Original Article

The Role Of Genetic Markers In Predicting Preterm Birth

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Abstract

Annotation

The problem of preterm birth (PB) is relevant in obstetrics and perinatology, since prematurity causes a significant proportion of perinatal morbidity and mortality. Great hopes are placed on modern immunological and, to a greater extent, genetic methods, which will allow us to assess the risk of preterm birth. We have studied the course of pregnancy and childbirth in 223 women in the gestation period of 22-36 weeks, of which 65 were women with threatened miscarriage, 52 with premature birth, 106 women were included in the control group. In all women of the observed main group, a genetic study of the polymorphism of the genes of interleukins IL-1b (T31C) and IL10 (G1082A) was carried out.

Our scientific studies have shown that the polymorphism of the IL-1β, IL-10 genes is a prognostic marker of the threat of miscarriage and premature birth for women in the Uzbek population. IL-10 (rs1800896) and IL-1b (rs1143627) gene polymorphisms are predictors of threatened miscarriage and preterm birth

The problem of preterm birth (PB) is relevant in obstetrics and perinatology, since prematurity causes a significant proportion of perinatal morbidity and mortality (3).

The main principle of organizing medical care for this pathology is timely prediction and early subclinical diagnosis of PB, determination of pregnancy management tactics, choice of drug therapy in order to prolong pregnancy and achieve intrauterine fetus maturity (1.5).

Despite the large number of known methods of prediction and biomarkers, more than half of the cases of PB remain unpredictable.

Great hopes are placed on modern immunological and, to a greater extent, genetic methods, which will allow us to assess the risk of developing preterm birth. (ten)

In recent years, the role of genetic factors in the formation of the mechanisms of the innate immune response has been actively studied. Of fundamental importance in the genesis of preterm birth is given to intracellular changes, disruption of processes that provide the functions of individual myocytes, for them the characteristic changes are a decrease in the volume density of myocytes, their degeneration, and disturbances in the organization in the stroma (13).

Many researchers note the presence of a hereditary predisposition, and recently there have been works indicating an increase in PB in the presence of undifferentiated connective tissue dysplasia in a pregnant woman, which leads to the study of gene polymorphisms associated with a violation of its development (2,4).

The study of gene polymorphism allows the use of genetic markers as non-invasive predictors of risk group formation and possible prediction of preterm birth.

Dysfunctions of maternal immune adaptations are associated with a number of pregnancy pathologies, including preterm birth (PB). Much research evidence is now available to support that PB is the result of impaired fetal and maternal tolerance and excessive premature inflammation (8,9). In this case, the balance between pro-inflammatory and anti-inflammatory cytokines is critical for fetal implantation, placental preparation, and pregnancy outcome (12,15,20). An imbalance between pro-inflammatory and anti-inflammatory cytokines, such as overexpression of pro-inflammatory cytokines and underexpression of anti-inflammatory cytokines, or vice versa, can initiate immune dysfunction. Both conditions can contribute to preterm labor.

Cytokines are major immune regulators during pregnancy, mediating gametogenesis, uterine receptivity, implantation responses, embryogenesis and fetal development and onset of labor, inducing inflammation or inducing immune tolerance. Aberrant expression of proinflammatory cytokines during pregnancy contributes to preterm birth, preeclampsia, and gestational diabetes mellitus (14.17). Based on the foregoing, it is more promising to determine the polymorphisms of their genes, when studying the immunogenetic pathogenesis of preterm birth, genetic polymorphisms IL-1b (T31C) and IL10 (G1082A) are of particular interest, which determine the ratio of the activities of the T-cytotoxic and B-cell immunity systems .

The study of the genetic predisposition to miscarriage is of great importance due to the potential possibility of determining the risk from early gestation and even before conception, which makes it possible to determine the risk group and predict pregnancy termination. Numerous scientific studies have identified more than 80 potential candidate genes for preterm birth and established gene polymorphisms associated with a high risk of preterm birth. According to Suprun S. V. et al. 2017. From their results, in pregnant women with PROM, mutations C592A were 1.5 times more common, and mutations C819T were 3.3 times more common, which largely determined the ratio of the activities of the T-cytotoxic and B-cell immunity systems.

