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ГІПЕРГОМОЦИСТЕЇНЕМІЯ У ДІТЕЙ ТА ЛІСОВІ ПОЖЕЖІ У ЧОРНОБИЛЬСЬКІЙ ЗОНІ ВІДЧУЖЕННЯ

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HYPERHOMOCYSTEINEMIA IN CHILDREN AND FOREST FIRES IN THE CHORNOBYL EXCLUSION ZONE

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Relevance of the problem. Studies conducted during the implementation of projects of the European Commission and the Rhône-Alpes Region (France) have found increased blood levels of homocysteine – a metabolic product of the essential amino acid methionine – in a significant number of examined adolescent children living in raions located near the Chornobyl nuclear power plant [1].

Given that hyperhomocysteinemia is associated with the development of severe pathological processes, including cardiovascular diseases and cancers, it is important to determine the cause of its occurrence [2, 3].

Hyperhomocysteinemia is known to be linked to genetic defects of folate metabolism, primarily to the MTHFR:C677T polymorphism [4].

Among environmental factors that can cause hyperhomocysteinemia, one should also mention products of burning of wood and peat in the form of finely dispersed particles with a size of 0.1-2.5 μm (PM2.5), including black carbon, that can penetrate into the respiratory tract, reaching the bronchioles and alveoli.

Long-term exposure to PM2.5 can cause the development or exacerbation of cardiometabolic disorders [6].

An association has been found between wood burning and increased blood homocysteine levels in the group of elderly men [7].

It should be noted that fires in the Chornobyl exclusion zone (ChEZ) are quite common. The largest forest and meadow fire in Ukraine over the past 25 years on an area of 10127 hectares occurred on April 26-29, 2015.

The maximum contamination density of the territory in the areas of ground forest fires in separate sections of Lubianka Forest Division was 1040

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Мета дослідження. *Визначити роль генотипів фолатного циклу у виникненні гіпергомоцистеїнемії у дітей після лісової пожежі у Чорнобильській зоні відчуження (ЧЗВ).*
Методи: *імунохімічний, ПЛР у режимі Real-time, математико-статистичний.*
Результати. *Обстеження 84 дітей підліткового віку із Поліського району Київської області дозволило виявити у них зміни метаболічних процесів у вигляді підвищеного продукування гомоцистеїну у зв'язку з лісовою пожежею у ЧЗВ 26-29 квітня 2015 року. Проведений порівняльний аналіз питомої ваги генотипів фолатного циклу у підгрупах дітей з гіпергомоцистеїнемією до і після лісової пожежі показав непричетність до цього явища ендогенного фактора у вигляді генетичного апарату, що контролює синтез ферментів фолатного циклу.*
Висновки. *Пожежі лісу і торфовищ у ЧЗВ є однією з основних причин підвищення рівня гомоцистеїну у крові дітей з прилеглих районів незалежно від стану генетичної системи фолатного циклу. Контроль над рівнем гомоцистеїну у крові дітей і дорослих, які перебувають під впливом продуктів горіння деревини, що містить радіоактивні елементи, є основним компонентом програми профілактики онкологічних і серцево-судинних захворювань.*

Ключові слова: фолатний цикл, гомоцистеїн, підлітки, лісові пожежі, Чорнобильська зона відчуження.

kBq/sq.m for ^{137}Cs; 368 kBq/sq.m for ^{90}Sr; 11.4 kBq/sq.m for $^{238-240}$Pu and 14.4 kBq/sq.m for ^{241}Am [8].

Thus, along with a genetic predisposition, forest fires in the ChEZ can be viewed as a source of hyperhomocysteinemia in residents of the districts adjacent to it.

In order to find out the main cause of hyperhomocysteinemia among the population of the districts located near the ChEZ, it is necessary to conduct a comparative assessment of homocysteine levels in the same group of subjects before and after a forest fire, taking into account the proportions of folate metabolism genotypes.

The aim of this study was to determine the role of folate metabolism genotypes in the occurrence of hyperhomocysteinemia in children after a forest fire in the ChEZ.

In this case, it is necessary to carry out a comparative assessment of proportions of folate metabolism genotypes in subgroups with hyperhomocysteinemia before and after a forest fire in the ChEZ in the same group of children.

Material and methods. The study was conducted within projects of the European Commission and the Rhône-Alpes Regional Council (France) implemented in Ukraine in 2013-2017.

The examined group comprised 84 children (39 boys and 45 girls) living on the territory of Ukraine after the Chornobyl accident (Polessky district, Kiev region) with a ^{137}Cs soil contamination level of <2 Curie/sq.km and bordering the ChEZ [9].

