ПОРІВНЯЛЬНА ОЦІНКА МЕТАБОЛІЧНИХ ПРОЦЕСІВ У ДІТЕЙ, ЯКІ ПРОЖИВАЮТЬ У РАЙОНАХ, ЩО ПОСТРАЖДАЛИ ВІД АВАРІЇ НА ЧОРНОБИЛЬСЬКІЙ АЕС

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COMPARATIVE ASSESSMENT OF METABOLIC PROCESSES IN CHILDREN LIVING IN THE AREAS AFFECTED BY THE CHERNOBYL NUCLEAR POWER PLANT ACCIDENT

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Keywords: homocysteine, folate metabolism, thyroid and pituitary hormones, folic acid, vitamin B_{12}, adolescents, radiation-contaminated areas.

The level of metabolic processes is one of the main markers of the health of the human body. It depends on the state of the genetic apparatus and the impact of external environment. It is important to determine the nature of the impact of external environment in areas affected by the Chornobyl nuclear power plant (CNPP) accident in order to organise effective preventive measures for radiation exposure related diseases. So, in our opinion, it is reasonable to carry out a comparative assessment of metabolic processes in the population living after the Chornobyl accident in various districts.

Purpose of the study: comparative assessment of metabolic processes in children continually living in Polissia and Ivankiv districts, Kyiv region, in the areas contaminated with radioactive elements as a result of the CNPP accident.

Material and methods. The study was carried out in Polissia and Ivankiv districts which territory is contaminated with radionuclides as a result of the CNPP accident (the ^{137}Cs soil contamination density is <2 Curie/km^2 [1]). The age of children at the time of examination was (15.2 ± 0.9) years (95% CI 15.0-15.4 years).

Gamma-emitting radionuclide (^{134}Cs and ^{137}Cs) concentrations were measured in 176 children from Polissia district and 222 children from Ivankiv district using a three-detector whole-body counter manufactured by Atom Komplex Prylad (Ukraine).

158 children from Polesia district and 179 children from Ivankiv district were examined in 2015. For this purpose, levels of thyroid stimulating hormone (TSH), free triiodothyronine (T_3), free thyroxine (T_4), homocysteine (Hc), vitamins B_9 and B_{12} in the blood and state of the genetic system of folate metabolism were assessed.

All children had blood drawn from the ulnar vein on an empty stomach in the morning to carry out above analyses. All of them at the time of blood draw attended school.

Blood samples were analysed in a laboratory certified under quality standards with the financial support of the Rhфne-Alpes Regional Council (France) and the agreement of parents.

TSH, T_3 and T_4 concentrations

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***Мета** — порівняльна оцінка метаболічних процесів у дітей, які тривалий час проживають у Поліському та Іванківському районах Київської області на території, забрудненій радіоактивними елементами внаслідок аварії на Чорнобильській атомній електростанції.*

***Матеріал і методи дослідження.** У підлітків визначався вміст радіонуклідів ^{137}Cs, оцінювався вміст у крові тиреотропного гормону гіпофіза, трийодтироніну, тироксину, гомоцистеїну, вітамінів B_9, B_{12}, а також стан генетичної системи фолатного циклу. Використовувалися імунохімічний і математико-статистичний методи.*

***Результати роботи.** Питома вага дітей досліджуваної вікової групи з концентрацією ^{137}Cs в організмі вище 5 Бк/кг маси тіла склав у Поліському районі 34,1% і був достовірно вищим ($p<0,05$), ніж в Іванківському районі (14,4%). Геном фолатного циклу дітей з обох районів не мав істотних відмінностей. Алелі ризику генетичних поліморфізмів фолатного циклу були відсутніми у 3,2% випадків у дітей з Поліського району і в 1,1% випадків — у дітей з Іванківського району. Рівень гомоцистеїну перевищував фізіологічний критерій (стан гіпергомоцистеїнемії) у дітей з Поліського району у 79,8% випадків, у дітей з Іванківського району — у 73,2% випадків. У дітей з Поліського району вміст фолієвої кислоти і тироксину у крові був вірогідно меншим, ніж у дітей з Іванківського району.*

***Висновки**. Причинами підвищеного вмісту радіонуклідів в організмі, гіпергомоцистеїнемії, порушення продукції гормонів щитоподібної залози, дефіциту фолієвої кислоти і вітаміну B_{12} у дітей Поліського району порівняно з дітьми Іванківського є незадовільні соціально-економічні умови проживання. Інкорпорація радіонуклідів ^{137}Cs в організм і щитоподібну залозу, дефіцит фолієвої кислоти можуть призводити до порушення продукції тироксину щитоподібної залозою у дітей, які проживають на території, забрудненій радіонуклідами внаслідок аварії на Чорнобильській атомній електростанції.*

Ключові слова: гомоцистеїн, фолатний цикл, гормони щитоподібної залози і гіпофіза, фолієва кислота, вітамін B_{12}, підлітки, радіаційно забруднені райони.

were determined using the immunochemical method with electrochemiluminescent detection (ECLIA), an analyser and a test systems: Cobas 6000; Roche Diagnostics (Switzerland).

