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RISK OF REPRODUCTIVE SYSTEM PATHOLOGY IN FEMALE PAINTERS VIA CONTACT WITH AROMATIC HYDROCARBONS

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Abstract. Genetic polymorphism of xenobiotics is a key factor that determines individual sensitivity to hazardous industrial chemicals and predisposition to occupational pathologies. Studies have shown that the maximum risk of reproductive system pathologies in female painters working with aromatic hydrocarbons is caused by lack of glutathione-transferase TI and glutathione-transferase MI enzymes encoded by the genes (genotype GSTM1«0»/GSTT1«0»).
Key words: xenobiotics biotransformation gene; glutathione-transferase; genetic polymorphism; reproductive system diseases.

Introduction. Aromatic hydrocarbons (AH) are among the most common xenobiotics widely used for industrial purposes as lacquer solvents, paint solvents, etc. In an industrial environment, benzene and its homologs enter a human body through inhalation (via respiratory organs) and via unbroken skin resulting in acute and chronic intoxications. Good solvability in lipids allows them to enter a body and get detoxicated in many organs and tissues. The basis for AH intoxication regardless the route of entry are processes of activation of free radical oxidation of lipids and oxidation of proteins in liver and brain cord; their detoxication takes places mainly in liver microsomes with the involvement of a system of enzymes including glutathione S-transferase through intensification of mono-oxygenase responses [1].

Under continuous impact of various pathological factors on the reproductive system, individual sensitivity is important which includes the condition of the pituitary-hypothalamic-ovarian, hypothalamic-pituitary-adrenal, and pituitary-hypothalamic- thyroid systems, lipid peroxidation processes, and the antioxidant protection capacity. Research suggests that among the causes of reproductive disorders are specific combinations of allelic variants of genes of the glutathione-S-transferase superfamily the products of which are responsible for

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biotransformation and detoxication of xenobiotics. The damaging effect of xenobiotics can take place at the stages of hematogenesis, fertilization, implantation and embryogenesis [2].

Predictive diagnostics of diseases is widely explored today; it is based on studying the genes of biotransformation of xenobiotics of glutathione-transferases T1, M1, and P1and microsomal epoxide hydrolase [3].

This study focuses on the relationship between the probability of a reproductive system pathology and polymorphism of the genes of glutathione S-transferase MI (GSTMI) and TI (GSTI) in the employees of a paint shop at Minsk Automobile Plant that work with benzene homologs – toluene, xylene, and styrene widely used as lacquer and paint solvents.

Materials and Research Methods. We conducted a genotype analysis by the GSTMI and GSTTI genes of the DNA samples of the painting and metal-coating shop employees at Minsk Automobile Plant – 119 women that have contact with benzene homologs (toluene, styrene) and a history of reproductive system diseases (inflammations, uterine fibroid, endometriosis, and sterility). The control group was composed of 162 IT employees that do not experience chemical exposure in their professional day-to-day operations.

DNA purification was conducted with the help of a phenol- chloroform extraction method from the peripheral blood leukocytes. Determination of the deletion polymorphisms of the genes of glutathione S-transferase MI (GSTMI) and TI (GSTI) was conducted using a multiplex PCR method with specific primers combined with the CYP1A1 gene which served as internal control (homozygotes for the normal allele and heterozygotes were not differentiated – «+» allelic condition, homozygotes for the deletion allele – «0» allelic condition).

For the statistical processing of the obtained data, we used the χ^2 criterion or in the event when one of the analyzed values was below five, we referred to Fischer's Exact Test. We also determined the risk ratio (OR) and its 95% confidence intervals (95% CI). We used SPSS Statistics 17.0.1 and MS Excel 2003 for all the statistical calculations.

Results and Discussion. Based on the results of mandatory medical examinations, we revealed a significant excess in the incidence of the reproductive system diseases in the female workers exposed to aromatic hydrocarbons as compared to the control group. The most common pathologies of the reproductive system included chronic hysteritis, adnexal affections, and benign tumors.

It is reported that many repeated disease are provoked by unfavorable combinations of biotransformation genes, more precisely, the presence of functionally defective versions of these genes in the body that leads to the synthesis of excessive activity or, conversely, more commonly, functionally weakened forms of these enzymes. Along with the impact of high doses

of hazardous substances to the body, a change in the flow rate of biotransformation processes can also have a much more significant impact on the probability of certain disorders as compared to normal conditions.

When comparing the frequency of mutant genotypes in female painters with the reproductive system pathologies, it was determined that the frequency of carriers of deletion in the GSTT1 gene in homozygous condition in the group of patients exceeded the corresponding figure for the control sample by more than half (35.4% and 20, 1%, respectively, P = 0.005, OR = 2.17, CI 1.25-3.77). The results are shown in Table 1 below.

