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ROLE OF TOLL-LIKE RECEPTOR GENETIC VARIABILITY IN NEONATAL HERPESVIRUS INFECTIONS

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Abstract
Toll-like receptors (TLRs) are pattern recognition receptors expressed by various immune cells that play a crucial role in initiating inflammatory processes and shaping the course of infectious diseases. *Objective:* To determine the role of polymorphisms in the TLR2 Arg753Gln (rs5743708) and TLR6 Ser249Pro (rs5743810) genes in newborn children with herpesvirus infections. *Materials and Methods:* A total of 134 newborns were examined. The main study group consisted of 68 neonates born to mothers with herpesvirus infections (HSV, CMV), while the control group included 66 healthy newborns from women with a physiological course of pregnancy and delivery. Clinical, genetic, and statistical methods were utilized for the study. *Results:* In the Uzbek population, newborn children with herpesvirus infections were found to possess several predisposing genetic markers, specifically the allelic variants -753Gln TLR2 Arg753Gln (rs5743708) and -249Ser TLR6 Ser249Pro (rs5743810). Predisposing values were also observed for the genotypes -753Arg/Arg TLR2 Arg753Gln (rs5743708) and -249Ser/Ser TLR6 Ser249Pro (rs5743810).

Keywords: Newborns, herpesvirus infections, sepsis, Toll-like receptors, gene polymorphism.

1. INTRODUCTION

Infection is a primary factor influencing changes in TLR expression during both the intrauterine and postnatal periods of development. Herpesviruses, in particular, involve the penetration of microorganisms into fetal tissues, leading to infection. The level of TLR expression directly correlates with the severity of the disease process, allowing them to be considered early markers of infection. Depending on the nature of the pathogen, an enhanced expression of specific TLRs may be observed. The highest risk of developing intrauterine infection is associated with primary viral

infections that are accompanied by significant changes in the immune status, such as a deficiency in subpopulations of mature lymphocytes, T-helper cells, and natural killer cells. Perinatal mortality from sepsis may also be linked to primary viral infection.

In cases of continuously persistent viral infections, the risk of fetal damage is tenfold lower than during primary infections. However, a persistent viral infection can contribute to the onset of nonspecific intrauterine infection by bacterial flora. Primary infection of the mother-to-be, for instance, with herpes simplex virus type 2 or CMV, or the reactivation of a latent virus during pregnancy, can result in intrauterine infection of the fetus, miscarriage, the development of a generalized infection in the newborn, or fetal death [2, 10].

Pro-inflammatory reactions induced by TLRs are regarded as the first line of defense, not only eliminating the threat of infection but also accelerating the process of restoring immune homeostasis. However, a disruption in the regulation of TLR activity often leads to the development of immunopathologies, including those associated with herpesvirus infections. The hyperactivation or inactivation of TLRs is primarily caused by mutations that affect the function of these receptors [11, 12].

According to research, dysfunction of innate immunity in women with herpesvirus infections during pregnancy—due in part to impaired immunological reactivity and defects in the TLR system—can contribute to fetal infection and the formation of congenital infection in newborns [3, 4]. Syncytiotrophoblast plays a significant role in these processes, as it expresses various types of TLRs throughout pregnancy and can participate in both virus recognition and its vertical transmission [5]. Among the known TLRs, TLR2 and TLR4 are of the greatest importance in this context [6], as they facilitate the formation of an antiviral immune response at the maternal-fetal interface.

It has been noted that in the first trimester of pregnancy, high expression of TLR2 and TLR4 is predominantly observed on the surface of cytotrophoblasts. In the third trimester, however, TLR2 is mainly localized on the endothelial cells of placental blood vessels and macrophages, while TLR4 is found on syncytiotrophoblasts and fibroblasts [7]. It is hypothesized that the absence of TLR expression on the outer layer of the trophoblast in the early stages of pregnancy serves a protective function, limiting the placental immune response until the barrier is compromised. Conversely, heightened TLR expression in later stages may have adverse consequences, activating inflammatory processes and increasing the risk of intrauterine virus transmission to the fetus.

Furthermore, an unfavorable pregnancy outcome and the likelihood of developing an intrauterine infection may be linked to genetic variations of TLRs that determine the functional activity of the receptors and the specifics of their signaling. For example, a study by Marco Antônio et al. [7] identified an association between the CC genotype of the rs3804100 polymorphism in the TLR2 gene and the risk of intrauterine CMV infection during pregnancy, and the AG genotype of the rs1898830 polymorphism with congenital herpesvirus infection in newborns, which points to the possibility of vertical virus transmission [9]. In a study by Marco Antônio and colleagues [7], a link was shown between the rs4696480, rs3804100, and rs1898830 genotypes of the TLR2 gene, rs3775291 of the TLR3 gene, and rs179008 of the TLR7 gene with the development of intrauterine CMV infection in women infected in the second trimester. The rs1898830 TLR2 genotype was associated with the risk of intrauterine virus transmission, while the rs3804100 and rs1898830 genotypes were associated with congenital herpesvirus infection in newborns. The TLR3 and TLR7 variants, however, showed no significant associations.

