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Research article

EFFECTS OF GENETIC POLYMORPHISMS OF GSTM1, GSTT1 AND GSTP1 GENES ON BLOOD METAL LEVELS IN NON-FERROUS METAL ALLOY SMELTER OPERATORS

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Heavy metal ions are known to induce generation of a large number of reactive oxygen species (ROS). Glutathione S-transferases (GSTs) play an important role in adaptation and response to oxidative stress. GSTM1, GSTT1, and GSTP1 genes have numerous described polymorphisms, the most significant being GSTM1, GSTT1, and GSTP1 Ile105Val deletion ones.

Our objective was to study the relationship between the genetic polymorphism of GSTM1, GSTT1, GSTP1 genes and blood levels of metals in smelter operators engaged in crude lead refining.

We examined 55 male lead-refining furnace operators working at a non-ferrous metal alloy plant. Blood metal concentrations were measured by inductively coupled plasma mass spectrometry. GSTM1 and GSTT1 deletion polymorphisms were determined using real-time SYBR Green qPCR and that of GSTP1 Ile105Val – using a commercial SNP Screening Kit. Statistical data processing was carried out using the Mann – Whitney U-test.

Blood levels of industry-specific metals were not statistically different between the workers with GSTT1 and GSTP1 genotypes. We established, however, that men with the null genotype of GSTM1 had significantly higher blood arsenic levels.

Our findings indicate that a high blood arsenic level associated with occupational exposure may be attributed to the GSTM1 null genotype. This observation can be used to identify the most vulnerable groups of individuals at risk of overexposure to arsenic.

Keywords: *xenobiotics, GSTM1, GSTT1, GSTP1, glutathione S-transferases, heavy metals, arsenic, polymorphisms.*

Chemical pollution of the environment remains a priority sanitary-epidemiological health risk factor. It is especially true for workers employed at harmful productions.

For example, workplace air in a zone where lead refining takes place contains large quantities of metals that can be found in lead cake such as lead, antimony, cop-

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per, sulfur, bismuth, arsenic, tin and some others.

Heavy metal ions are known to be able to stimulate formation of reactive oxygen species (ROS) in large quantities. These species induce oxidative stress in a cell thereby damaging cellular structures [1, 2].

Glutathione-S-transferases (GST) play an important role in adaptation and response to oxidative stress. Many polymorphisms have been described for GSTM1, GSTT1 and GSTP1 genes; however, the deletions in GSTM1, GSTT1 and Ile105Val for GSTP1 gene are the most significant ones. These polymorphisms lead to either total absence of an enzyme or to a reduction in its activity. As a result, the described mutations can lead to weaker resistance to effects produced by heavy metals [3–6].

Studies with their focus on workers' genetic predisposition provide more data on pathogenesis of a disease and make it possible to identify groups that are more susceptible to exposure to harmful occupational factors. This is vital for developing and implementing activities aimed at managing health risks at a workplace, personified medical and prevention programs included.

Our research goal was to examine an association between genetic polymorphism of GSTM1, GSTT1, and GSTP1 genes and metal contents in blood of smelters dealing with crude lead refining.

Materials and methods. We examined 55 males who worked as smelters in the refining section of a metallurgic (smelting) workshop at an enterprise that produced non-ferrous alloys. Their age was from 28 to 56 years (an average age was 40.94 ± 7.04 years). The sampling was ethnically homogenous. Mass concentrations of metals (Pb, N = 54; Cd, N = 52; Sb, N = 43; As, N = 43) were identified in blood by mass spectrometry with inductively coupled plasma. DNA was extracted from peripheral blood according to conventional procedures and polymorphisms were identified as per the procedure, which we described in our previous studies [7].

Since genotype analysis does not allow distinguishing between the normal homozygote (I/I) and heterozygote (I/D) for GSTT1 and GSTM1 genes, we took a recessive model with using I/* (I/I or I/D) and DD (null allele) variables. We applied a dominant genetic model (Ile/Ile against Val/*) for GSTP1 Ile105Val polymorphism and combined Ile/Val and Val/Val into one group. Differences in the identified indicators were estimated with Mann – Whitney test and χ^2 with Yates's correction. Critical significance in testing the null hypothesis was taken as equal to 0.05. We applied Kolmogorov – Smirnov test to check whether data were normally distributed. The results were statistically analyzed with Statistica 12 software package (StatSoft Inc, USA).