Blood homocysteine levels were determined using an immunochemical method with chemiluminescent detection (CLIA). An analyser and a test system: Architect 1000 (ABBOT Diagnostics (USA). Blood homocysteine levels of over 10 μmol/L in the children were defined as hyperhomocysteinemia.

Vitamin B_9 (folacin) concentrations were determined using the immunochemical method with electrochemiluminescent detection (ECLIA). An analyser and a test system: Cobas e411; Roche Diagnostics (Switzerland).

Vitamin B_{12} (holotranscobalamin, active vitamin B_{12}) concentrations were determined using the immunochemical method with chemiluminescent detection (CLIA). An analyser and a test system: Architect 1000 (Abbott Diagnostics (USA).

The allelic variants C677T and A1298C of the MTHFR gene (synthesis of the methylenetetrahydrofolate reductase enzyme), A2756G of the MTR gene (synthesis of the B_{12}-dependent methionine synthase enzyme) and A66G of the MTRR gene (synthesis of the methionine synthase reductase enzyme) were determined during genetic analysis of folate metabolism.

A real-time PCR method was used. An analyser and a test system: DT-96 detecting thermocycler, DNA-Technology (Russia).

The statistical processing of the obtained results was performed using the IBM SPSS Statistics 22 software (USA). The arithmetic mean (M) ± standard error of mean (m), confidence interval for the average value (95% CI), median (Me), interquartile range (IR), minimum and maximum parameter values and percentiles were calculated for the variables under analysis. The distribution hypothesis was tested (a Kolmogorov-Smirnov test). All the parameters under study did not conform to the normal distribution law, thus, a non-parametric U Mann-Whitney test was used to compare values. The statistical significance of variables was assessed by determining a significance level for p with the help of a statistical software programme. The Student's t-test was used to compare relative values. The critical level of significance for the null hypothesis (p) was set at 0.05. The relationship between blood thyroxin, folic acid and vitamin B_{12} levels was established with the help of the Spearman's rank correlation coefficient (r_{xy}). Strength of correlation was assessed by a typical scale: weak – 0 to 0.299; moderate – 0.3 to 0.699; strong – 0.7 to 1.0.

Table 1

Percentage of cases that include risk alleles of folate metabolism genetic polymorphisms in the groups of examined children

Subgroup №	Number of polymorphisms	Polissia district		Ivankiv district	
		Absolute number	Percentage, %	Absolute number	Percentage, %
1	0	5	3.2	2	1.1
2	1	32	20.2	25	14.0
3	2	66	41.8	85	47.5
4	3	49	31.0	52	29.0
5	4	6	3.8	15	8.4
Total	158	100	179	100	

Table 2

Percentage of polymorphic alleles of folate metabolism genes in the children from Polissia district, (n=158)

Gene, polymorphism	"Neutral" allele		"Heterozygous genotype" risk allele		"Homozygous genotype" risk allele	
	Absolute number	%	Absolute number	%	Absolute number	%
MTR:A2756G	104	65.8	45	28.5	9	5.7
MTHFR:A1298C	82	51.9	60	38.0	16	10.1
MTHFR:C677T	79	50.0	60	38.0	19	12.0
MTRR:A66G	32	20.2	72	45.6	54	34.2

Table 3

Percentage of polymorphic alleles of folate metabolism genes in the children from Ivankiv district, (n=179)

Gene, polymorphism	"Neutral" allele		"Heterozygous genotype" risk allele		"Homozygous genotype" risk allele	
	Absolute number	%	Absolute number	%	Absolute number	%
MTR:A2756G	106	59.2	62	34.6	11	6.2
MTHFR:A1298C	90	50.3	80	44.7	9	5.0
MTHFR:C677T	81	45.2	83	46.4	15	8.4
MTRR:A66G	27	15.1	94	52.5	58	32.4

Results and discussion. The percentage of children of the age group under study with ^{137}Cs levels of over 5 Bq/kg of body weight was 34.1% in Polissia district (60 cases out of 176) and was significantly higher ($p<0.05$) than in Ivankiv district – 14.4% (32 cases out of 222).

There were no differences between the groups of children from Polissia and Ivankiv districts in terms of the percentage of cases that include risk alleles of folate metabolism genetic polymorphisms. The absence of risk alleles of folate metabolism genetic polymorphisms was detected in 3.2% of the children from Polissia district and in 1.1 % of the children from Ivankiv district. The highest percentage was held by the subgroups with two polymorphisms (table 1).

