

The significance of TNF- α gene polymorphisms in preterm delivery

Znaczenie polimorfizmów genu TNF- α w porodzie przedwczesnym

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Abstract

Introduction: Nowadays the strong genetic background of preterm delivery (PTD) in connection with immune answer has been indicated. The purpose of the study was the assessment of frequency of TNF- α -238G>A, -308G>A, -376G>A gene polymorphisms in the etiology of preterm delivery.

Material and methods: The study group consisted of 150 women with PTD (22+0 - 36+6 gw.), the controls of 150 women who delivered at term (>37 gw.). PTD group was divided into subgroups: a/ delivery between 22-28 gw., b/ 28-32 gw., and c/ 32-36+6 gw. Genetic analysis was performed by PCR/RLFP method.

Results: Overrepresentation of -238GA genotype (12.7 vs. 4.7%, $p=0.011$) and -238A allele (7.7 vs. 2.3%, $p=0.002$) in PTD group has been observed. In PTD 28-32 gw. subgroup, higher frequency of -238GA genotype (31.6 vs. 4.7%, $p=0.00095$), and mutated -238A allele (21.1 vs. 2.3%, $p=0.00004$) was noted. Moreover in PTD 28-32 gw. subgroup we have noted higher presence of heterozygous -376GA genotype (10.5 vs. 1.3%, $p=0.063$) and mutated -376A allele (5.3 vs. 0.7%, $p=0.064$). Analysis of TNF- α polymorphisms co-occurrence showed statistically significant overrepresentation of genotypes containing mutated -238A allele in PTD group (-238GA/-308GG/-376GG: 8.0 vs. 2.7%, $p=0.035$). Haplotype analysis revealed statistically significant difference between PTD and controls in the incidence of -376G/-308G/-238A haplotype containing mutated -238A allele (0.063067 vs. 0.016634, $p=0.030$).

Conclusion: The study indicated the strong association of mutated -238A allele of TNF- α gene with increased risk of PTD. Analysis of genotypes and alleles prevalence in PTD women divided according to gestational age suggests the possible role of mutated variants of -238G>A and -376G>A TNF- α polymorphisms in Polish women delivering between 28 and 32 gw.

Key words: **preterm delivery / TNF-alpha / genetic polymorphism /**

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Streszczenie

Cel pracy: Obecnie w etiologii porodu przedwczesnego (PTD – preterm delivery) wskazuje się na silny udział czynników genetycznych w połączeniu z odpowiedzią immunologiczną. Celem pracy była ocena częstości występowania polimorfizmów -238G>A, -308G>A, -376G>A genu TNF- α w etiologii porodu przedwczesnego.

Materiał i metody: Grupę badaną stanowiło 150 kobiet z PTD (22+0-36+6 tc.), grupę kontrolną natomiast 150 kobiet, które urodziły po 37 tc. Grupę PTD podzielono na podgrupy: a/ 22-28 tc., b/ 28-32 tc., c/ 32-36+6 tc. Analizę genetyczną przeprowadzono z zastosowaniem metody PCR/RFLP.

Wyniki: Obserwowano znacząco wyższą częstość występowania genotypu -238GA (12,7 vs. 4,7%, $p=0,011$) oraz allele -238A (7,7 vs. 2,3%, $p=0,002$) w grupie PTD. W podgrupie PTD 28-32 tc. odnotowano wyższą częstość genotypu -238GA (31,6 vs. 4,7%, $p=0,00095$) oraz zmutowanego allele -238A (21,1 vs. 2,3%, $p=0,00004$). Co więcej, w grupie PTD 28-32 tc. obserwowano wyższą częstość genotypu heterozygotycznego -376GA (10,5 vs. 1,3%, $p=0,063$) oraz zmutowanego allele -376A (5,3 vs. 0,7%, $p=0,064$). Analiza polimorfizmu genu TNF- α wykazała statystycznie istotną przewagę współwystępowania genotypów zawierających zmutowany allele -238A w grupie PTD (-238GA/-308GG/-376GG: 8,0 vs. 2,7%, $p=0,035$). Ponadto odnotowano statystycznie istotną różnicę pomiędzy grupą PTD oraz kontrolną w częstości występowania haplotypu -376G/-308G/-238A zawierającego zmutowany allele -238A (0,063067 vs. 0,016634, $p=0,030$).