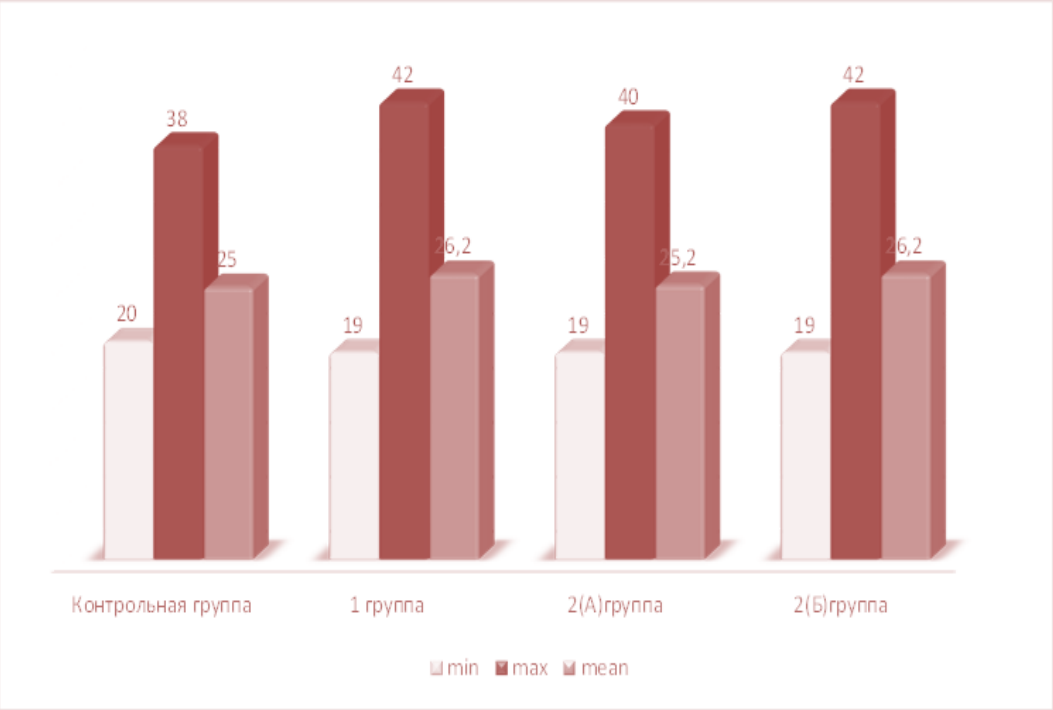
Based on the data obtained, many authors believe that IL-10 gene polymorphisms can be considered a predictor marker of PDRPO and a risk factor predicting the development of preterm birth (18,19).

In this connection, the scientific search for new markers of immunogenetic disorders remains an urgent promising program of modern obstetrics in order to determine the risk group, predict miscarriage and reduce perinatal losses. (6.11). In connection with the foregoing, the aim of this work was to study the polymorphism of the genes of interleukins IL-1b (T31C) and IL10 (G1082A) in the blood serum of women with threatened miscarriage and preterm birth.

Materials and methods of examination

In accordance with the purpose and objectives, the research work was carried out in the city maternity complex Mokhi-Khosa and the regional perinatal center of the city of Bukhara. We studied the course of pregnancy and childbirth in 223 women at 22-36 weeks' gestation, 65 of them were women with threatened miscarriage, 52 with premature birth, 106 women were included in the control group. Genetic studies were carried out at the Republican Scientific Research Medical Center of Hematology in the Department of Molecular Cellular Medical Technology, headed by Doctor of Medical Sciences, Professor Boboev K.T. PCR method (real time)

The studies were carried out on the device "Rotor-Gene Q". The material was DNA isolated from the peripheral venous blood of pregnant women. In all women of the observed main group, a genetic study of the polymorphism of the genes of interleukins IL-1b (T31C) and IL10 (G1082A) was carried out. When women were included in the sample, they did not have a decisive role: professional, social and psychological status. The selected clinical groups are comparable in number. It should be noted that the age of women in the main group ranged from 19 to 42 years, in group 1 26.2 ± 0.8 (19-42) years, in group 2a 25.2 ± 0.8 (19-40), in group 2 group 26.2±0.8(19-42), the control group was 25.7±0.7(20-38) years. (Fig. 1)



Rice. 1. Distribution of patients by age.