There were no differences between the groups of children from Polissia and Ivankiv districts in terms of the percentage of polymorphic alleles of folate metabolism genes (tables 2-3). In the both groups of children, the proportion of cases with a genotype that includes only a neutral allele for the MTR:A2756G and MTHFR:A1298C polymorphisms exceeded the proportion of cases with genotypes that include a risk allele. In the case of the MTHFR:C677T and MTRR:A66G polymorphisms, the proportion of cases with a risk allele was over or

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***Objective**. We performed a comparative assessment of metabolic processes in the children living for a long time in Polissia and Ivankiv districts of Kyiv oblast, at the territories contaminated with radioactive elements as a result of the accident at the Chornobyl NPP.*

***Materials and methods.** We determined a content of ^{137}Cs radionuclides, a content of thyrotropic pituitary hormones, triiodothyronine, thyroxin, homocysteine, vitamins B_9, B_{12} in the blood of the adolescents, and a state of folate metabolism genetic system as well.The immunochemical and mathematical-and-statistical methods were applied.*

***Results**. The percentage of the children in the age group under study with ^{137}Cs concentration in the organism over 5 Bq/kg of body weight was 34.1% in Polissia district and was significantly higher ($p<0.05$) than in Ivankiv district — 14.4%. The genome of folate metabolism didn't have significant differences in the children of both districts. There were no risk alleles of folate metabolism genetic polymorphisms in 3.2% of the cases in the group of the children from Polissia district and in 1.1% in the group of the children from Ivankiv district. A level of homocystenia exceeded a physiological criterion (state of hyperhomocysteinemia) in 79.8% of cases in the group of the children from Polissia district and in 73.2% of cases in the group of the children from Ivankiv district. A content of folic acid and thyroxine in the blood was significantly lower in the group of children from Polissia district than in Ivankiv district.*

***Conclusions**. The poor socio-economic conditions are the reasons for a higher content of radionuclides, hyperhomocysteinemia, disruption of the production of thyroid gland hormones, deficiency of folic acid and vitamin B_{12} in the children from Polissia district in comparison with the children from Ivankiv district. The incorporation of ^{137}Cs radionuclides in the organism and thyroid gland, and folic acid deficiency can lead to disruption of thyroxine production by the thyroid gland in the children living at the territory contaminated with radionuclides as a result of the accident at the Chornobyl NPP.*

Keywords: homocysteine, folate metabolism, thyroid and pituitary hormones, folic acid, vitamin B_{12}, adolescents, radiation-contaminated areas.

equal to the proportion of cases with a neutral allele. The proportion of cases with heterozygous variants that include a risk allele exceeded the proportion of cases with homozygous variants with the same allele.

Hyperhomocysteinemia was observed in 79.8% of cases (67 out of 84 cases) in the group of children from Polissia district and in 73.2% of cases (131 out of 179 cases) in the group of children from Ivankiv district.

The comparative analysis did not reveal any statistically significant differences between blood homocysteine (Hc) values in the children from the both districts. At the same time, it was established that blood vitamin B_9, triiodothyronine and thyroxine levels were significantly higher in the group of children from Ivankiv district than in the group of children from Polissia district (tables 4-5).

The reference range for folic acid (vitamin B_9) in the blood specified by the laboratory where the analysis was done was 4.6-18.7 ng/ml, for vitamin B_{12} – 191.0-663.0 pg/ml.

The interval between the extreme values of vitamin B12 in the blood of the children from Polissia district was 160.9-902.5 pg/ml and 136.1-853.4 pg/ml for the children from Ivankiv district. The interval between the extreme values of vitamin B_9 in the blood of the children from Polissia district was 2.14-13.23 ng/ml and 2.34-15.89 ng/ml for the children from Ivankiv district.

The percentage of cases with folic acid and thyroxine levels below the reference values was significantly lower in the group of children from Ivankiv district than in the group of children from Polissia district (table 6).

A direct weak correlation was established between thyroxine and folic acid levels (r_{xy}=0.166, p=0.026, n=179), and between thyroxine and vitamin B_{12} levels (r_{xy}=0.230, p<0.02, n=179) in the group of children from Ivankov district.