Wnioski: Wyniki badań wskazały na silną korelację pomiędzy zmutowanym allele -238A genu TNF- α a wzrostem ryzyka wystąpienia PTD. Analiza częstości genotypów i alleli badanych polimorfizmów u kobiet z PTD podzielonych w zależności od wieku ciążowego sugeruje możliwą rolę zmutowanych wariantów polimorfizmów -238G>A oraz -376G>A TNF- α w etiologii tego powikłania u kobiet polskich, u których poród odbył się pomiędzy 28 a 32 tc.

Słowa kluczowe: poród przedwczesny / TNF-alfa / polimorfizm genetyczny /

Introduction

Etiology of preterm delivery (PTD) seems to be connection of genetic and environmental factors. The key part of etiological concerns is contribution of immune cytokine network in this condition. Tumor necrosis factor-alpha (TNF- α) is one of the main cytokine of inflammatory and immunological response. It is produced in the answer to stimuli that are bacterial wall lipopolysaccharides (LPS). During pregnancy TNF- α , after LPS stimulation followed by IL-1 and IL-6 modulation, is synthesised by trophoblastic cells, thus enhances uterus contractions [1, 2]. This process activates also secretion of proteolytic enzymes by neutrophils what leads to cervix dilatation. Additionally increased cytokines concentration is related to higher expression of oxytocin receptor in uterus [2]. Contemporary analysis revealed increased concentration of TNF- α in serum and amniotic fluid in women with high risk of PTD. Differences in activity and concentration of TNF- α may be explained by the presence of polymorphic variants in the gene coding this immunologic factor [3, 4, 5].

In PTD pathogenesis research mainly genetic variants of cytokines, including TNF- α are investigated [6, 7, 8]. Gene encoding TNF- α is located in chromosome (locus 6p23-6q12). In the promoter region of TNF- α gene few polymorphic loci are found. Some of these polymorphisms are most promising to be involved in PTD etiology and commonly analysed [9, 10]. Thus increased cytokines level in amniotic fluid and cervical mucous as well as some polymorphisms of cytokine genes could be a marker of PTD risk [2, 8].

The aim of the study was to assess the -238G>A, -308G>A, and -376G>A polymorphisms of gene coding for TNF- α in the PTD etiology in Polish women.

Material and methods

Patients

Study group consisted of 150 women (mean age 29,2 \pm 5,6 years, mean gestational age 33,7 \pm 2,8 gw.) with preterm delivery (22+0 - 36+6 gw.). To the control group 150 healthy pregnant women (mean age 29,0 \pm 3,7 years, mean gestational age 39,3 \pm 1,2 gw.) who delivered >37 gw. were enrolled. Patients were recruited into the study in Division of Perinatology and Women's Diseases, Poznan University of Medical Sciences, Poznan, Poland.

All patients gave their informed written consent to participate in the project. The goals of the investigation were approved by Bioethical Committee of Poznan University of Medical Sciences.

Inclusion criteria to study group were as follow: delivery within 22+0 to 36+6 gw., clinical symptoms of preterm delivery (regular utero contractions, abdominal pain, cervical dilatation) and/or preterm premature rupture of membranes, singleton pregnancy, Caucasian race, Polish origin.

Inclusion criteria to control group were as follow: delivery >37+0 gw., singleton pregnancy, proper course of pregnancy, Caucasian race, Polish origin.

All patients with multifetal pregnancy, gestational hypertension, preeclampsia, uterus anomalies, cervical insufficiency, fetal anomalies, gestational diabetes, polyhydramnion, oligohydramnion, general infections, intrauterine fetal death and chronic internal diseases were excluded from the study and control groups.

Genetic analysis

Genomic DNA was extracted from blood leucocytes using QIAamp DNA Blood Mini Kit (QIAGEN Inc., Germany). Genotyping was performed using polymerase chain reaction (PCR) and restriction length fragment polymorphism (RLFP) procedures.