Table 1

The frequency of carriers of mutant genotypes in female workers diagnosed with the reproductive system diseases (the group of patients), and the corresponding control group

Genotype	Control group n=162	Group of patients n=119	P	OR (95% CI)
GSTM1«0»	50,6%	60,2%	0,12	1,47 (0,9-2,41)
GSTT1«0»	20,1%	35,4%	0,005*	2,17 (1,25-3,77)
GSTM1«0»/GSTT1«0»	11,0%	21,2%	0,022*	2,17 (1,11-4,27)

*Differences are significant (P<0,05)

We also revealed an almost two-fold increase in the frequency of double deletion of GSTM1«0»/GSTT1«0» genotype in the group of patients as compared to the control sample (21.2% against 11.0%, P=0.022, OR=2.17, CI 1.11-4.27). The obtained results suggest a relatively higher predisposition to the reproductive system pathologies in the employees that work with AH and have GSTT1«0» and GSTM1«0»/GSTT1«0» genotypes.

When analyzing the data on the workers in contact with AH and a length of employment of 5-10 years, we revealed a displacement of the frequency ratio of allelic state GSTT1 «0» towards the individuals diagnosed with the reproductive system diseases (14.3% in the control group and 30.2% in the group of patients); their differences are close to significant (p = 0.064, OR = 2.6, CI 0.93-7.29) (Table 2). The share of the GSTT1 gene deletion carriers in the homozygous state in individuals with the length of employment of over ten years in the group of patients exceeded those for the control group by more than two and a half times (46.5% and 16.7%, respectively, P = 0,02, OR = 4,35, CI 1,65-11,44). Such distribution of frequencies of allelic state of GSTT1 «0» indicates that its negative impact on the development of the reproductive system pathology appears after five years of exposure to the AH and increases significantly with further contact.

Table 2

Dependency of the frequency of some mutant genotypes on the length of employment in individuals diagnosed with the reproductive system diseases (the group of patients) and in the corresponding control group

Genotype	Length of employment	Control group, N (%)	Group of patients, N (%)	P	OR (95% CI)
GSTT1«0»	<5 years	16 (32,7)	4 (22,2)	0,55*	0,59 (0,17-2,08)
	5-10 years	7 (14,3)	13 (30,2)	0,064	2,6 (0,93-7,29)
	>10 years	8 (16,7)	20 (46,5)	0,02	4,35 (1,65-11,44)
GSTM1«0»/ GSTT1«0»	<5 years	9 (18,4)	3 (16,7)	0,84*	0,89 (0,21-3,73)
	5-10 years	4 (8,2)	9 (20,9)	0,13*	2,98 (0,85-10,49)
	>10 years	4 (8,3)	12 (27,9)	0,025*	4,26 (1,26-14,44)

*The value was obtained with the help of Fischer's Exact Test

Additionally, a more than three-fold excess of the frequency of occurrence of a combination of two deletion genotypes of GSTM1«0» and GSTT1 «0» was found in individuals with the reproductive system diseases and more than ten years of employment, as compared to the corresponding control group (27.9% vs 8, 3%, P = 0,025, OR = 4,26, CI 1,26-14,44). These results support the fact that the allelic state of GSTM1 «0» in the setting of the absence of a properly functioning GSTT1 genotype is also a risk genotype during contact with AH.

To determine the relative risk of the reproductive system diseases, it is necessary to take into account that the main impact on this indicator is made by the allelic state of the GSTT1 gene, GSTM1 gene polymorphism contributes significantly less, however, the maximum risk is caused by the lack of activity of the two enzymes encoded by these genes (genotype GSTM1 «0 »/ GSTT1« 0 ») (Table 3).

Table 3

Relative risk of the reproductive system diseases in female painters based on a genotype analysis based on the GSTMI and GSTTI genes

Genotype	Probability	OR (95% CI)
GSTM1«+»/GSTT1«+»	Very low	0,51 (0,3-0,87)
GSTM1«0»/GSTT1«+»	Low	0,97 (0,59-1,6)
GSTM1«+»/GSTT1«0»	High	1,65 (0,77-3,54)
GSTM1«0»/GSTT1«0»	Very high	2,17 (1,11-4,27)

Based on the conducted studies, we developed an algorithm to identify the "at risk" groups; it takes into account the results of molecular genetic testing of genes responsible for the biotransformation of xenobiotics in workers in contact with the arenes. Determination of the

GSTM1, GSTT1 gene polymorphism gives a doctor an opportunity to predict a eproductive system disease and, therefore, to properly select preventive measures.

Thus, this area of research is of current interest since it is instrumental in developing a course of preventive measures at the stage of hiring staff as well as at early stages of its development. It is necessary to use genetic tests in combination with all the other results of a medical exam.

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