute and chronic hepatic diseases (eg, toxic hepatitis) are used. The impact of chemicals and their metabolites brings about pronounced functional and structural changes in hepatocytes and activation of cytotoxic immune T cells. The toxic effect of xenobiotics depends on a variety of factors—the compound chemical

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structure, blood flow in the liver, protein bonds, genetic factors, age, dietary habits, and drug and alcohol abuse.¹⁻³

The body susceptibility to environmental hazards is known to largely depend on the activity of enzymes in the xenobiotic detoxification system. The xenobiotic detoxification process usually involves two successive phases: phase I enzymes bind xenobiotics to form intermediate genotoxic metabolites. The main function of phase II enzymes is to detoxify and neutralize the hydrophilic and toxic products of phase I. Glutathione S-transferases as representatives of detoxification phase II enzymes are present in various tissues and organs.⁴ The genes controlling the synthesis of these enzymes are characterized by a significant population polymorphism. Functionally defective alleles of glutathione S-transferase genes play an important role in the pathogenesis of many diseases. The contribution of these genetic polymorphisms to human susceptibility to hepatitis of various etiologies and liver cirrhosis has already been shown.^{5,6}

We conducted this study to elucidate

the association between polymorphism in *glutathione S-transferase genes (GSTM1, GSTT1 and GSTP1)* and the risk of toxic liver damage in a group of petrochemical workers.

Materials and Methods

The study population consisted of Caucasian individuals from Bashkortostan Republic, Russia. Of 228 male petrochemical workers included in this study, 156 were healthy workers and 72 patients had toxic hepatitis. Toxic hepatitis was diagnosed based on the following criteria: history of occupational exposure preceding liver damage; presence of clinical hepatotoxicity-related symptoms (*eg*, vomiting, nausea, fatigue, right upper quadrant abdominal pain, and jaundice); and having liver enzymes activities at least twice the upper limit of the reference range. The diagnosis was confirmed morphologically. Those with serious infectious, liver diseases, and consumption of genotoxic drugs during the last six months were excluded from the study. Patients with viral hepatitis and history of alcohol intake were also excluded. A control group consisted of 322 healthy individuals selected from the general population with no history of exposure to any kind of toxic chemicals, any serious medical problems and intake of drugs, was also studied. The groups examined were matched for age, sex, and ethnicity.

To identify genotypes that determine the workers' sensitivity to toxic occupational factors, the group of healthy workers was compared with the control group of individuals without occupational toxic burden. To identify genotypes associated with susceptibility to toxic liver damage, a comparison was made between patients and healthy workers.

DNA Extraction and Genotyping

DNA was isolated from peripheral blood

TAKE-HOME MESSAGE

- In petrochemical industries, a number of hazardous substances with hepatotropic properties that cause both acute and chronic occupational diseases, including toxic hepatitis, are used.
- The body susceptibility to environmental hazards largely depends on xenobiotic detoxification gene system functioning.
- Several reports show that functionally defective alleles of glutathione S-transferase genes play an important role in the pathogenesis of many diseases, including hepatitis of various etiologies and cirrhosis.
- Carriage of the Val/Val genotype of the rs1695 polymorphic locus of the *GSTP1* gene can be a protective factor for the development of toxic liver damage.

samples taken from the study participants by proteinase K treatment, phenol-chloroform-isoamyl alcohol extraction, and ethanol precipitation. The *GSTP1* rs1695 gene polymorphism was evaluated using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, as described previously.⁷ The polymerase chain reaction (PCR) primers were: 5'-ACC CCA GGG CTC TAT GGG AA-3' (F), and 5'-TGA GGG CAC AAG AAG CCC CT-3' (R). The PCR conditions included an initial denaturation at 95 °C for 10 min, followed by 29 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 30 s, and finally, one cycle of final extension step at 72 °C for 10 min. The PCR product was digested with the restriction endonuclease Alw26I restriction enzyme and put at 37 °C overnight. The deletion polymorphisms of the *GSTM1* and *GSTT1* genes were performed using PCR, as described previously.^{8,9} Primers for *GSTM1* were: 5'-GAA CTC CCT GAA AAG CTA AAG C-3' (F), and 5'-GTT GGG CTC AAA TAT ACG GTG G-3' (R); those for *GSTT1* were: 5'-TTC CTT ACT GGT CCT CAC ATC TC-3' (F), and 5'-CAC CGG ATC ATG GCC AGC A-3' (R). An initial denaturation at 94 °C for 10 min followed by 35 cycles of denaturation at 94 °C for 60 s, annealing at 61 °C for 60 s and extension at 72 °C for 60 s, followed by a final extension at 72 °C for 7 min were performed for *GSTM1*. Similarly, an initial denaturation at 95 °C for 10 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 61 °C for 15 s and extension at 72 °C for 60 s, followed by a final extension at 72 °C for 7 min for *GSTT1*. PCR products were subjected to electrophoresis on 3% agarose gel and stained with ethidium bromide.

Ethics

Written informed consent was obtained from all the included participants. The

study was approved by the Ethics Committee of the Ufa Research Institute of Occupational Health and Human Ecology, Russia. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Statistical Analysis

SPSS® for Windows® ver 17.0 (SPSS Inc, Chicago, IL, USA) was used for data analysis. The difference in the frequencies of alleles and genotypes between studied groups was assessed by χ^2 test. A p value <0.05 was considered statistically significant.

Results

We examined 72 patients with toxic liver damage with a mean age of 45.1 (SD 13) years, 156 healthy workers with a mean age of 45.7 (11.7) years, and 322 healthy controls with a mean age of 45.6 (10.6) years. All participants were male. There was no significant difference in the distribution of age, sex, and ethnicity among the studied groups.