To conduct a clinical and statistical analysis of women in the main group, anamnestic data, somatic and gynecological diseases, the menstrual cycle, and the onset of sexual activity were taken into account. And also the menstrual cycle of women of the main group, its beginning, nature and duration were evaluated.

We have studied the frequency distribution of alleles and genotypes of the IL-1b (T31C) gene polymorphism in the group of pregnant women with threatened preterm labor, preterm labor and normal pregnancy included in our study. The frequency distribution of alleles and genotypes has been studied. Several studies have correlated an increase in pro-inflammatory cytokines, including IL1b, with the risk of PVR [7; 102-107-st.]. Because IL1b expression was found to be increased in preterm birth.

Regarding the study of T31C polymorphism in the genesis of IL1b and the expression of the cytokine IL1b, there are many studies that correlate with a non-wild-type allele and a high concentration of IL1b. Therefore, women carrying this allele may be at risk of overexpressing IL1b, leading to endothelial dysfunction and even preterm birth. In our study, we found that in patients of the main group, homozygous TT or wild-type genotype of the IL-1b (T31C) gene was in 31.6%, heterozygous TC genotype in 43.6%, CC homozygous CC or non-wild-type genotype was detected in 24 .8% of patients (Figure 2).

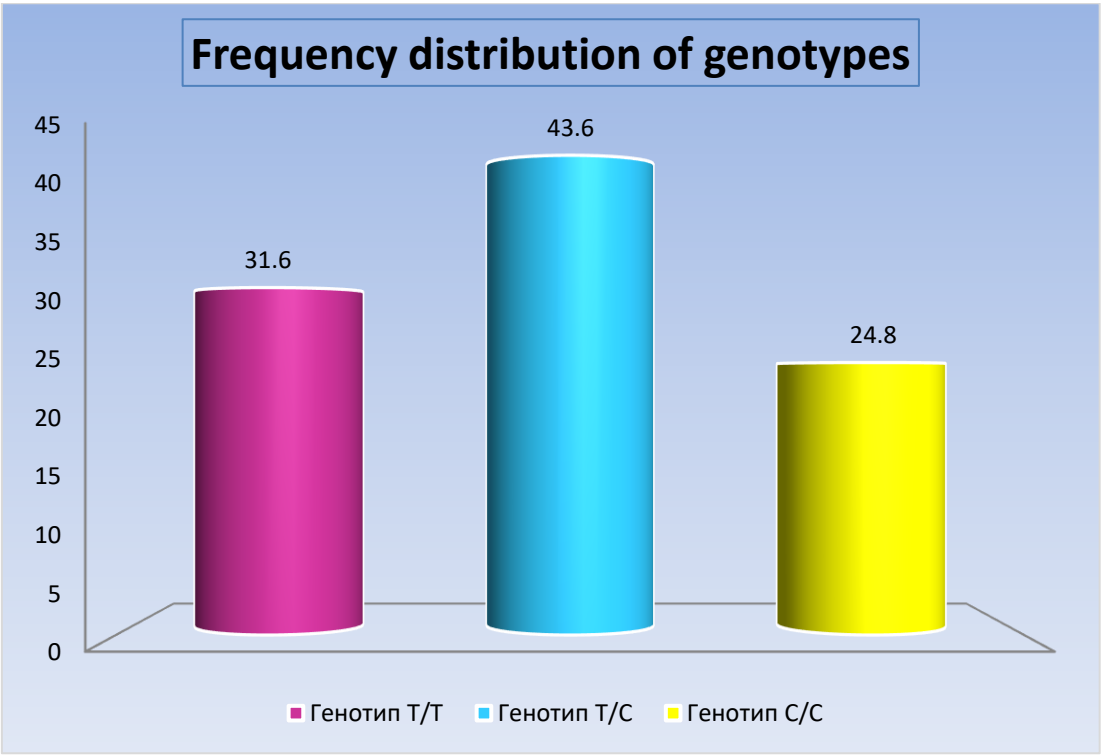


Fig. 2. Distribution of genotypes of T31C polymorphism in the IL-1b gene in pregnant women of the main group.