-238G>A, -308G>A, -376G>A *TNF-α* gene polymorphisms

For detected of *TNF-α* -376G>A mutation PCR was amplified with starters: 5'-AAg AAT CAT TCA ACC AgC gg and 5'-CCT CAA Cgg ACT CAg CCT TC (TibMolBiol, Poland) (PCR product 393 bp long) and hydrolysed with *TasI* (*Tsp509I*) restriction enzyme.

For detected of *TNF-α* -238G>A and -308G>A mutations PCR was amplified with starters: 5' - AAA Tgg Agg CAA Tag gTT TTg Agg ggC TTg and 5' - TAC CCC TCA CAC TCC CCA TCC TCC CTg ATC (TIBMolBiol, Poland) (PCR product 131 bp long). PCR products were hydrolysed for -238G>A polymorphism with *BspPI* (*AlwI*) restriction enzyme and for -308G>A polymorphism with *FaqI* (*BsmFI*) restriction enzyme, respectively.

Statistical analysis was performed by SPSS 17.0 PL for Windows. As a statistically significant we have considered *p* value lower than 0,05. Frequencies of genotypes were compared by chi-square test. Expected genotype frequencies were calculated from allele frequencies applying Hardy-Weinberg equilibrium. For evaluation of haplotypes frequency of investigated polymorphisms PHASE (2.1) and Haploview (4.2) programs were used.

Results

-238G>A, -308G>A, and -376G>A *TNF-α* polymorphism

The most interesting results were connected with -238G>A polymorphism of *TNF-α* gene. The overrepresentation of heterozygous -238GA genotype in PTD group (12.7 vs. 4.7%, *p*=0.011) has been observed. Furthermore, in two patients from PTD group mutated -238AA genotype was noted, but it was not observed in any patient from the control group (1.3 vs. 0.0%). Mutated -238A allele also was overrepresented in PTD group (7.7 vs. 2.3% in control group, *p*=0.002).

The frequencies of -308GG, -308GA and -308AA genotypes were as follows: 68.7 vs. 30.0 vs. 1.3% in PTD group and 72.0 vs. 26.0 vs. 2.0% in controls (*ns*). The frequency of alleles of investigated polymorphism was similar in both analysed groups: -308G (83.7 vs. 85.0%, *ns*) and -308A (16.3 vs. 15.0%, *ns*).

Mutated -376AA genotype have not been observed in any subject from the study and control groups. The frequency of heterozygous -376GA genotype was slight higher in PTD group than in controls (2.7 vs. 1.3%, *ns*). Mutated -376A allele was observed more frequently in PTD group (1.3 vs. 0.7% in controls, *ns*). (Table I).

Polymorphisms of investigated genes in relation to gestational week at delivery in PTD group

The study group of women with PTD was divided into 3 subgroups in relation to gestational week at delivery: a/ 22-28 gw., b/ 28-32 gw., c/ 32-36 gw. In each of subgroup the frequency of genotypes and alleles of investigated *TNF-α* gene polymorphisms has been analysed.

The most interesting observation was noted in relation to frequency of heterozygous -238GA genotype in PTD 28-32 gw. subgroup (31.6 vs. 4.7%, *p*=0.00095). Similar, frequency of mutated -238A allele in PTD 28-32 gw. subgroup also was much higher (21.1 vs. 2.3% in the control group, *p*=0.00004).

Also in the PTD 32-36 gw. subgroup statistically significant differences of heterozygous -238GA *TNF-α* genotype (10.6 vs. 4.7%, *p*=0.033) and mutated -238A *TNF-α* allele (6.1 vs. 2.3%, *p*=0.022) have been observed. (Table II).

Analyzing the -308G>A *TNF-α* polymorphism in the subgroups divided according to the gestational week at delivery any differences in presence of genotypes and alleles between PTD and control groups has been observed. (Table III).

Relating to the -376G>A *TNF-α* polymorphism in the PTD 28-32 gw. subgroup higher frequency of heterozygous -376GA *TNF-α* genotype was observed (10.5 vs. 1.3%, *p*=0.063). Similar observation was noted in the same subgroup as to mutated -376A *TNF-α* allele (5.3 vs. 0,7%, *p*=0.064). (Table IV).