Table 4

Statistical characteristics of blood variables of metabolic processes in the children examined

Variables	Polissia district		Ivankiv district	
	Me	IR	Me	IR
HC, μMOL/L	12.25	10.43-14.48	11.61	9.69-13.35
B_9, NG/ML	5.43	4.34-6.92	6.53	5.10-7.95
B_{12}, PG/ML	318.65	256.53-437.13	309.10	255.10-422.40
TSH, μIU/ML	1.86	1.41-2.48	1.80	1.30-2.40
T_3, PG/ML	4.1	3.79-4.62	4.40	3.99-4.75
T_4, NG/DL	1.18	1.07-1.28	1.23	1.14-1.31

Table 5

Results of statistically significant differences when comparing blood variables of metabolic processes in the examined children from Polissia[1] and Ivankiv[2] districts

Variables	Groups of comparison	Comparison group size	Average rank	U test value, Significance level, p
B_9, ng/ml	1	84	109.76	U = 5650.0; p = 0.001
	2	179	142.44	
T_3, pg/ml	1	158	150.69	U = 11247.5; p = 0.001
	2	179	185.16	
T_4, ng/dl	1	158	152.88	U = 11594.5; p = 0.004
	2	179	183.23	

Note: 1 – group 1; 2 – group 2.

Thus, during the studies conducted it was found that a significantly larger number of children from Polissia district in comparison with Ivankiv district had ^{137}Cs levels of over 5 Bq/kg of body weight. The main reason for this may be the consumption by the children of food products of local production, mushrooms, forest berries and the meat of wild animals containing radioactive elements.

No differences were found in terms of the genome of folate metabolism in the children from the districts under study. However, the genome of the children examined contained risk alleles. Only 3.2% of the children from Polissia district and 1.1% of the children from Ivankiv district had no risk alleles of folate metabolism genetic polymorphisms.

Homocysteine levels exceeded the physiological range in more than 70.0% of the children from both groups. An inverse association was observed between blood homocysteine and B_9 and B_{12} levels in the children living in the areas affected by the Chornobyl accident [2].

A folic acid deficiency is more pronounced in the children from Polissia district comparing to the children from Ivankiv district. First of all, it is associated with an alimentary factor.

The associations between thyroxine and folic acid levels, and thyroxine and vitamin B_{12} levels suggest that a folate and vitamin B_{12} deficiency may be the reason for inadequate thyroxine production by the thyroid gland.

Considering that the thyroid gland intensively incorporates ^{137}Cs radionuclides [3], one can say that the latter participate in the disruption of thyroxine production.

Thus, the main differences between the children from Polissia and Ivankiv districts are associated with blood folic acid and thyroxine levels.

The children from Polissia district are in conditions of a more pronounced folic acid deficiency in comparison with the children from Ivankiv district located closer to Kyiv. The reason for this, first of all, is a poor socio-economic situation in Polissia district [4]. As a result, its inhabitants resort to consuming wild mushrooms and berries and meat of local wild animals containing large doses of radionuclides. Radiation exposure and folic acid deficiency result in abnormal thyroid function and decreased thyroxine production.

Conclusions

1. There was a considerably larger percentage of cases with body ^{137}Cs levels of over 5 Bq/kg in the group of children from Polissia district than in the group of children from Ivankiv district, though the both districts had ^{137}Cs soil contamination levels of <2 Cu/km^2.

2. The genome of folate metabolism of the children from the both districts had no significant differences. The risk alleles of folate metabolism genetic polymorphisms were absent in 3.2% of cases in the group of children from Polissiaidistrict and in 1.1% of cases in the group of children from Ivankiv district.

3. Homocysteine levels exceeded the physiological range (hyperhomocysteinemia) in 79.8% of cases in the group of children from Polissia district and in 73.2% of cases in the group of children from Ivankiv district.

4. Blood folic acid and thyroxine levels were significantly lower in the children from Polissia district than in the children from Ivankiv district.

5. The poor socio-economic conditions are one of the main reasons for inadequate intake of folic acid in the children from Polissia district. The incorporation of ^{137}Cs radionuclides into the body and thyroid gland, and folic acid deficiency can lead to disruption of thyroxine production by the thyroid gland in children living in the areas contaminated with radionuclides as a result of the CNPP accident.

Table 6

Percentage of cases with values different from the reference ones

Variables	Polissia district			Ivankiv district		
	N[1]	Number of cases	%	N	Number of cases	%
Hc, μmol/L	84	67	79.8	179	131	73.2
B_9, ng/ml	84	25	29.8	179	29	16.2*
B_{12}, pg/ml	84	4	4.8	179	9	5.0
TSH, μIU/mL	158	4	2.5	179	4	2.2
T_3, pg/ml	158	18	11.4	179	26	14.5
T_4, ng/dl	158	49	31.0	179	35	19.6*

*Note: * — statistical differences between groups ($p<0.05$);*
*** — values below the reference values were taken into account when assessing B_9, B_{12}, T_4, above the reference values — when assessing Hc, TSH, T_3; N[1] — group size.*

ЛІТЕРАТУРА

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