On the other hand, in the control group, a marked difference was found in the percentage of genotype differences. In particular, the proportion of the homozygous TT genotype was 35.8%, and in the control group, the heterozygous TC genotype and the homozygous CC genotype showed the result of 44.3% and 19.8%, respectively. Thus, it can be concluded that the mutant homozygous genotype is more common in the main group, while in the normal group the control group dominated by definition of the normal homozygous genotype. With regard to the heterozygous genotype, although in both groups the percentage of the heterozygous genotype is higher compared to other forms of the genotype, the difference in proportions between the two groups was not significant.

When we divided patients at risk for preterm birth into two subgroups, the first subgroup had a T allele prevalence of 46.9% (while this result was 58% in the control group, as mentioned earlier). It was found that the minor allele - C is significantly higher, 53.1% in the first group, compared with the control group, 42%.

Interestingly, when statistically processing the results of the second subgroup, we found that the proportion of the normal wild type allele (T allele) was even greater in patients of this group compared to the control group (61.5% and 58%, respectively), and the minor allele was C was 38.5%. (Figure 3)

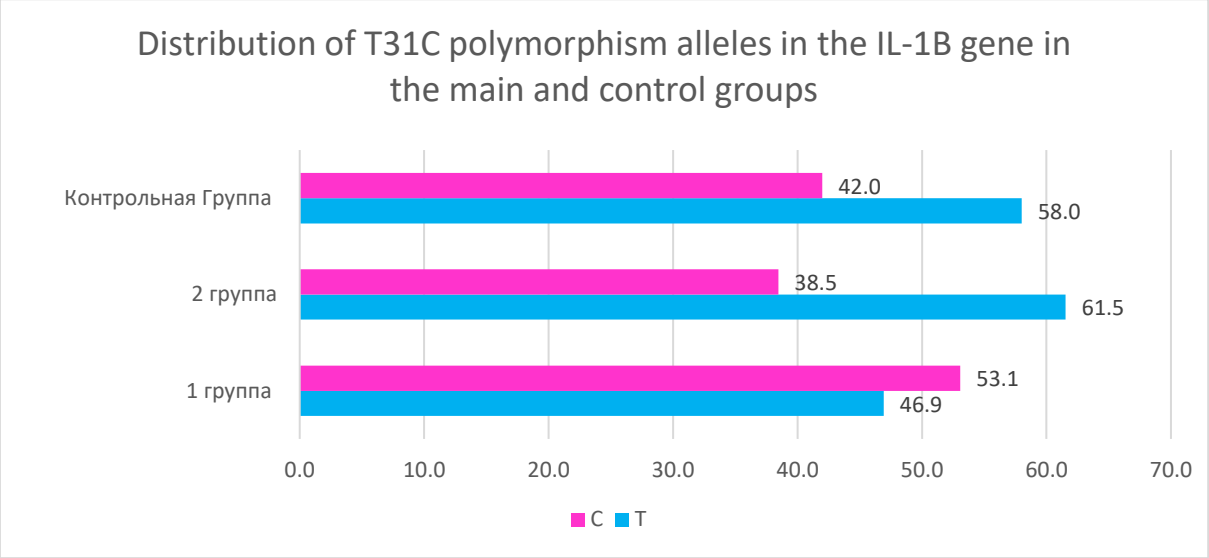


Fig. 3. The frequency of distribution of alleles of the genotypes of the IL1b (T31C) gene polymorphism in the main and control groups

As for the proportion of genotypes, in the first subgroup, the homozygous non-wild-type variant (CC genotype) and the heterozygous genotype were statistically significantly higher than in the control group (27.7% and 50.8% in the first subgroup; 19.8% and 44.3 % in the control group, respectively), while the percentage of the homozygous normal variant (TT genotype) prevailed in the control group, 35.8% (while in the first subgroup this figure was 21.5%). On the other hand, the distribution of genotypes in the second subgroup was 44.2%, 34.6%, and 21.2% according to T/T, T/S, and S/S, respectively (Fig. 4).

