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Genetic Polymorphism Of Some Cytokines In Newborn Infants With Hypoxic-Ischemic Encephalopathy

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ABSTRACT

Background. It is now known that cytokine production directly depends on the genes encoding their activity. The study of the relationship between the polymorphism of these genes and cytokine production will make it possible to predict the risk of pathology development, the nature and severity of its course. The aim of the study was to investigate the polymorphism of cytokine genes IL-1β; IL-10, TNFα in preterm infants with hypoxic-ischemic encephalopathy.

Materials and Methods. During the research a total of 112 premature infants with hypoxic-ischemic encephalopathy and 95 healthy preterm infants were examined. Genotyping of polymorphic regions of immune response genes was carried out by polymerase chain reaction (PCR) with allele-specific primers (NPF Litech, Moscow) and electrophoretic detection of reaction products in agarose gel. Clinical, neurosonographic, and statistical studies were performed.

Results. The distribution of allele and genotype frequencies of cytokine polymorphisms has been established, indicating that the -308(G/A) TNF polymorphism as well as the IL-1-511T/C TT polymorphism contribute to the predisposition to hypoxic-ischemic encephalopathy (HIE) development in preterm infants.

Conclusions: The findings may be one of the prognostic factors for the development of hypoxic-ischemic encephalopathy in premature infants.

Keywords: newborns, cerebral ischemia, cytokines, gene polymorphism.

INTRODUCTION

Hypoxic-ischemic encephalopathy (HIE) is a set of neurological symptoms developing in newborns as a result of intrauterine hypoxia. To date, many pathogenetic mechanisms of HIE have been studied, which eventually lead to edema and death of brain neurons [1,2,3]. The problem of ischemic brain damage in the fetus and neonate is highly relevant in neonatology, as it entails high morbidity and mortality [4,5,6]. The study of pro- and anti-inflammatory cytokines plays a significant role. A number of studies have shown that a significant increase in some interleukins (ILs), such as IL-1β IL-4, IL-6, IL-8, TNF-α, is directly related to the severity of ischemic brain damage [7,8]. Increased levels of IL-1β in children with cerebral ischemia play a very significant role in neurodamage [8,9]. TNF-α is known to be a key proinflammatory cytokine [8]. Elevated levels of TNF-α in microglia and high levels in cerebrospinal fluid correlate with periventricular leukomalacia and posthemorrhagic ventriculomegaly, and lead to neurological deficits [10].

The generation of cytokines is now recognized to be directly dependent on the genes encoding their activity. The association between these genes' polymorphisms and cytokine production will be studied in order to predict the probability of disease development, as well as the nature and severity of its course [11].

Interleukin genes are highly polymorphic, and the number of polymorphic sites in one gene sometimes reaches several dozens [11,12]. Currently available data suggest a close association of gene polymorphisms with a variety of pathologies (including CNS lesions) and can be used as markers in the diagnosis of various diseases [13,15]. The study of the cytokine system in preterm infants with cerebral ischemia revealed some regularities in the distribution of allelic variants of TNF-α - T-1031C, C-857T, G-308A, G-238A, and IL-1β - C-511T, C-3954T [16,17].

A definite association between TNF-α-1031T/C, IL-1β- 511C/T, and IL-10-1082 G/A polymorphisms and the development of periventricular leukomalacia has also been detected [14]. In a study in a Chinese population, patients with the genetic polymorphism of IL-1β- 1470 G/C, 511 T/C, 31 C/T were more prone to the occurrence of inflammation [15,18]. The gene encoding IL-10 is located on chromosome 1 (1q31-1q32). A number of polymorphisms of the 5-promoter region of the IL-10 gene have been characterized. They include several point mutations (G-1082A, C-819T, C-592A), the realization of which occurs through mRNA transcription amplification [15,16,17].

Thus, the issue of studying the molecular genetic mechanisms of hypoxic-ischemic encephalopathy in newborn infants is an important and urgent direction that will allow predicting the occurrence of the pathology before its clinical symptoms appear.