Studies by Teräsjärvi J. et al. [11] confirmed that the heterozygous rs187084 genotype of the TLR9 gene correlates with a high level of viremia. In turn, Wang K and colleagues [12] showed that the presence of heterozygous rs3775291 genotypes of the TLR3 gene and rs5741880 of the TLR7 gene increases the risk of developing congenital herpesvirus infection.

Thus, further investigation into the characteristics of TLR expression and their genetic variations will provide a deeper understanding of the mechanisms behind the formation of congenital herpesvirus infection and help to identify key targets for predicting and preventing this pathology. This can also lead to the development of reliable prognostic criteria for disease severity and outcomes, which is of practical importance for timely diagnosis, selecting optimal therapy, and reducing adverse consequences [14].

2. MATERIALS AND METHODS

Our research was conducted at the Republican Perinatal Center and in the neonatology departments of the 5th City Children's Hospital. A total of 134 newborns were examined. The main group consisted of 68 newborns born to mothers with herpesvirus infections (HSV, CMV), of whom 32 children had monoinfections and 36 had a combined CMV+HSV infection. The control group comprised 66 healthy newborns born to women with a physiological course of pregnancy and delivery.

The inclusion criteria for the study were the presence of one or more clinical signs of intrauterine infection (IUI) in the newborn, such as intrauterine growth restriction,

pneumonia, hepatitis, meningoencephalitis, micro- or hydrocephaly, cerebral calcifications, early or prolonged jaundice, fever, severe neurological and respiratory distress, seizures, or gastrointestinal tract disorders.

The diagnosis of IUI was established according to ICD-10, block P35-P39, which covers infectious diseases specific to the perinatal period. The diagnosis was confirmed by a combination of clinical and laboratory data, along with the results of specific tests (PCR, ELISA).

The exclusion criteria were genetic pathologies, metabolic disorders, and the presence of isolated or multiple congenital malformations.

We conducted a clinical examination of the newborns. All observed newborns were in serious condition. To assess the state of the newborns at birth, their Apgar scores were analyzed. Among children born with a risk of CMV, the Apgar score was 4.4±0.1 points at 1 minute and 6.7±0.3 points at 5 minutes. For children with a risk of HSV, the Apgar score was 3.8±0.1 points at 1 minute and 5.7±0.3 points at 5 minutes. The lowest Apgar scores were noted in children with a risk of combined CMV+HSV infections, with scores of 2.7±0.1 points at 1 minute and 5.2±0.3 points at 5 minutes. This suggests that newborns with combined intrauterine infections are born in a more severe condition than those with monoinfection.

Genetic studies were performed at the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan. Venous blood from the cubital vein was used for DNA extraction. Genotyping of polymorphic regions of the immune response genes was performed using polymerase chain reaction (PCR) and electrophoretic detection of the reaction products in an agarose gel. The distribution of allelic variants and genotypes for TLR2 Arg753Gln (rs5743708) and TLR6 Ser249Pro (rs5743810) was studied in newborns with herpesvirus infections. The distribution of genotypes in the studied polymorphic loci was analyzed using logistic regression and was checked for compliance with the Hardy-Weinberg equilibrium using the Fisher's exact test. Statistical significance was considered at $p<0.05$.

3. RESULTS AND DISCUSSION

During our research, we performed genotyping of TLR2 (Arg753Gln) in the main group of sick newborns with herpesvirus infections (CMV, HSV, CMV+HSV) and conducted a comparative analysis with a group of healthy children.

We studied the distribution of allelic variants and genotypes for TLR2 Arg753Gln (rs5743708) in newborns with herpesvirus infections (Table 1). As the data shows, we found that the Gln allele occurred significantly more frequently (13.97%) in the study

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group compared to the control group (3.03%), with an OR of 5.197 (95%CI:1.718–15.721), indicating a predisposing value.

A high level of statistical significance in patients with IUI was also noted for the heterozygous -753Arg/Gln genotype (27.9%), which was identified as a predisposing genotype (OR = 6.01, 95%CI:1.919–18.82, χ2=11.278, p=0.000784). In the control group, this genotype was present with a frequency of 6.06%.

For the -753Arg/Arg genotype, a significant decrease in frequency was observed in the patient group, although true significance was not found (OR = 0.166, 95%CI:0.053–0.521, χ2=11.278, p=0.000784). Similarly, the -753Arg allele also achieved a true significance of χ2=10.219 (p=0.00139).

Table 1. Distribution of allelic variants and genotypes of TLR2 Arg753Gln (rs5743708) in newborn children with herpesvirus infections.