Coexistence of -238G>A, -308G>A and -376G>A *TNF-α* polymorphisms in both analysed groups

Coexistence of genotypes containing mutated -238A allele was more frequent at patients from the PTD group. There has been noted higher frequency of coexistence of following genotypes in the study group: -238GA/-308GG/-376GG (8.0 vs. 2.7%, *p*=0.035), -238GA/-308GG/-376GA (2.7 vs. 1.3%, *ns*), -238GA/-308GA/-376GG (2.0 vs. 0.7%, *ns*) and -238AA/-308GG/-376GG (1.3 vs. 0.0%). These results may suggest participation of mutated variant -238A in increased risk of preterm delivery. In patients from the control group the frequency of coexistence of homozygotic genotypes -238GG/-308GG/-376GG without mutated variants was higher than in PTD group (68.0 vs. 57.3%, *p*=0.037). (Table V).

The haplotypes frequency of *TNF-α* polymorphisms

Among investigating *TNF-α* polymorphisms 6 haplotypes triplets for position -376, -308, -238 (-376G/-308G/-238G, -376G/-308G/-238A, -376G/-308A/-238G, -376G/-308A/-238A, -376A/-308G/-238G, -376A/-308G/-238A) in both analysed groups of women have been noted. There has been observed statistically significant higher frequency of -376G/-308G/-238A haplotype containing mutated -238A allele (0,063067 vs. 0,016634 in the control group, *p*=0.030) in PTD group.

There has been also revealed overrepresentation of -376A/-308G/-238A haplotype (haplotype containing mutated -376A and -238A alleles) in the group of women with PTD (0,013300 vs. 0,006400, *ns*). (Table VI).

Discussion

***TNF-α* gene polymorphisms**

To our knowledge this is the first study in the population of Polish women focused on collective analysis of -238G>A, -308G>A and -376G>A *TNF-α* polymorphisms. There are few interesting studies on *TNF-α* polymorphisms and its correlation with PTD which, because of its location in the regulatory region, may be very important in the *TNF-α* gene expression.

-238G>A, -376G>A *TNF-α* polymorphism

In our research the most valuable results concern -238 *TNF-α* gene polymorphism. It is noteworthy that in this study higher frequency of -238GA genotype in PTD group (12.7 vs. 4.7%, *p*=0.011) has been observed. Moreover at two patients with PTD mutated homozygotic -238AA genotype was noted and it was not observed at any patient from the control group (1.3 vs. 0.0%).

Elżbieta Drews-Piasecka et al. *The significance of TNF- α gene polymorphisms in preterm delivery.***Table I.** The frequency of genotypes and alleles of -238G>A, -308G>A, -376G>A TNF-alpha gene polymorphism.

TNF- α Genotypes	Study group PTD (n=150)		Control group (n=150)		OR	95 % CI	p
	Observed value n(%)	Expected value (%)	Observed value n(%)	Expected value (%)			
-238G>A							
GG	129 (86.0)	85.2	143 (95.3)	95.4	0.30	0.10-0.76	0.004
GA	19 (12.7)*	14.2	7 (4.7)*	4.6	2.96	1.14-8.59	0.011
AA	2 (1.3)	0.6	0 (0.0)	0.0	-	-	-
Total	150 (100.0)	100.0	150 (100.0)	100.0	-	-	-
Alleles							
G	277 (92.3)	-	293 (97.7)	-	0.29	0.10-0.71	0.002
A	23 (7.7)*	-	7 (2.3)*	-	3.48	1.41-9.72	0.002
Total	300 (100.0)	-	300 (100.0)	-			
-308G>A							
GG	103 (68.7)	70.0	108 (72.0)	72.3	0.85	0.50-1.44	0.31
GA	45 (30.0)	27.3	39 (26.0)	25.5	1.22	0.71-2.08	0.26
AA	2 (1.3)	2.7	3 (2.0)	2.2	0.66	0.05-5.87	0.50
Total	150 (100.0)	100.0	150 (100.0)	100.0			
Alleles							
G	251 (83.7)	-	255 (85.0)	-	0.90	0.57-1.44	0.37
A	49 (16.3)	-	45 (15.0)	-	1.11	0.69-1.76	0.37
Total	300 (100.0)	-	300 (100.0)	-			
-376G>A							
GG	146 (97.3)	97.4	148 (98.7)	98.7	0.49	0.04-3.51	0.34
GA	4 (2.7)	2.6	2 (1.3)	1.3	2.03	0.28-22.68	0.34
AA	0 (0.0)	0.0	0 (0.0)	0.0	-	-	-
Total	150 (100.0)	100.0	150 (100.0)	100.0			
Alleles							
G	296 (98.7)	-	298 (99.3)	-	0.50	0.04-3.49	0.34
A	4 (1.3)	-	2 (0.7)	-	2.01	0.29-22.39	0.34
Total	300 (100.0)	-	300 (100.0)	-			