The objective of the investigation is to study the polymorphism of cytokine genes IL-1β; IL-10, TNF-α in preterm infants with hypoxic-ischemic encephalopathy.

MATERIALS AND METHODS

During the study, 207 newborn infants were examined. The study group included 112 premature infants with hypoxic-ischemic encephalopathy admitted to the Department of Pathology of Newborn of City Children's Hospital No.5 of Tashkent whereas the control group included 95 full-term infants without perinatal CNS lesions and birth trauma. The patients were selected in accordance with the established criteria: children with chromosomal and genetic diseases were not included in the study group.To establish the diagnosis, a clinical examination of newborn infants was performed with the assessment of neurological status and neurosonography.

Material for DNA extraction was venous blood from the ulnar vein (Beckton-Dickinson vacutainers were used for blood sampling) with anticoagulant/ preservative 15% tricalic EDTA (Ethendianin-

tetraacetic acid). The blood was stored for further processing for up to 24 hours at a temperature not higher than +4°C.

A two-step method of blood cell lysis was used to obtain genomic DNA. Further purification of leukocyte lysates was based on the method of alcohol-salt treatment according to S. Miller et al. (1988) in a modification proposed by the Stanford University laboratory.

Genotyping of polymorphic regions of immune response genes was performed by polymerase chain reaction (PCR) with allele-specific primers (NPF Litech, Moscow) and electrophoretic detection of reaction products in agarose gel. Three SNPs were tested: IL-1β (T-511C); IL-10 (G-1082A), TNF (G-308A), all these SNPs have been previously confirmed and have a minor allele frequency of 1% or more (NCBI dbSNPdatabase, http://www.ncbi.nlm.nih.gov/projects/SNP/index.html).Identification of the amplification products and their distribution with respect to the length marker was performed in ultraviolet light (310 nm) after electrophoresis for 15 minutes at 300 V (in both cases the run was 3-4 cm) and staining with ethidium bromide.

The distribution of genotypes in the polymorphic loci under study was studied using logistic regression analysis and testing for Hardy-Weinberg equilibrium using Fisher's exact test. The correspondence of patients and controls by sex and age was taken into account. Differences at $p<0.05$ were considered statistically significant.

Statistical processing of the obtained data was performed using Microsoft Excel, SISA9.17® and statistical software package SISA, Arlequin 3.5.2. and a number of formulas.

RESULTS AND DISCUSSION

The mean gestational age of the observed preterm infants ranged from 34.3±0.5 to 36.0±0.2 weeks. In the main group of neonates, the highest Apgar scores on the 1st minute of life were in infants with 1st degree GIE and were 6.0±0.2 points, and 7.3±0.2 points at the 5th minute of life. The lowest Apgar scores on the Apgar scale were found in children with HIE of the 3rd degree, 3.7±0.3 at the 1st minute and 5.2±0.3 at the 5th minute.

IL-1 rs16944 genotyping was performed in a group of 112 newborn infants with HIE and a comparative analysis of the results compared with the control group was performed.

Table 1 shows the results of genotype allele distribution. As shown in the table, we found that the T allele was significantly more frequent (1.5-fold) in preterm infants with HIE compared to controls with OR=1.097, 95%CI=1.097 >1.763> 2.832. In its turn, the homozygous TT genotype of IL-1- 511T/C in children with HIE was 2.6 times more frequent than in the control group and showed the highest level of reliability, which, according to OR indices, was registered as a predisposing genotype OR=2.982, 95%CI=1.071 >2.982> 8.303, $\chi2=4.699$.

No true significance was found for the CC and CT genotype in infants with HIE relative to controls, whereas the C allele reached true significance, suggesting that the CC genotype may be reaching true values when the sample is expanded.