Results and discussion. In the present study, we examined an association between genetic polymorphism of GSTM1, GSTT1, and GSTP1 genes and metal contents in blood of smelters who worked in the refining section of a metallurgic (smelting workshop) at an enterprise that produced non-ferrous alloys. Alleles of the examined genotypes were distributed according to an average pattern typical for a European population (Figure). Therefore, the analyzed sampling was homogeneous. It was then divided into two groups as per presence or absence of predisposition for each genotype: normal genotype/heterozygote and deletion genotype for GSTM1 and GSTT1; normal genotype and mutant genotype / heterozygote for GSTP1.

Metal contents in blood as per genotype frequencies (for better illustration, mean values are used) and authenticity of differences are given in Table.

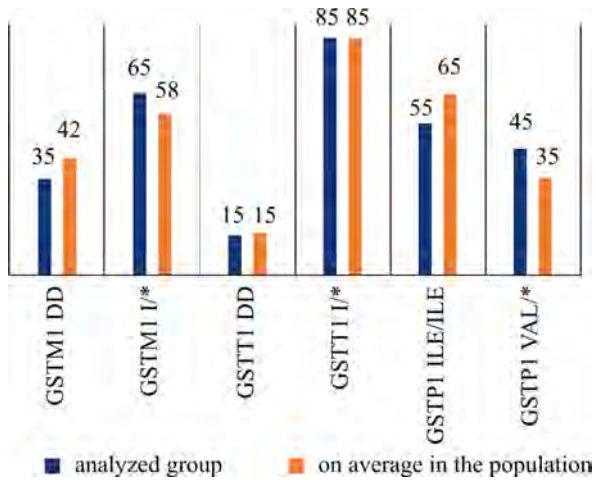

Figure. Distribution of GSTM1, GSTT1, GSTP1 genotype frequencies in smelters working in the refining section of a metallurgic (smelting) workshop at an enterprise that produces non-ferrous alloys and average frequencies in the population: I/* is the normal genotype/heterozygote, DD is the deletion (mutant) genotype, Ile/Ile is the normal genotype, Val/* is the mutant genotype/heterozygote (GSTM1: $\chi^2 = 0.76$, $p = 0.3833$; GSTT1: $\chi^2 = 0.04$, $p = 0.84$; GSTP1: $\chi^2 = 1.69$, $p = 0.1939$)

Overall, we did not detect any statistically authentic differences regarding GSTT1 and GSTP1 genotypes in metal contents in blood of workers under the same exposure. This is probably due to mutant alleles of these genes being less frequent than mutant GSTM1 and since the sampling was rather small, it also prevented us from identifying any significant differences for these two genotypes.

Heavy metals are excreted rapidly from the body due to their hydrophilic properties growing because of enzymatic activity by glutathione-transferases. Probably, compensatory detoxification pathways become involved to excrete lead, antimony and cadmium, and they induce activity of other enzymes.

Although we did not detect any statistically significant differences for all the aforementioned metals, we established an association with the deletion in GSTM1 gene and

Mean metal contents in blood of smelters with different GSTs genotypes

	GSTT			**GSTM**			GSTP		
	I/*	DD	P-value	I/*	DD	P-value	Ile/Ile	Val/*	P-value
Cd, μg/dm^3	0.362 ± 0.06 (N =44)	0.408 ± 0.18 (N = 8)	0.91	0.386 ± 0.07 (N = 34)	0.338 ± 0.1 (N = 18)	0.69	0.4 ± 0.08 (N = 24)	0.342 ± 0.08 (N = 28)	0.56
As, μg/dm^3	7.07 ± 1.78 (N = 36)	4.771 ± 2.67 (N = 7)	0.48	**4.00 ± 0.91 (N = 27)**	**11.24 ± 3.66 (N = 16)**	**0.02**	6.448 ± 3.03 (N = 19)	6.893 ± 1.46 (N = 24)	0.21
Sb, μg/dm^3	8.87 ± 0.71 (N = 36)	8.776 ± 0.87 (N = 7)	0.78	9.15 ± 0.94 (N = 27)	8.34 ± 0.44 (N = 16)	0.66	8.908 ± 1.21 (N = 19)	8.809 ± 0.58 (N = 24)	0.67
Pb, μg/dm^3	369.39 ± 23.8 (N = 46)	416.01 ± 51.6 (N = 8)	0.49	392.04 ± 28.3 (N = 35)	347.33 ± 32.44 (N = 19)	0.55	366.7 ± 37.87 (N = 24)	384 ± 25.02 (N = 30)	0.76

N o t e : I/* is the normal genotype / heterozygote, DD is the deletion (mutant) genotype, Ile/Ile is the normal genotype, Val/* is the mutant genotype / heterozygote. The table contains mean values and error of the mean; statistically authentic differences are given in bold ($p \leq 0.05$).

arsenic contents in blood. People with the mutant genotype had arsenic in their blood in a concentration that was by 3 times higher against the same indicator in people with the normal genotype ($p = 0.02$).