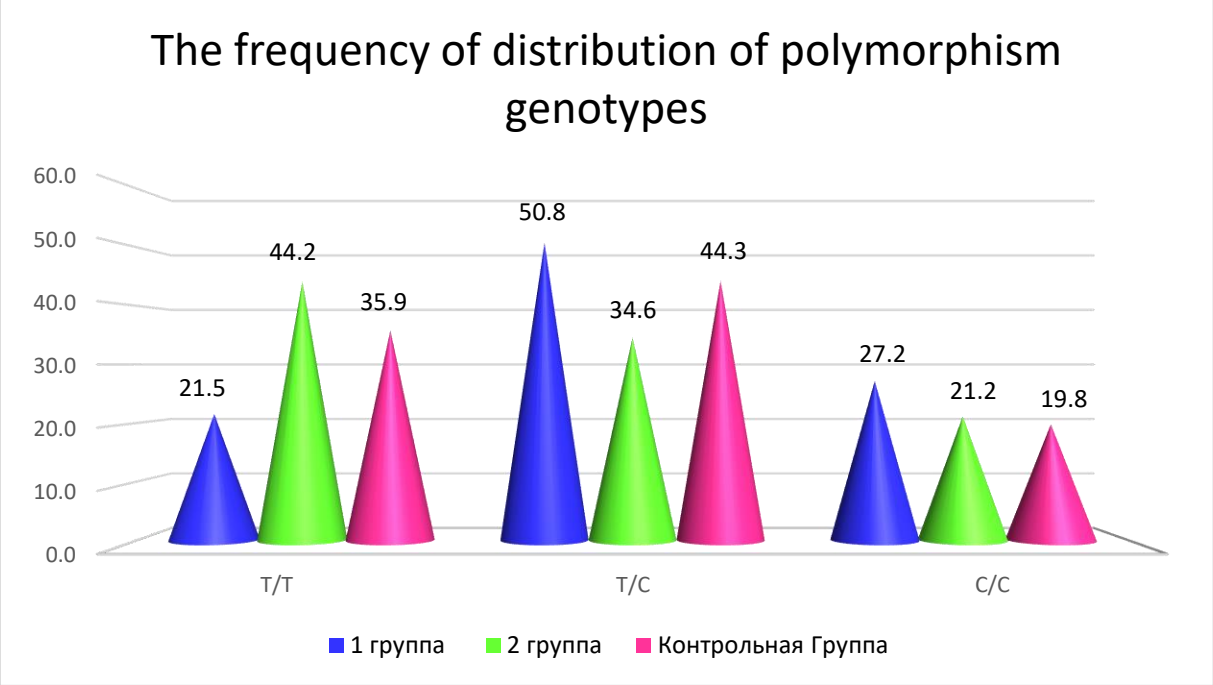


Fig-4 Distribution of genotypes of poymorphism

The distribution of genotypes in the studied polymorphisms of the IL1b (T31C) gene was checked for compliance with the Hardy-Weinberg equation. In the main group, the index of homozygous genotypes - T / T and C / C according to empirically observed and theoretically expected levels was 0.32 / 29; 0.25/0.22, respectively (Table 1). In the control group, the T frequency of these genotypes was 0.36/0.34 and 0.2/0.18.

It follows that although a comparative difference between the observed and expected prevalence of genotypes in both

groups was found, they were not statistically significant ($\chi2<3.85$; $P>0.05$) (Table 1).

Table 1 Correspondence of the genoptype of the IL1b (T31C) gene polymorphism in patients of the main and control groups.

