БІОЛОГІЧНІ НАУКИ

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RECURRENT PREGNANCY LOSS ASSOCIATION WITH ALLELIC VARIANTS OF IL8 AND IL10 GENES

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The study evaluated IL8 -781C/T and IL10 -592C/A, -1082A/G polymorphisms association with recurrent pregnancy loss (RPL) (n=110 case, n=106 control).
IL10 -592AA genotype and -1082A allele carriers frequency was higher in RPL group (6.6 %, 87.3 %) comparing to control (0.9 %, 76.4 %). The -1082A allele carriers have twice increased RPL risk (OR=2.12; CI95 %: 1,03–4,34).
Keywords: IL8 gene/ IL10 gene/ interleukin/ recurrent pregnancy loss.

У дослідженні оцінювали асоціацію поліморфізмів генів IL8 -781C/T та IL10 -592C/A, -1082A/G зі звичним невиношуванням вагітності (НВ) (n=110 дослідна група, n=106 контроль).
Частота носіїв генотипа -592AA і алелю - 1082A гена IL10 була вище в групі НВ (6,6 %, 87,3 %) порівняно з контрольною (0,9 %, 76,4 %). Ризик невиношування у носіїв алелю -1082A вдвічі вище (OR = 2,12; CI95 %: 1,03-4,34).
Ключові слова: IL8 ген / IL10 ген / інтерлейкін / звичне невиношування вагітності.

1. Introduction

The loss of three or more sequential pregnancies in the first trimester of gestation is defined as recurrent pregnancy loss (RPL). Up to 5 % of reproductive age women are affected by this pathology [1]. Amongst the variety of RPL etiologies (cytogenetic, endocrine, immunological, anatomical) the idiopathic is the most common [2]. Knowledge accumulation in this field made it clear that successful pregnancy is a result of dynamic interaction between immunological and immunogenetic factors. It was shown that the disruption of these networks may often result in reproductive failure [2]. Multiple studies confirmed that the maternal immune system is crucial for pregnancy establishment and maintenance [3]. Periods of pro-inflammatory and anti-inflammatory environment prevalence in gestational tissues occur in precise sequence to provide pregnancy tolerance [4]. Cytokines are essential molecules to provide this type of interchange.

2. Purpose

The aim of this study was to evaluate the association of *IL8* gene -781C/T, *IL10* gene -592C/A and -1082A/G polymorphisms with RPL pathogenesis in group of patients from Ukraine.

3. Literature review

Cytokines are crucial participants in the processes of gametogenesis, implantation, trophoblast invasion, placental development and immunological tolerance of fetus [2]. In particular, pro-inflammatory cytokines (IL-1, IL-6, IL-8, IL-15) promote cytotrophoblast invasion of decidua, inflammatory and embriotoxic reaction. On the other hand, anti-inflammatory cytokines (IL-4, IL-10) prevent embriotoxic reaction of mother's organism [3]. One of the primary factors affecting cytokine network function is altered regulation of respective genes expression. Numerous studies were concentrated on the impact of allelic variants in cytokine genes on their expression and, as a result, the association of some polymorphisms with pathologies [5-7].

Chemokine IL-8 is a crucial player in the process of implantation. It promotes trophoblastic cells migration and invasion [8]. *IL8* gene is located on chromosome 4 in position 4q13-q21. It consists of 4 exons and 3 introns and encloses 235 SNPs [9]. 3'-UTR region and intron 1 are the most enriched with SNPs [9]. Interleukin 10 is an anti-inflammatory cytokine which is a major suppressor of embryo elimination and a promoter of trophoblastic growth [3]. *IL10* gene is located on chromosome 1 in position 1q31-q32. It consists of 5 exons and 4 introns. The total amount of 187 SNPs is reported for this gene, predominantly located inside intron 1 and within 2 Kb prior to coding region (including promoter region [10]. Several SNPs have been reported to influence *IL8* and *IL10* gene expression [11, 12]. Some of them were found to be associated with reproductive pathologies, though these data remain controversial and require further investigation [13].