Arsenic metabolism has several ways; binding to certain proteins is one of them [8], and conjugation with glutathione is another [9]. After consequent stages in methylation are completed, this way leads to formation of two end products, methylarsonic acid (MMA) and dimethylarsinic acid (DMA). MMA and DMA metabolites are less toxic than non-organic compounds and are easier to excrete with urine [10, 11]. Such members of the GST family as GSTP1, GSTT1 and GSTM1 can influence an ability to metabolize arsenic depending on their expression and various allele types [12–19]. Thus, González-Martínez with colleagues showed that the deletion variant of GSTM1 produced negative effects on arsenic excretion by the kidneys due to its lower enzymatic activity. Contents of arsenic metabolites in urine went down proportionate to an increase in overall arsenic contents in case the deletion GSTM1 genotype was present. No such regularities were established for GSTT1 and GSTP1 genotypes [20]. This fact indicates that GSTM1 makes a significant contribution to arsenic metabolism and its deletion genotype can lead to high arsenic concentrations in blood due its lower enzymatic activity.

Conclusion. We established in this study that elevated arsenic concentrations in blood detected under exposure to harmful occupational factors might be caused by the deletion GSTM1 genotype. In future, this fact can be used to identify population groups that are the most susceptible to exposure to arsenic in high concentrations as well as to implement prevention activities in due time.

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References

1. Ercal N., Gurer-Orhan H., Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Curr. Top. Med. Chem.*, 2001, vol. 1, no. 6, pp. 529–539. DOI: 10.2174/1568026013394831

2. Flora S.J.S., Mittal M., Mehta A. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J. Med. Res.*, 2008, vol. 128, no. 4, pp. 501–523.

3. Nourozi M.A., Neghab M., Bazzaz J.T., Nejat S., Mansoori Y., Shahtaheri S.J. Association between polymorphism of GSTP1, GSTT1, GSTM1 and CYP2E1 genes and susceptibility to benzene-induced hematotoxicity. *Arch. Toxicol.*, 2018, vol. 92, no. 6, pp. 1983–1990. DOI: 10.1007/s00204-017-2104-9

4. Klusek J., Głuszek S., Klusek J. GST gene polymorphisms and the risk of colorectal cancer development. *Contemp. Oncol. (Pozn.)*, 2014, vol. 18, no. 4, pp. 219–221. DOI: 10.5114/wo.2014.41388

5. Farmohammadi A., Arab-Yarmohammadi V., Ramzanpour R. Association analysis of rs1695 and rs1138272 variations in GSTP1 gene and breast cancer susceptibility. *Asian Pac. J. Cancer Prev.*, 2020, vol. 21, no. 4, pp. 1167–1172.

6. Sirivarasai J., Wananukul W., Kaojarern S., Chanprasertyothin S., Thongmung N., Ratanachaiwong W., Sura T., Sritara P. Association between inflammatory marker, environmental lead exposure, and glutathione S-transferase gene. *Biomed Res. Int.*, 2013, vol. 2013, pp. 474963. DOI: 10.1155/2013/474963

7. Shaikhova D.R., Amromina A.M., Sitnikov I.A., Sutunkova M.P., Gurvich V.B., Astakhova S.G. Features of GSTM1, GSTT1 and GSTP1 Genetic Polymorphism in Nizhny Tagil Metallurgical Plant Workers with Cardiovascular Diseases. *Zdorov'e naseleniya i sreda obitaniya – ZNiSO*, 2021, vol. 1, no. 12, pp. 36–40. DOI: 2219-5238/2021-29-12-36-40 (in Russian).