Main groups					
alleles	Rate of alleles				
T	0,53				
C	0,47				
Genotypes	Rate of genotypes		$\chi2$	p	Df
	observable	expected			
T /T	0,32	0,29	0,39		
T /C	0,44	0,5	0,9		
C /C	0,25	0,22	0,51		
Total	1	1	1,8	0,176	1

Controlled group					
alleles	Rate of alleles				
T	0,58				
C	0,42				
genotypes	Rate of genotypes		$\chi2$	p	Df
	observable	expected			
T /T	0,36	0,34	0,15		
T /C	0,44	0,49	0,42		
C /C	0,2	0,18	0,29		
Total	1	1	0,85	0,342	1

As for the heterozygous genotype, in the main group of patients for T31C polymorphism in the IL1b gene, in the main group the expected results were higher than the observed ones (0.44/0.50; D=-0.12), in the main group the expected results were higher than the observed ones (0.44/0.49; D=-0.09), translation results means that in our study, people who had heterozygous results had a minor deficiency compared to the population average. (tab1 and 2).

Tab 2

Groups	Ho	He	D*
Main group	0,44	0,5	-0,12
Controlled group	0,44	0,49	-0,09

Note: D = (Ho - He)/He

The chance of developing PVR was higher in carriers of the homozygous mutant genotype (OR=1.2; 95% CI: 0.83-1.75), while the wild-type allele reduced it, playing a protective role (OR=0.8; 95%CI: 0.57-1.21). However, the statistical results did not show a significant relationship between the non-wild-type allele and the inducible role, as well as the wild-type allele and the protective role ($\chi2<3.84$; $p>0.05$) (Table 3)

To elucidate the pathogenetic role of different genotypes in the development of preterm birth, we calculated the odds ratio that the T/T odds ratio is 0.9, which means that it plays a protective role in the pathogenesis of preterm birth. On the other hand, the odds ratio T/C was 1.0, and the homozygous mutant C/C genotype was 1.3, which means that the latter increases the risk of preterm birth by 30%. However, the chi-square score showed results below 3.84 ($p>0.05$)

In our study, the distribution of genotype frequencies in the IL1b (T31C) gene in the main and control groups obeyed the Hardy–Weinberg law. However, we were able to find a statistically significant association between polymorphism and an increased risk of preterm birth, for the wild-type and wild-type alleles, as well as for other genotypes. Except for the second group, when the wild-type (T) allele and the non-wild-type (C) allele showed a significant and significant result as a proactive and proactive role.

In our study, the distribution of G and A polymorphism alleles located in the 1082G>A promoter region (rs1800896) of the IL10 gene showed that the proportion of the G allele was significantly higher in the main group compared to the control group (37.6% and 19.3%, respectively).), while allele A of the IL-10 gene was more common in the control group (80.7% and 62.4%, respectively). When we examined the distribution of the G1082A polymorphism allele in the IL10 gene, we found that in the first group, the A allele is 63.1% and the G allele is 36.9%, while in the second group, the distribution of the A allele is 61.5% and the Gallele 38.5%

The following results on the distribution of genotypes in the main and control groups were obtained: homozygous genotype G/G 17.9% and 3.8%; heterozygous genotype G/A 39.3% and homozygous genotype A/A 42.7% and 65.1%, respectively. This means that the control group in terms of the homozygous A/A result significantly dominated the main group, while the homozygous G/G genotype was common in the main group. Interestingly, when we divided the patients into two subgroups, we found that in the first subgroup the frequency of occurrence of the homozygous form of the genotype (G / G - 20.0% in the first subgroup and 3.8% in the control group) was significant, the heterozygous variant was noticeable (GA - 33.8% in the first subgroup and 31.1% in the control group) prevailed in comparison with the control group. However, in the control group, the homozygous A/A genotype prevailed with 65.1%, while in the first subgroup this figure was 46.2%.

However, in the second subgroup, the frequency of the G allele was more common in the main group - 38.5%, and the A allele - in the control group. When it comes to the distribution of genotypes, the G/G and G/A genotypes significantly predominated in the second subgroup compared to the control group (15.4% and 3.8%; 46.2 and 31.1%, respectively), and the control group prevailed over the second group in homozygous A/A genotype (65.1% and 38.5%). The distribution of genotypes in the studied polymorphic loci was no genotypes from the Hardy-Weinberg equation in the control group were found at all (Table 13 and 14), while in the main group these indicators had a slight difference between observed and expected. More specifically, in relation to homozygous G/G and A/A genotypes, the observed results were slightly higher than the expected results (0.18 and 0.14; 0.43 and 0.39, respectively), while the frequency of occurrence for the heterogeneous genotype was low in the observed result compared to the expected result (D=-0.16) (Table 14). A negative indicator (-0.16) means that in our study the heterozygous genotype was deficient. But still, these results were not significant ($\chi2<3.85$; $P>0.05$), which means that during the analysis the probability of occurrence of systematic errors is low.