* p<0.05, all women from PTD group were compared to the control group

Table II. The frequency of genotypes and alleles of -238G>A polymorphism of TNF- α gene in the PTD group in relation to gestational week.

TNF- α -238G>A	Study group PTD									Control group n (%)
	22-28 gw.			28-32 gw.			32-36 gw.			
	n (%)	OR	p	n (%)	OR	p	n (%)	OR	p	
Genotypes										
GG	8 (100.0)	-	-	12 (63.2)	0.08	0.00016*	109 (88.6)	0.38	0.033*	143 (95.3)
GA	0 (0.0)	-	-	6 (31.6)	9.43	0.00095*	13 (10.6)	2.62	0.033*	7 (4.7)
AA	0 (0.0)	-	-	1 (5.3)	-	-	1 (0.8)	-	-	0 (0.0)
Total	8 (100.0)			19 (100.0)			123 (100.0)			150 (100.0)
Allele										
G	16 (100.0)	-	-	30 (78.9)	0.09	0.00004*	231 (93.9)	0.37	0.022*	293 (97.7)
A	0 (0.0)	-	-	8 (21.1)	11.16	0.00004*	15 (6.1)	2.72	0.022*	7 (2.3)
Total	16 (100.0)			38 (100.0)			246 (100.0)			300 (100.0)

* p<0.05, all women from PTD group were compared to the control group

Table III. The frequency of genotypes and alleles of -308G>A polymorphism of TNF- α gene in the PTD group in relation to gestational week.

TNF- α -308G>A	Study group PTD									Control group
	22-28 tc.			28-32 tc.			32-36 tc.			
	n (%)	OR	p	n (%)	OR	p	n (%)	OR	p	
Genotypy										
GG	6 (75.0)	1.16	0.61	12 (63.2)	0.67	0.29	85 (69.1)	0.87	0.34	108 (72.0)
GA	2 (25.0)	0.94	0.65	7 (36.8)	1.66	0.23	36 (29.3)	1.18	0.32	39 (26.0)
AA	0 (0.0)	-	-	0 (0.0)	-	-	2 (1.6)	0.81	0.59	3 (2.0)
Suma	8 (100.0)			19 (100.0)			123 (100.0)			150 (100.0)
Allele										
G	14 (87.5)	1.23	0.56	31 (81.6)	0.78	0.36	206 (83.7)	0.91	0.38	255 (85.0)
A	2 (12.5)	0.81	0.56	7 (18.4)	1.28	0.36	40 (16.3)	1.10	0.38	45 (15.0)
Suma	16 (100.0)			38 (100.0)			246 (100.0)			300 (100.0)

* $p < 0.05$, all women from PTD group were compared to the control group.

Table IV. The frequency of genotypes and alleles of -376G>A polymorphism of TNF- α gene in the PTD group in relation to gestational week.

TNF- α -376G>A	Study group PTD									Control group
	22-28 gw.			28-32 gw.			32-36 gw.			
	n (%)	OR	p	n (%)	OR	p	n (%)	OR	p	
Genotypes										
GG	8 (100.0)	-	-	17 (89.5)	0.12	0.063	121 (98.4)	0.81	0.61	148 (98.7)
GA	0 (0.0)	-	-	2 (10.5)	8.71	0.063	2 (1.6)	1.22	0.61	2 (1.3)
AA	0 (0.0)	-	-	0 (0.0)	-	-	0 (0.0)	-	-	0 (0.0)
Total	8 (100.0)			19 (100.0)			123 (100.0)			150 (100.0)
Alleles										
G	16 (100.0)	-	-	36 (94.7)	0.12	0.064	244 (99.2)	0.82	0.61	298 (99.3)
A	0 (0.0)	-	-	2 (5.3)	8.28	0.064	2 (0.8)	1.22	0.61	2 (0.7)
Total	16 (100.0)	-	-	38 (100.0)			246 (100.0)			300 (100.0)

* $p < 0.05$, all women from PTD group were compared to the control group

Table V. Genotypes co-occurrence of -238 G>A, -308 G>A, -376 G>A polymorphisms of TNF- α gene.