8. Naranmandura H., Suzuki N., Suzuki K.T. Trivalent arsenicals are bound to proteins during reductive methylation. *Chem. Res. Toxicol.*, 2006, vol. 19, no. 8, pp. 1010–1018. DOI: 10.1021/tx060053f

9. Hayakawa T., Kobayashi Y., Cui X., Hirano S. A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. *Arch. Toxicol.*, 2005, vol. 79, no. 4, pp. 183–191. DOI: 10.1007/s00204-004-0620-x

10. Vahter M. Mechanisms of arsenic biotransformation. *Toxicology*, 2002, vol. 181–182, pp. 211–217. DOI: 10.1016/s0300-483x(02)00285-8

11. Tseng C.-H. Arsenic methylation, urinary arsenic metabolites and human diseases: current perspective. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev.*, 2007, vol. 25, no. 1, pp. 1–22. DOI: 10.1080/10590500701201695

12. Schuliga M., Chouchane S., Snow E.T. Upregulation of glutathione-related genes and enzyme activities in cultured human cells by sublethal concentrations of inorganic arsenic. *Toxicol. Sci.*, 2002, vol. 70, no. 2, pp. 183–192. DOI: 10.1093/toxsci/70.2.183

13. Quiñones L., Lee K., Varela F.N., Escala M., García K., Godoy L., Castro A., Soto J. [et al.]. Cancer pharmacogenetics: study of genetically determined variations on cancer susceptibility due to xenobiotic exposure. *Rev. Med. Chil.*, 2006, vol. 134, no. 4, pp. 499–515. DOI: 10.4067/s0034-98872006000400015 (in Spanish).

14. Agusa T., Fujihara J., Takeshita H., Iwata H. Individual variations in inorganic arsenic metabolism associated with AS3MT genetic polymorphisms. *Int. J. Mol. Sci.,* 2011, vol. 12, no. 4, pp. 2351–2382. DOI: 10.3390/ijms12042351

15. Janasik B., Reszka E., Stanislawska M., Wieczorek E., Fendler W., Wasowicz W. Biological monitoring and the influence of genetic polymorphism of As3MT and GSTs on distribution of urinary arsenic species in occupational exposure workers. *Int. Arch. Occup. Environ. Health*, 2015, vol. 88, no. 6, pp. 807–818. DOI: 10.1007/s00420-014-1009-7

16. De Chaudhuri S., Ghosh P., Sarma N., Majumdar P., Sau T.J., Basu S., Roychoudhury S., Ray K., Giri A.K. Genetic variants associated with arsenic susceptibility: study of purine nucleoside phosphorylase, arsenic (+3) methyltransferase, and glutathione S-transferase omega genes. *Environ. Health Perspect.*, 2008, vol. 116, no. 4, pp. 501–505. DOI: 10.1289/ehp.10581

17. Zhong S., Zhou S.-F., Chen X., Chan S.Y., Chan E., Ng K.-Y., Duan W., Huang M. Relationship between genotype and enzyme activity of glutathione S-transferases M1 and P1 in Chinese. *Eur. J. Pharm. Sci.*, 2006, vol. 28, no. 1–2, pp. 77–85. DOI: 10.1016/j.ejps.2006.01.002

18. Agusa T., Iwata H., Fujihara J., Kunito T., Takeshita H., Minh T.B., Trang P.T.K., Viet P.H., Tanabe S. Genetic polymorphisms in glutathione S-transferase (GST) superfamily and arsenic metabolism in residents of the Red River Delta, Vietnam. *Toxicol. Appl. Pharmacol.*, 2010, vol. 242, no. 3, pp. 352–362. DOI: 10.1016/j.taap.2009.11.007

19. Ramprasath T., Murugan P.S., Prabakaran A.D., Gomathi P., Rathinavel A., Selvam G.S. Potential risk modifications of GSTT1, GSTM1 and GSTP1 (glutathione-S-transferases) variants and their association to CAD in patients with type-2 diabetes. *Biochem. Biophys. Res. Commun.*, 2011, vol. 407, no. 1, pp. 49–53. DOI: 10.1016/j.bbrc.2011.02.097

20. González-Martínez F., Sánchez-Rodas D., Varela N.M., Sandoval C.A., Quiñones L.A., Johnson-Restrepo B. As3MT and GST Polymorphisms Influencing Arsenic Metabolism in Human Exposure to Drinking Groundwater. *Int. J. Mol. Sci.*, 2020, vol. 21, no. 14, pp. 4832. DOI: 10.3390/ijms21144832

Shaikhova D.R., Amromina A.M., Bereza I.A., Shastin A.S., Gazimova V.G., Sutunkova M.P., Gurvich V.B. Effects of genetic polymorphisms of GSTM1, GSTT1 and GSTP1 genes on blood metal levels in non-ferrous metal alloy smelter operators. Health Risk Analysis, 2022, no. 3, pp. 176–181. DOI: 10.21668/health.risk/2022.3.17.eng

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