The children's average age at the time of examination was (15.5±0.1) years (95% CI 15.4-15.7 years).

In order to further determine homocysteine concentrations and genotype of folate metabolism (FM), each child had blood drawn from the ulnar vein after fasting in the morning twice on 02/04/2015 and 18/12/2015.

The blood samples were analysed at a laboratory certified under international quality standards with the agreement of the parents.

Blood homocysteine concentrations were measured using a chemiluminescent immunoassay (CLIA) method. Analyser and test kit: Architect 1000 (ABBOT Diagnostics (USA)). Blood homocysteine levels of over 10 µmol/L were defined as hyperhomocysteinemia.

The following allelic variants were identified during genetic analysis of FM: C677T and A1298C of the MTHFR gene (methylenetetrahydrofolate reductase), A2756G of the MTR gene (B_{12}-dependent methionine synthase) and A66G of the MTRR gene (methionine synthase reductase). A real-time PCR method was used. Analyser and test kit: DT-96 detecting thermocycler, DNA-Technology (Russia).

The statistical processing of the results obtained was performed using the IBM SPSS Statistics 22 software (USA). Arithmetic mean (M), ± standard error of mean (m), confidence interval for a mean value (95% CI), median (Me), interquartile range (IR), minimum and maximum parameter values and percentiles were calculated for the variables analysed.

The statistical significance of variables was assessed by determining a significance level for p with the help of the 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.

Results and discussion. A statistically larger number of cases of hyperhomocysteinemia was found in the total group of children and a group of boys during the measurement on 02/04/2015 compared with 18/12/2015 (table 1). There were no significantly statistical differences between hyperhomocysteinemia rates during two measurements in the group of girls (table 1).

The comparison of proportions of similar genotypes of FM

Table 1

Dynamics of cases of hyperhomocysteinemia in groups of children

Date of examination of children	Number of cases of hyperhomocysteinemia in groups of children					
	Total (n = 84)		Boys (n = 39)		Girls (n = 45)	
	Abs. number	%	Abs. number	%	Abs. number	%
02/04/2015	48	57.1	23	59.0	25	55.6
18/12/2015	67	79.8*	35	89.7**	32	71.1***

*Note: * – statistical differences between hyperhomocysteinemia rates in the total group of children: t = 3.26; p = 0.001481.*
*** – statistical differences between hyperhomocysteinemia rates in the group of boys: t = 3.30; p = 0.001689.*
**** – statistical differences between hyperhomocysteinemia rates in the group of girls: t = 1.54; p = 0.128839.*

Table 2

Frequency of polymorphic alleles of folate metabolism genes in children with blood homocysteine levels of over 10 µmol/L during the measurement on 02/04/2015 (n = 48)

Gene, polymorphism	«Neutral» allele		Risk allele, heterozygous mutation form		Risk allele, homozygous mutation form	
	Abs. number	%	Abs. number	%	Abs. number	%
MTR:A2756G	35	72.9	11	22.9	2	4.2
MTHFR:A1298C	26	54.2	15	31.3	7	14.5
MTHFR:C677T	20	41.6	19	39.6	9	18.8
MTRR:A66G	7	14.6	20	41.7	21	43.7

polymorphisms in a total group of children, groups of boys and girls with hyperhomocysteinemia reported on 02/04/2015 and 18/12/2015 showed no statistical differences (tables 2-7). A similar result was obtained when proportions of the MTHFR:677 CT/MTHFR:1298 AC heterozygous association were compared (table 8).

The results obtained indicate that a significant increase in the number of cases of hyperhomocysteinemia was observed in the group of adolescent children living in the district bordering the ChEZ during the period between the measurements of this metabolite in the blood (02/04/2015-18/12/2015). Statistically significant differences between relative coefficients were reported in the total group of children and in the group of boys.

Statistical analysis showed that an endogenous factor in the form of a genetic apparatus regulating the synthesis of FM enzymes is not involved in this phenomenon. The proportions of genotypes including risk alleles did not differ statistically in these groups of children with hyperhomocysteinemia reported on 02/04/2015 and 18/12/2015.

