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Glutathione-S-transferase M1 (GSTM1), P1 (GSTP1), and T1 (GSTT1) gene polymorphisms in children having got obstructive lung disorders when residing at Cs^{137} contaminated territories of Ukraine

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Ionizing radiation and a complex of other negative factors of the Chornobyl disaster have a significant impact on the functional state of the respiratory system, causing a high incidence of bronchial hyperreactivity in children living under chronic intake of radionuclides with a long half-life, which is a risk factor for the formation of chronic bronchopulmonary pathology [1- 3].

Currentstudiesare focused on studyingthe molecular and genetic bases of hereditary predisposition and are to define the role of certain genes and enzymes encoded by them in the pathogenesis of bronchoobstructive diseases [4 - 6].

The reaction of each individual organism to theenvironment influencedepends on the genetically determined features of functional enzyme systems in the phaseI and II of xenobioticdetoxification. Thus, glutathione-S-transferases (GSTs) are a large group of enzymes that are directly involved in the second phase of biotransformation, characterized by broad substrate specificity and the ability to metabolize many substances . A wide isomorphic spectrum of GSTs which is determined by the polymorphism of the genes encoding themhas been established. Differences in the structure of isoenzymes lead to different ability to metabolize xenobiotics and oxidative stress products. This causes a different degree of susceptibility of each individual to the occurrence of multifactorial diseases, including respiratory pathology [5].

Isoenzymes encoded by genes of the glutathione-S-transferase family (GSTT1, GSTM1, GSTP1) are an important part of antioxidant protection at the cellular level. The expression of these genes occurs from the early stages of embryonic development and given their established influence on the development of respiratory dysfunction in children [4, 6], so it is advisable to determine their polymorphism.

Materials And Methods: TheGSTT1, GSTM1 gene deletion polymorphismand theGSTP1 geneA313G polymorphism were studied in the molecular genetic laboratory of the State Institution "Reference Center for Molecular Diagnostics of the Ministry of Health of Ukraine".

There were examined 48 schoolchildren, aged from 10 to 17 years. All the children permanently (from birth) lived in radioactively contaminated areas (RCA) of the Narodychi, Ovruch, Olevskyand Korosten'districts of Zhytomyr region with density of 137Cs contamination of soil from 185 to 555 kBq/m2. The content of 137Cs in the body of children ranged from 74 to 8806 Bq.

All examined children had no clinical signs of respiratory pathology. Functional disorders of the gastrointestinal tract, chronic compensated tonsillitis and dental caries were found in them.

Total genomic DNA was isolated from blood according to a standard protocol using proteinase K and sodium dodecyl sulfate as detergent. The GSTT1 and GSTM1 genes deletions weredetected by multiplex polymerase chain reaction (PCR). PCR results were analyzed by the method of electrophoresis in 2% agarose gel. The expected DNA fragment lengths (431 bp for GSTT1 and 120 bp for GSTM1) were calculated with the DNASTAR computer data processing software package using the GSTT1 and GSTM1 gene sequences available in the Genbank database. Homozygous forms with deletion of both copies of the GSTT1 and GSTM1 genes were identified by the absence of a corresponding fragment on the electrophoregram. Such genotypes were designated as T1del and M1del. Accordingly, the presence of these fragments on electrophoregrams indicated homo- or heterozygosity by a normal gene copy. The genotype of such patients was designated as T1+ and M1+.

Genomic DNA for molecular genetic study ofGSTP1 geneA313G polymorphism was isolated from peripheral blood by a commercial test system "innuPREP Blood DNA Mini Kit" ("Analytik Jena", Germany) using centrifugal filters. Modified protocols with oligonucleotide primers were used to determine the GSTP1 (A313G) rs1695 gene polymorphic variants by PCR and subsequent restriction fragment length polymorphism (RFLP) analysis. The study region of the gene was amplified using specific primers ("Metabion", Germany) for GSTP1 (GTAGTTGCCCAAGGTCAAG– forward, AGCCACCTGAGGGGTAAG– reverse). The molecular weight of the amplified fragment was 433 bp. Restriction analysis was performed using AlwI restriction endonuclease (manufactured by Thermo Fisher) according to the manufacturer's instructions. Visualization of the formed restriction products was performed in 2% agarose gel, depending on the molecular weight of the formed fragments. In the presence of the AA genotype in the gel after cleavage, 2 fragments with a molecular weight of 328 and 105 bp were visualized. When a single nucleotide substitution appeared, an additional restriction site was formed and as a result two additional fragments with a molecular weight of 222 bp and 106 bp, so the heterozygous variant GA was characterized by the formation of 4 fragments – 328 bp, 222 bp, 106 bp and 105 bp, and homozygous variant GGby 3 fragments (222, 106 and 105 bp). Fragments with a molecular weight of 106 bp and 105 bp were visualized in agarose gel as a single fragment.