From the point of view of the influence of the polymorphism variant in the promoter locus 1084 of the IL10 gene on preterm birth, we found that the odds ratio (OR) of the risk of developing the disease in the main group for carriers of the A allele was - 0.4 (95% CI: 0.26-0 ,61), which means the A allele plays a significant and significant protective role against preterm birth in the Uzbek population ($\chi2=18.1$; $P=0.01$). On the other hand, in our study, the G allele played a significant and strong initiatory role in preterm birth (OR=2.5; 95%CI: 1.64-3.85; $\chi2=18.1$; $P=0.01$) (Table 5.15) In addition, analysis of the distribution of genotypes in our study showed that the homozygous G/G genotype increased the risk of preterm birth (OR=5.6; 95% CI: 2.04-15.25), while genotype A/A suppressed this risk (OR=0.4; 95%CI: 0.23-0.68), playing a protective role. And these results were statistically significant ($\chi2=11.2$; $P=0.01$). On the other hand, we could not find a statistically significant association between the heterozygous genotype and preterm birth ($\chi2=1.6$; $P=0.3$), despite the fact that the odds ratio showed that the heterozygous genotype increases the likelihood of preterm birth.

In the first subgroup for allele G and A, a statistically significant relationship with preterm birth was revealed, when the first allele increased, and the second allele reduced the risk of preterm birth (OR=2.4; 95% CI: 1.5-3.97; $\chi2= 12.9$; $P=0.01$ and OR=0.9; 95% CI: 0.25-0.67; $\chi2=12.9$; $P=0.01$, respectively by alleles).

The result obtained for the homozygous G/G genotype, this genotype significantly and significantly increased the risk of PVR in patients of the first subgroup (OR=8.8; 95% CI: 2.22-18.3; $\chi2=11.8$; P=0 .01), while the homozygous genotype A/A significantly reduced the risk of preterm birth, which was also statistically significant and significant (OR=8.5; 95% CI: 0.25-0.86; $\chi2=5.9$; $P=0.025$).

We found that the G allele of the second group plays an initiating role (OR=2.6; 95% CI: 1.56-4.36) in the pathogenesis of preterm birth, and the A allele plays a protective role (OR=0.4; 95% CI: 0.23-0.64). Our results were statistically significant ($\chi 2>3.84$; $P<0.05$.

As a key regulator of immunity, IL-10 not only has immunomodulatory activity, but also has a direct beneficial effect on the vascular system and promotes successful cellular communication at the border between mother and fetus [16; 11-19-st.]. Compared to the other genes mentioned above, with respect to the IL10 G1082A gene polymorphism, we found a statistically significant association between the polymorphic gene variant and preterm birth. More specifically, in our study, the G allele played an initiatory role in the pathogenesis of preterm birth, while the A allele protected women from preterm birth in the Uzbek population. In addition, the homozygous G/G genotype significantly increased the risk of preterm birth, while the homozygous A/A reduced this risk. All of these results were reliable and significant ($\chi 2>3.84$; $P<0.05$). On the other hand, although the heterozygous G/A genotype slightly increased the risk of preterm birth.

Conclusion

1. As signs of the threat of termination of pregnancy increased, an increase in genetic indicators was noted, which led to premature birth and perinatal complications.

2. Our scientific studies have shown that the polymorphism of the IL-1β, IL-10 genes is a prognostic marker of the threat of abortion and preterm birth for women of the Uzbek population

3. Polymorphism of the IL-10 rs (1800896) and IL-1b (rs1143627) genes are predictors of threatened miscarriage and preterm birth

4. Timely diagnosis of the threat of miscarriage and preterm birth, based on genetic markers, made it possible to choose treatment tactics in a timely manner, prolong pregnancy and improve perinatal outcomes

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