Forest and peat fires officially reported in Sections 306-308 of Lubyanka Forest Division in the ChEZ during the periods 26-27/04/2015 and 28-29/04/2015 on the territory with an area of 10127 hectares [8] and during the period from 29/06/2015 to 05/07/2015 on the territory with an area of 130 hectares should be probably considered as one of the causes of this phenomenon [10]. The maximum contamination density of the territory amounted to 1040 kBq/sq.m for ^{137}Cs; 368 kBq/sq.m for ^{90}Sr; 11.4 kBq/sq.m for $^{238-240}$Pu and 14.4 kBq/sq.m for ^{241}Am [8].

At the same time, finely dispersed burning particles with a size of 0.1-2.5 μm (PM2.5), including black carbon, as well as radionuclides, being spread with air currents over long distances from the fire site were penetrating into the respiratory tract of residents of localities situated near the ChEZ, reaching the bronchioles and alveoli [5].

Oxidative stress occurring with the involvement of homocysteine may be one of the main links in the pathogenesis of cardiovascular diseases [11], which are widespread among the population of the districts affected by the Chornobyl accident [12].

Thus, the negative effect of burning of forests containing radioactive elements on methionine metabolism resulted in an increase in blood homocysteine levels even in those children who did not have a genetic predisposition to this.

Experimental studies using laboratory animals conducted during the first years after the Chornobyl nuclear power plant accident also confirm the negative effect of incorporated radioactive agents on methionine metabolism.

Daily feeding of white rats for 28 days with whole oats containing ^{137}Cs with the activity concentration of 445.7 Bq/kg and ^{90}Sr with the activity concentration of 15.5 Bq/kg (animals of a control group received daily as food whole oats where ^{137}Cs concentration was 44.2 Bq/kg and ^{90}Sr concentration amounted to 1.7 Bq/kg) led to a significant decrease in methionine concentrations in the myocardium

Table 3

Frequency of polymorphic alleles of folate metabolism genes of children with blood homocysteine levels of over 10 μmol/L during the measurement on 18/12/2015 (n = 67)

Gene, polymorphism	«Neutral» allele		Risk allele, heterozygous mutation form		Risk allele, homozygous mutation form	
	Abs. number	%	Abs. number	%	Abs. number	%
MTR:A2756G	47	70.1	18	26.9	2	3.0
MTHFR:A1298C	41	61.2	19	28.4	7	10.4
MTHFR:C677T	31	48.3	25	37.3	11	16.4
MTRR:A66G	13	19.4	29	43.3	25	37.3

Table 4

Frequency of polymorphic alleles of folate metabolism genes of boys with blood homocysteine levels of over 10 μmol/L during the measurement on 02/04/2015 (n = 23)

Gene, polymorphism	«Neutral» allele		Risk allele, heterozygous mutation form		Risk allele, homozygous mutation form	
	Abs. number	%	Abs. number	%	Abs. number	%
MTR:A2756G	17	73.9	5	21.7	1	4.4
MTHFR:A1298C	10	43.5	10	43.5	3	13.0
MTHFR:C677T	9	39.1	9	39.1	5	21.8
MTRR:A66G	3	13.0	6	26.1	14	60.9

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Objective: *We determined a role of folate metabolism genotypes in the occurrence of hyperhomocysteinemia in children after a forest fire in the Chornobyl exclusion zone (ChEZ).*
Material and methods: *In the study, we applied immunochemical, Real-time PCR, mathematical and statistical methods.*
Results: *A survey of 84 adolescents from the Poliske district, Kiev region revealed changes in their metabolic processes in the form of increased homocysteine production in connection with a forest fire in the ChEZ which was recorded during April 26-29, 2015. A comparative analysis of the specific gravity of the folate cycle genotypes in the subgroups of the children with hyperhomocysteinemia before and after a forest fire showed that an endogenous factor in the form of a genetic apparatus, controling synthesis of folate cycle enzymes, was not involved in this phenomenon.*
Conclusions: *Forest and peat fires in the Chernobyl exclusion zone are one of the main causes for the elevation in blood homocysteine levels in children from the adjacent districts, regardless of the state of folate metabolism genetic system. Monitoring of the blood homocysteine levels in children and adults living under conditions of the exposure to wood combustion gases, containing radioactive elements, is a key component of the programmer for the prevention of cancer and cardiovascular diseases.*

Keywords: folate metabolism, homocysteine, adolescents, forest fires, Chornobyl exclusion zone.

and skeletal muscles tissues comparing to the control group [13]. In our opinion, it is linked to the disturbance of its resynthesis from homocysteine.

The findings indicate that there is a necessity to monitor blood homocysteine levels in children affected by air currents that include products of burning of forests and peats in the ChEZ.