-238G>A/-308G>A/-376G>A TNF- α polymorphisms	Study group PTD n(%)	Control group n(%)	p
-238GG/-308GG/-376GG	86 (57.3)	102 (68.0)	0.037*
-238GG/-308GA/-376GG	41 (27.3)	38 (25.3)	0.396
-238GG/-308AA/-376GG	2 (1.3)	3 (2.0)	0.500
-238GA/-308GG/-376GG	12 (8.0)	4 (2.7)	0.035*
-238GA/-308GG/-376GA	4 (2.7)	2 (1.3)	0.342
-238GA/-308GA/-376GG	3 (2.0)	1 (0.7)	0.311
-238AA/-308GG/-376GG	2 (1.3)	0 (0.0)	-
Total	150 (100.0)	150 (100.0)	

* $p < 0.05$, all women from PTD group were compared to the control group.

Table VI. The frequency of haplotypes of analysed TNF- α gene polymorphisms.

-376/-308/-238 TNF-α haplotypes	Study group PTD n=150		Control group n=150		p
	frequency	standard mistake	frequency	standard mistake	
-376G/-308G/-238G	0.760266	0.001519	0.826699	0.001492	ns
-376G/-308G/-238A	0.063067*	0.001519	0.016634*	0.001492	p=0,030
-376G/-308A/-238G	0.163034	0.001475	0.149701	0.000953	ns
-376G/-308A/-238A	0.000300	0.001475	0.000299	0.000953	ns
-376A/-308G/-238G	0.000034	0.000333	0.000266	0.001293	ns
-376A/-308G/-238A	0.013300	0.000333	0.006400	0.001293	ns

* $p < 0.05$, all women from PTD group were compared to the control group.

Furthermore, mutated -238A allele was present at 7.7% women from the study group and only at 2,3% women from the control group ($p=0.002$).

In our previous study concerning genetic variability in Polish women with PTD, preterm premature rupture of membranes (PPROM) and intraamniotic infection (IAI) similar results were revealed. Although the small number of investigated patients (53 subjects) the heterozygous -238GA genotype was observed in 32,1% women with PTD/IAI and only in 15,2% healthy pregnant women from the control group (OR=2.64, $p=0.014$). Mutated -238AA genotype was noted in 3,8% patients with PTD/IAI, and it was not observed in any woman from the control group. Analysis of mutated -238A allele revealed its significantly more frequent presence in women with PTD with former premature preterm rupture of membranes and intraamniotic infection (19.8 vs. 7.6%, OR=3.0, $p=0.002$). This polymorphism may play an important role in immunological response modulation in women with PTD, PPROM and IAI [4, 11].

The study focused on -238G>A TNF- α polymorphism was also performed by Annels et al. in Australia. This research confirmed that some cytokines influence the increased PTD risk. The study group in this analysis consisted of 202 Caucasian women who delivered before 35 gw. and 185 white women with term birth. Results of the research revealed that presence of -488A/-238G/-308A haplotypes of TNF- α gene is strongly correlated to increased PTD risk [12]. In contrast to our results, the haplotype presenting by Annels et al. contains the wild-type -238G TNF- α allele.

The interesting results were also connected with -376G>A TNF- α gene polymorphism. The frequency of -376GA genotype was slightly higher in PTD group (2.7 vs. 1.3% in controls, $p=ns$). The frequency of mutated -376A allele was 1,3% in PTD group and 0,67% in control group ($p=ns$). These results are difficult to interpret because of the lack of analysis concerning the contribution of -376G>A TNF- α gene polymorphism in PTD etiology in other populations.