SNP	Group	Allele	Allele frequency,%	χ2 (p)	OR (95% CI)	Genotype	Genotype frequency,%	χ2 (p)	OR (95% CI)
TLR2 (Arg753 Gln)	Study group	-753 Arg	86,03	10.219 (0.00139)	0.192 (0.064 -0.582)	-753 Arg/Arg	72,06	11.278 (0.000784)	0.166 (0.053 - 0.521)
		-753 Gln	13,97		5.197 (1.718 -15.721)	-753 Arg/Gln	27,94		6.01 (1.919 - 18.82)
						-753 Gln/Gln	0	0	
	Control group	-753 Arg	96,97			-753 Arg/Arg	93,94		
		-753 Gln	3,03			-753 Arg/Gln	6,06		
						-753 Gln/Gln	0	0	

For the -753Arg/Arg genotype, a significant decrease in frequency was observed in the patient group, though true significance was not detected, with an OR of 0.166 (95%CI:0.053–0.521), χ2=11.278 (p=0.000784). Similarly, the -753Arg allele reached true significance with χ2=10.219 (p=0.00139).

TLR6 Ser249Pro (rs5743810). Next, a comparative study was conducted on the distribution of allelic and genotypic frequencies for TLR6 Ser249Pro in the group of sick newborns with herpesvirus infections and in the control group (Table 2). We found a statistically significant increase in the frequency of the -249 Ser allele in newborns with intrauterine infections compared to the control group (69.12% and 48.48%, respectively; OR = 2.378; 95%CI:1.718–15.721; χ2=11.785 (p=0.000597).

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Table 2. Distribution of allelic and genotypic frequencies of TLR6 Ser249Pro (rs5743810) in newborn children with herpesvirus infections.

SNP	Group	Allele	Allele frequency ,%	χ2 (p)	OR (95% CI)	Genotype	Genotype frequency, %	χ2 (p)	OR (95% CI)
TLR6 (Ser249Pro)	Study group, n-68	-249Ser	69,12	11.785 (0.000597)	2.378 (1.444 - 3.916)	-249 Ser/Ser	51,47	13.22 (0.000277)	3.939 (1.846 - 8.406)
		-249Pro	30,88		0.421 (0.255 - 0.693)	-249 Ser/Pro	35,29	5.02 (0.025057)	0.455 (0.227 - 0.91)
						-249 Pro /Pro	13,24	2.674 (0.102008)	0.477 (0.194 - 1.172)
	Control group, n-66	-249Ser	48,48			-249 Ser/Ser	21,21		
		-249Pro	51,52			-249 Ser/Pro	54,55		
						-249 Pro/Pro	24,24		

At the same time, the -249 Pro allele of the studied polymorphism was found to be significantly less frequent in newborns with intrauterine infections compared to the control group (30.88% and 51.52%, respectively; OR = 0.421; 95%CI:0.255–0.693; χ2=11.785 (p=0.000597)).

Upon comparative analysis of TLR6 Ser249Pro genotypes, significant differences were identified between sick and control newborns for the -249 Ser/Ser genotype. This homozygous genotype was found to be 2.4 times more frequent in children of the main group (51.4% vs 21.21% in the control group; OR = 3.939; 95%CI:1.846–8.406; χ2=13.22 (p=0.000277)).

Analysis of the heterozygous -249 Ser/Pro genotype revealed differences in frequency between the patient and control groups (35.29% vs 54.55%, respectively; OR = 0.455; 95%CI:0.227–0.91; χ2=5.02 (p=0.025057)). As mentioned earlier, a significant difference was found in the frequency of the -249 Pro allele for the TLR6 Ser249Pro polymorphism. In the genotypic analysis, the homozygous -249 Pro/Pro genotype was less frequent in sick children (13.24%) than in the control group (24.24%).

4. CONCLUSIONS

Thus, it was established that in the Uzbek population, the -753Arg TLR2 Arg753Gln (rs5743708) allele and the -249Pro TLR6 Ser249Pro (rs5743810) allele had a significant protective effect against herpesvirus infections in newborns. Protective values were also found for the -753Arg/Gln TLR2 Arg73Gln (rs5743708) and -249Ser/Pro TLR6 Ser249Pro (rs5743810) genotypes.

In newborns of the Uzbek population with herpesvirus infections, a number of predisposing markers were identified, such as the allelic variants -753Gln TLR2 Arg753Gln (rs5743708) and -249Ser TLR6 Ser249Pro (rs5743810). Predisposing values were also found for the genotypes -753Arg/Arg TLR2 Arg753Gln (rs5743708) and -249Ser/Ser TLR6 Ser249Pro (rs5743810).

The established genetic markers of predisposition and resistance of TLR2 and TLR6 to the development of mono- and co-infected herpesvirus infections in newborns of the Uzbek population can be used as prognostic criteria for the development of this disease.

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