Conclusions

1. Forest and peat fires in the Chernobyl exclusion zone are one of the main causes for the elevation in blood homocysteine levels in children from the adjacent districts, regardless of the state of folate metabolism geneticsystem.

Table 5

Frequency of polymorphic alleles of folate metabolism genes of boys with blood homocysteine levels of over 10 μmol/L during the measurement on 18/12/2015 (n = 35)

Gene, polymorphism	«Neutral» allele		Risk allele, heterozygous mutation form		Risk allele, homozygous mutation form	
	Abs. number	%	Abs. number	%	Abs. number	%
MTR:A2756G	24	68.6	10	28.6	1	2.8
MTHFR:A1298C	17	48.6	13	37.1	5	14.3
MTHFR:C677T	17	48.6	13	37.1	5	14.3
MTRR:A66G	7	20.0	13	37.1	15	42.9

Table 6

Frequency of polymorphic alleles of folate metabolism genes of girls with blood homocysteine levels of over 10 μmol/L during the measurement on 02/04/2015 (n = 25)

Gene, polymorphism	«Neutral» allele		Risk allele, heterozygous mutation form		Risk allele, homozygous mutation form	
	Abs. number	%	Abs. number	%	Abs. number	%
MTR:A2756G	18	72.0	6	24.0	1	4.0
MTHFR:A1298C	16	64.0	5	20.0	4	16.0
MTHFR:C677T	11	44.0	10	40.0	4	16.0
MTRR:A66G	4	16.0	14	56.0	7	28.0

2. Monitoring blood homocysteine levels in children and adults living under conditions of exposure to wood burning products containing radioactive elements is the key component of the programme for the prevention of cancer and cardiovascular diseases.

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ГИПЕРГОМОЦИСТЕИНЕМИЯ У ДЕТЕЙ И ЛЕСНЫЕ ПОЖАРЫ В ЧЕРНОБЫЛЬСКОЙ ЗОНЕ ОТЧУЖДЕНИЯ

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Цель исследования. *Определить роль генотипов фолатного цикла в возникновении гипергомоцистеинемии у детей после лесного пожара в Чернобыльской зоне отчуждения (ЧЗО).*
Методы: *иммунохимический, ПЦР в режиме Real-time, математико-статистический.*
Результаты. *Обследование 84 детей подросткового возраста из Полесского района Киевской области позволило выявить у них изменения метаболических процессов в виде повышенной продукции гомоцистеина в связи с лесным пожаром в ЧЗО, который регистрировался в течение 26-29 апреля 2015 года. Проведенный сравнительный анализ удельного веса генотипов фолатного цикла в подгруппах детей с гипергомоцистеинемией до и после лесного пожара показал непричастность к данному явлению эндогенного фактора в виде генетического аппарата, контролирующего синтез ферментов фолатного цикла.*
Выводы*. Пожары леса и торфяников в ЧЗО являются одной из основных причин повышения уровня гомоцистеина в крови детей из прилежащих районов вне зависимости от состояния генетической системы фолатного цикла. Контроль за уровнем гомоцистеина в крови детей и взрослых, проживающих в условиях воздействия продуктов горения древесины, содержащей радиоактивные элементы, является основным компонентом программы профилактики онкологических и сердечно-сосудистых заболеваний.*

Ключевые слова: фолатный цикл, гомоцистеин, подростки, лесные пожары, Чернобыльская зона отчуждения.

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Table 7

Frequency of polymorphic alleles of folate metabolism genes of girls with blood homocysteine levels of over 10 μmol/L during the measurement on 18/12/2015 (n = 32)

Gene, polymorphism	«Neutral» allele		Risk allele, heterozygous mutation form		Risk allele, homozygous mutation form	
	Abs. number	%	Abs. number	%	Abs. number	%
MTR:A2756G	23	71.9	8	25.0	1	3.1
MTHFR:A1298C	24	75.0	6	18.7	2	6.3
MTHFR:C677T	14	43.8	12	37.5	6	18.7
MTRR:A66G	6	18.7	16	50.0	10	31.3

Table 8

Proportions of the MTHFR:677 CT/MTHFR:1298 AC heterozygous associationin groups of children with hyperhomocysteinemia reported on 02/04/2015 and 18/12/2015

Date of examination of children	Proportions of a MTHFR:677 CT/ MTHFR:1298 AC heterozygous association					
	Total group		Boys		Girls	
	Abs.	%	Abs.	%	Abs.	%
02/04/2015	9	18.8	5	21.7	4	16.0
18/12/2015	8	11.9	5	14.3	3	9.4

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