Ventilation lung capacity was examinedby the pneumotachographic method, according to the analysis of "the flow-volume" loop using automated spirometer. There were determined the forced vital capacity (FVC) of the lungs, the peak of expiration flow (PEF), maximum expiratory flow (MEF) according to th levels of 25 %, 50 % and 75 % FVC – MEF25, MEF50, MEF75,forced expiratory volume during the first second (FEV1). An analysis of indices was carried out as a percentage of the predictable. The negative deviation of the indicator was considered to be the limit of the norm, moderate and pronounced pathological changes: for FVC: – 80, 79–65 and less than 65 and for PEF– 71, 70 – 40 and less than 47; for FEV1 – 81, 80–66; for MEF25– 74, 73–51 and less than 51; for MEF50– 72; 71–48 and less than 48; for MEF75– 62; 61–27 and less 27.

The pharmacologic inhalation test with bronchodilator drug, affecting the β2-adrenergic lung receptors was used to detect the early changes

in the ventilation lung capacity –thebronchialhyperreactivity (latent andnonlatent bronchospasm). Values of the ventilation lung capacity were recorded before 5–8 min and after oftwo inhalation doses of dosage salbutamol sulphate aerosol (one dose contains of 100 micrograms). An increase of bronchial patency values (FEV1, MEF25, MEF50, MEF75) by 12% and more compared with the initial values was taken as the criterion of the test positivity.

The content of 137Cs in the body of children was determined using a human radiation detector (HRD) Scanner-3M manufactured by the Institute of Human Ecology.

Results: To establish the probable effect of glutathione-S-transferase gene polymorphism (GSTT1, GSTM1 and GSTP1) on the functional state of the respiratory system, children-residents of RCA were divided into two subgroups depending on the presence (n = 29) or absence (n = 19) of bronchial hyperreactivity.

The average values of respiratory tract patency at different levels of the bronchial tree were not significantly different and were within physiological fluctuations in both selected subgroups. Thus, the indicators that integrally characterize the patency of the respiratory tracts were, respectively: PEF/PPEF ((91.4 ± 3.9)% and (88.8 ± 4.1)%, p> 0.05; FEV1/PFEV1 (89.2 ± 4.0)% and (91.3 ± 2.6)%, p> 0.05. There were no significant differences in the values of patency in proximal bronchi of large diameter MEF25 / PMEF25 (90.1 ± 3.6)% and (92.0 ± 5.1)%, p> 0.05. The patency parameters of proximal bronchi of the medium diameter: MEF50/PMEF50 did not significantly differ (90.8 ± 2.9)% and (91.7 ± 3.8)%, p> 0.05. There was no significant difference of patency value in the peripheral bronchi of the small diameter: MOSH75/NMOSH75 (96.2 ± 4.6)% and (99.2 ± 5.5)%, p> 0.05. No significant differences were noted between the value of elasticity and elongation of pulmonary tissue and respiratory apparatus of the chest FVC/PFVC (87.4 ± 4.7)% and (90.5 ± 5.9)%, p> 0.05. However, the frequency of bronchial hyperreactivity was detected in 29 of the 48 examined children (60.4%), and in children of the control group – 22.4% (p <0.01).

The results of investigations showed that among children with bronchial hyperreactivity (n = 29) GSTM1 gene deletion polymorphismwas found in 72.41% (n = 21) of children, and among children without bronchial hyperreactivity – in 36.84% (n = 7) of children, p <0.05. In groups of children with and without bronchial hyperreactivity the frequency of theGSTT1gene deletion genotype was, respectively, 31.03% (n = 9) and 21.05% (n = 4) and had not statistically significant differences (p> 0.05).

Therefore, bronchial hyperreactivity was more common found in children whose genotype characterized by the presence of the GSTM1 gene deletion variants.

Distribution of the GSTP1 gene polymorphic variants was analyzed in 48 children-residents of RCA, in the presence (29 children) or absence (19 children) of bronchial hyperreactivity.

It was found that in children-residents of RCAwith diagnosed bronchial hyperreactivity, the frequency of AA-genotype was – 27.59% (8 children), AG-genotype – 55.17% (16 children), GG-genotype – 17, 24% (5 children), the frequency of the A-allele –55.17%, and G-allele – 44.83%.

In children without bronchial hyperreactivity, the following frequencies of genotypes by the GSTP1 gene were detected: AA genotype – in 10 children (52.63%), AG genotype – in 5 children (26.32%), GG genotype – in 4 children (21.05%), the frequency of the A-allele – 25 (65.79%), G-allele – 13 (34.21%).

Comparative analysis showed that in the presence of bronchial hyperreactivity in children the genotype AG was more common - 55.17% than in children without bronchial hyperreactivity - 26.32%, p <0.05, and the frequency of genotype AA, on the contrary had a clear tendency to decrease (27.59% and 52.63%, p> 0.05).

Thus, in the GSTP1 gene AG-heterozygotesbronchial hyperreactivity was probably more common than in homozygotes with functional A-allele.

Conclusions: The GSTM1 gene deletion polymorphism and the GSTP1 gene313AG genotype were found more often in the subgroup of children-residents of RCA with bronchial hyperreactivity, than in children without bronchial hyperreactivity, the frequency of GSTT1 gene deletion polymorphism in both subgroups had not statistically significant differences.Increased frequency of early disorders of ventilation lung capacity as bronchial hyperreactivity in children-residents of RCA is associated with the presence of the GSTM1 gene deletion polymorphism and the GSTP1 gene 313AG genotype, which may be a risk factor for further development of chronic bronchial pathology.

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