Table 1. Distribution of alleles and genotypes of IL-1 -511T/C in newborn infants with hypoxic-ischemic encephalopathy

Genotype	HIE n= 112	HIE, %	Genotype	Control, n=66	Control, %	χ2	OR (95% CI)
C	139	62,05	C	98	74,24	5.545 (p=0.018533)	0.353 >0.567> 0.912

T	85	37,95	T	34	25,76		1.097 >1.763> 2.832
CC	49	43,75	CC	37	56,06	2.52 (p=0.112388)	0.33 >0.61> 1.125
CT	41	36,61	CT	24	36,36	0.001 (p=1)	0.537 >1.011> 1.901
TT	22	19,64	TT	5	7,58	4.699 (p=0.030172)	1.071 >2.982> 8.303

Note:χ2 – Pearson reliability index; OR – relative risk.

Further, when examining the distribution of alleles and genotypes of IL-10G-1082A (rs1800896) in HIE patients and controls, no significant differences were found, and all ORs were close to 1.0 (Table 2).

Table 2. Distribution of alleles and genotypes of the IL-10 gene G-1082A in newborn infants with hypoxic-ischemic encephalopathy

Genotype	HIE, n=112	HIE, %	Genotype	Control, n=66	Control, %	χ2	OR(95% CI)
G	149	66,52	G	88	66,67	0.001 (p=1)	0.63 >0.993> 1.567
A	75	33,48	A	44	33,33		0.638 >1.007> 1.588
GG	39	34,82	GG	23	34,85	0 (p=1) *	0.528 >0.999> 1.891
GA	71	63,39	GA	42	63,64	0.001 (p=1)	0.526 >0.99> 1.862
AA	2	1,79	AA	1	1,52	0.018 (p=1)	0.105 >1.182> 13.29

Note: χ2 – Pearson reliability index; OR – relative risk.

In a comparative study of the allele and genotype frequency distributions of polymorphic markers of the TNF-α -308G/A gene in infants in the observed groups (Table 3) there was a statistically significant increase in the frequency of the A allele in preterm infants with HIE compared with the control group (16.07% and 8.42%, respectively; OR =2.082; 95% CI: 1.116 >2.082>3.886; χ2=5.478 (p=0.019253)). At the same time, the G allele of the studied polymorphism occurred significantly less frequently compared

to controls (83.93% and 91.58%, respectively; OR = 0.48; 95% CI: 0.257>0.48> 0.896; χ2=5.478 (p=0.019253)).

Further, a comparative analysis of TNFα -308G/A genotypes for the GG genotype revealed significant differences between the main and control groups (67.86% and 83.16%, respectively; OR = 0.428; 95% CI: 0.219 >0.428> 0.834; χ2=6.397 (p=0.011429)). Analysis of the heterozygous GA genotype revealed a significantly higher frequency of its occurrence in neonates with HIE than in the control group (32.14% and 16.84%, respectively; OR = 2.339; 95% CI: 1.199 >2.339> 4.561; χ2=6.397 (p=0.011429)).

Table 3. Distribution of allele and genotype frequencies of TNF-α-308G/A gene in newborn infants with hypoxic-ischemic encephalopathy

Genotype	HIE, n= 112	HIE, %	Genotype	Control, n=95	Control, %	χ2	OR (95% CI)
G	188	83,93	G	174	91,58	5.478 (p=0.019253)	0.257 >0.48> 0.896
A	36	16,07	A	16	8,42		1.116 >2.082> 3.886
GG	76	67,86	GG	79	83,16	6.397 (p=0.011429)	0.219 >0.428> 0.834
GA	36	32,14	GA	16	16,84	6.397 (p=0.011429)	1.199 >2.339> 4.561
AA	0	0,00	AA	0	0,00		

Note: χ2 – Pearson reliability index; OR – relative risk.

As described above, there was a significant difference in the frequency of the A allele of the TNF-α-308G/A polymorphism under study, but no homozygous AA genotype was detected in the genotypic analysis.

CONCLUSION

Thus, the data obtained on the distribution of allele and genotype frequencies of cytokine polymorphisms indicate that the -308(G/A) TNF-α polymorphism as well as the IL-1-511T/C TT contribute to the predisposition to the development of HIE in preterm infants and may be one of the prognostic factors of hypoxic-ischemic encephalopathy. The presence of polymorphic genes may have a significant impact on the degree of production of pro-inflammatory and anti-inflammatory cytokines, which may play an important role in neural tissue damage.

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