Additionally, very remarkable part of our study is the analysis of the co-occurrence of all investigated TNF- α polymorphisms. What is noteworthy the combination of genotypes co-occurrence containing mutated -238A allele have been noted more often in PTD group with statistically significant difference.

-308G>A TNF- α polymorphism

Many researches were connected with -308G>A TNF- α polymorphism related to PTD. Roberts et al. performed one of the first studies on the -308G>A TNF- α gene polymorphism (57 women with PTD and 110 women as a control group). The frequency of mutated -308A allele in the whole PTD group was 44.0 vs. 30.0% in the control group (OR=1.81, $p=0.08$). The frequency of mutated -308A allele was significantly higher in the group of women who delivered after PPROM (OR=3.18, $p=0.008$). Results of this research suggest probable correlation between investigated TNF- α polymorphism and PTD related to PPROM [13]. In the other study 54 women with PTD and 79 women who delivered at term were involved. The Authors revealed statistically significant correlation between -308G>A polymorphism and the risk of PTD. Carriers of mutated -308AA genotype statistically more frequent delivered prematurely [14]. Results published by Speer et al. confirmed connection between -308G>A TNF- α polymorphism, histologically diagnosed chorionamnionitis and PTD. Furthermore, the Authors observed positive correlation of -308GG genotype with genito-urinary tract infection [5].

In one of the largest analysis performed on the group of 834 women with a positive PTD history few polymorphisms were investigated (-174G>C IL-6, -308G>A TNF- α and +3954 IL-1 β). At subjects with mutated -308AA TNF- α genotype time of duration of pregnancy was statistically shorter in comparison to women carriers of -308GG or -308GA genotypes ($p=0.03$). Moreover, among women with -308AA genotype strong tendency to spontaneous PTD before 28 gw. was noted. The whole analysis revealed significant correlation between -308G>A polymorphism and the PTD risk [15].

Other type of analysis was performed by Liang et al. in China. In the group of women with PTD, their children and fathers of prematurely born children (250 study and 260 control families) the genotypes and alleles frequency of -308G>A TNF- α polymorphism was investigated. The results revealed correlation of co-occurrence of genotype at mother and child with PTD. The coexistence of one -308G>A genotype in mother or in child increased the PTD risk. When heterozygous -308GA genotype was present simultaneously in mother and in child (-308GA/-308GA) the risk of PTD was decreased [16].

In contrary there have been also other results connecting $-308G>A$ *TNF-α* polymorphism with PTD. Research of Mattar et al. involved 45 women of Caucasian race, 81 women of mixed population from the USA (Afroamericans, Americans with Spanish origin) and 13 black women delivered before 37 gw. There were no statistically significant differences as to the genotypes and alleles frequency of $-308G>A$ polymorphism [17]. Similar results were obtained by Chauhan et al. The Authors did not observe correlation of $-308G>A$ *TNF-α* polymorphism with PTD risk [18]. Amory et al. investigated $-308G>A$ and $-863C>A$ *TNF-α* polymorphisms in group of 118 women and their newborns delivered before 34 gw. Mothers homozygotes $-863AA$ statistically more often delivered prematurely and more often were diagnosed for IAI. The Authors did not observe such a correlation with $-308G>A$ *TNF-α* polymorphism [19]. Moreover, Stonek et al. did not reveal any statistically significant differences in genotypes and alleles frequency of $-308G>A$ *TNF-α* polymorphism in a group of 1652 women with intrauterine fetal death, preeclampsia, preterm delivery or low birth weight [20]. These results are similar to ours, as we also have not observed any significant influence of $-308G>A$ *TNF-α* polymorphism on PTD etiology.

Conclusions

1. The study indicated the strong association of mutated $-238A$ allele of *TNF-α* gene with increased risk of PTD.
2. Analysis of genotypes and alleles prevalence in PTD women divided according to gestational age suggests the possible role of mutated variants of $-238G>A$ and $-376G>A$ *TNF-α* polymorphisms in Polish women delivering between 28 and 32 gw.
3. The $-308G>A$ polymorphisms of *TNF-α* gene probably is not connected with the risk of PTD in investigated population of Polish women.

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