A significant ($p=0.022$) difference was observed in the frequency of the null genotype of the *GSTM1* gene between healthy workers and the control group (Table 1). No significant ($p=0.947$) difference was observed in the frequency of deletion of the *GSTM1* gene between patients with toxic liver damage and healthy workers.

No significant difference in the frequency of the null genotype of the *GSTT1* gene was found between the healthy workers and controls. No significant difference was observed between the distribution of the null genotype of the *GSTT1* gene between patients and healthy workers (Table 1).

A significant difference was observed in the distribution of *GSTP1* genotypes between the groups examined (Table 1). The frequency of the Val/Val genotype of the *GSTP1* rs1695 gene polymorphism

Table 1: Frequency distribution of genotypes of *GSTM1*, *GSTT1* and *GSTP1* polymorphisms in the groups examined. Values are n (%).

Gene	Genotype	Toxic liver damage	Healthy workers	Controls	p*	p†
GSTM1	Non-null	41 (56.9)	88 (56.4)	144 (44.7)	0.947	0.022
	Null	31 (43.1)	68 (43.6)	178 (55.3)		
GSTT1	Non-null	60 (83.3)	130 (83.3)	248 (77)	0.849	0.142
	Null	12 (16.7)	26 (16.7)	74 (23)		
GSTP1	Ile/Ile	49 (68.1)	102 (65.4)	238 (73.9)	0.806	0.069
	Ile/Val	23 (31.9)	42 (26.9)	78 (24.2)		
	Val/Val	0 (0)	12 (7.7)	6 (1.9)		
	Ile	121 (84)	246 (78.8)	554 (86)		
	Val	23 (16)	66 (21.2)	90 (14)		

*p value for comparison of sick workers with healthy ones; †p value for comparison of healthy workers with controls.

in healthy workers was significantly ($p=0.005$) higher than that in the control group. The frequency of Val/Val genotype of the *GSTP1* rs1695 gene polymorphism was also significantly ($p=0.036$) higher in healthy workers compared with patients with toxic liver damage (7.7% vs. 0%). The Val/Val genotype might be a protective factor for toxic liver damage (OR 0.001, 95% CI 0.685 to 0.891).

Discussion

The glutathione S-transferase family belongs to enzymes of phase II xenobiotic biotransformation. The most important representatives of glutathione S-transferases are *GSTM1*, *GSTT1* and *GSTP1*, which are widely represented in all organs and tissues. The peculiarity of the *GSTM1* and *GSTT1* genes is the presence of null alleles with extended deletions, in which high-grade enzymes are not formed.¹⁰ In our study, we examined the association between glutathione S-transferase gene polymorphisms (*GSTM1*, *GSTT1* and *GSTP1*) and susceptibility to toxic liver damage in petrochemical workers. We discovered the

protective effect of the Val/Val genotype of the *GSTP1* rs1695 gene polymorphism on the risk of toxic liver damage.

The *GSTM1* enzyme is known to be involved in the antioxidant defense system of the body. The presence of two null alleles of the *GSTM1* gene leads to the absence of the protein product of this gene, which contributes to the development of oxidative stress, causes an increase in the level of DNA adducts and can provoke the development of various liver pathologies.^{11,12} A number of research findings show its important prognostic significance in the development of occupational diseases.^{13,14} In contrast to these results, our study showed that there was no significant difference in the distribution of the *GSTM1* gene genotypes among workers depending on the presence or absence of occupational pathology. However, in our case, the distribution of *GSTM1* gene polymorphism was significantly different between healthy workers and the control group.

The *GSTT1* gene is involved in the conversion of active toxic metabolites into their inactive forms. The significance of the *GSTT1* gene in detoxification processes

remains controversial and disputable.¹⁵ In our study, differences in the frequency of the null genotype of the *GSTT1* gene among patients and healthy workers were not significantly different. Similarly, previous studies have shown that there is no significant association between the *GSTT1* gene genotype and the risk of liver disease.^{6,16}

The *GSTP1* gene has several polymorphic variants; a functionally significant one is the replacement of adenine by guanine in the 5th exon of the gene, which leads to the replacement of isoleucine by valine in the 105th position of the peptide. The *GSTP1* gene polymorphism can result in a decrease in its activity and, consequently, an increase in the accumulation of toxicants in the body.^{17,18} There are studies on the polymorphic rs1695 locus of the *GSTP1* gene in occupational groups exposed to benzidine and styrene. They show the role of enzymes of xenobiotic metabolism in the formation of the individual sensitivity of workers in the context of hazardous industries.^{19,20} In the study by Ma, *et al*, it was found that the Ile/Val and Val/Val genotypes are associated with a predisposition to bladder cancer caused by benzidine.²¹ The results from this study indicate a selection of the homozygous Val/Val genotype in the group of healthy workers perhaps in favor of its protectivity, confirmed by Mapp, *et al*, indicating the protective value of the Val/Val genotype in workers exposed to isocyanate.²²

The *GSTP1* gene is characterized by selective expressive activity and substrate specificity, which results in the activation of polycyclic aromatic hydrocarbons with the formation of more reactive metabolites; that is probably why the Val allele, which reduces the catalytic activity of the enzyme, is preferred when exposed to aromatic hydrocarbons.

The main limitation of this study was the relatively small sample size. In order

to give more valuable results, further research is needed with larger sample size.

In conclusion, we demonstrated that in petrochemical workers, the carriage of the Val/Val genotype of the rs1695 polymorphic locus of the *GSTP1* gene could be a protective factor concerning the development of toxic liver damage. The study of the role of glutathione S-transferase genes in the formation of workers' responses to the influence of chemical factors of work environment is a prospective scientific trend, in order to substantiate the criteria for individual risks for developing occupational and general diseases among workers, as well as in solving problems of prevention, medical management and rational employment.

Conflicts of Interest: None declared.

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