4. IL8 and IL10 variants association with RPL

A case group comprised 110 unrelated women with history of idiopathic RPL (at least two cases of consequent miscarriage in the first trimester), patients of

the SI "Institute of Pediatrics, Obstetrics and Gynecology of NAMS of Ukraine" (aged 34,15 + 4,49 years; fetal losses 2,73 + 0,87; parity 0,29+0,55). In order to exclude other possible risk factors but immunogenetic the patients underwent thorough examinations and only the patients with following clinical features were chosen for the investigation: no family history of birth defects; no genital tract anatomic abnormalities (confirmed by ultrasonography or hysterosalpingography); no common immunologic risk factors (anti-nuclear antibodies, anti-phospholipid antibodies, lupus anticoagulant), defects of thyroid function, diabetes mellitus, hyperprolactinemia and infections such as chlamydia; neither inherited nor acquired thrombophilia, no Leiden (*F5* G1691A) and the prothrombin gene mutations (*F2* G20210A); normal karyotype of both patients and their partners (though not all the abortuses underwent cytogenetic analysis).

A control group consisted of 106 unrelated healthy women (aged 26,23±2,99 years) with no history of RPL, who have given birth to at least one child conceived in natural way. All participants have given their informed consent prior to clinical examination and genotyping. The study was approved by The Bioethical Committee of Institute of Molecular Biology and Genetics of NAS of Ukraine.

The material of the study was genomic DNA extracted from peripheral blood samples of patients and control group individuals using standard phenol–chloroform technique. Genotyping for *IL8* gene -781C/T, *IL10* gene -592C/A and -1082A/G polymorphic variants was performed by a PCR-based restriction fragment length polymorphism assay as described previously [11, 14]. Statistical analysis has been performed using GenePop and OpenEpi statistical packages [15, 16]. Fisher's exact test (Mid-P method) was used to estimate the difference in genotype and allelic distribution. In order to assess the association of certain genotype with RPL, OR index was calculated. A P-value of less than 0.05 was regarded as significant.

The results of genotyping for both studied polymorphic variants are presented in Table 1. Genotype frequencies for all studied variants showed no significant deviation from the ones expected according to Hardy-Weinberg equilibrium.

Table 1

Genotype and allelic distribution of *IL8* gene -781C/T, *IL10* gene -592C/A and -1082A/G gene polymorphic variants in studied groups

Locus		RPL group, n	RPL group, %	Control group, n	Control group, %	OR	CI 95%
IL8-781C/T	Genotype						
	CC	38	34,5	18	16,9	5,06	2,56 – 9,99
	CT	58	52,7	43	40,6		
	TT	14	12,7	45	42,5	0,20	0,10 – 0,39
	Allele						
	C	134	60,9	79	37,3	-	-
	T	86	39,1	133	62,7	-	-
IL10-592C/A	Genotype						
	CC	63	57,3	70	66,1	0,69	0,40 – 1,20
	CA	40	36,4	35	33,0	1,16	0,66 – 2,03
	AA	7	6,4	1	0,9	7,14	0,86 – 59,03
	Allele						
	C	166	75,5	175	82,5	-	-
	A	54	24,5	37	17,5	-	-
IL10-1082A/G	Genotype						
	AA	35	31,8	23	21,7	2,12	1,03 – 4,34
	AG	61	55,5	58	54,7		
	GG	14	12,7	25	23,6	0,47	0,23 – 0,97
	Allele						
	A	131	59,5	104	50,9	-	-
	G	89	40,5	108	49,1	-	-

No difference was found in *IL8* gene -781C/T genotype distribution in studied groups. As to *IL10* gene polymorphic variants, frequency of individuals with -592AA genotype was significantly higher in RPL group (6,6 %) comparing to control group (0,9 %). Frequency of *IL10* gene -1082A allele carriers was significantly higher in group of patients with RPL (87,3 %) comparing to control group (74,6 %). The -1082A allele carriers have RPL risk increased up to 2-fold (OR=2,12; CI 95 %: 1,03 – 4,34). Obtained results are supported by the existing knowledge of immunological processes during early stages of gestation.

The greatest challenge to the maternal immune response occurs at the outset of pregnancy, when the embryo implants into the maternal endometrium and development of the placenta commences. The inflammation process is crucial during this stage as it facilitates the process of implantation [17]. On the other hand, unbalanced inflammation during early stages of gestation may lead to negative consequences. IL-10 appears to be the most powerful immunosuppressive factor produced locally in gestational tissues [18, 19]. Studies on human decidua and endometrium have shown negative effect of IL-10 deficit on pregnancy maintenance [20]. Knock-out studies on mice models have established increased rate of fetal resorption and higher susceptibility of developing fetus to pathogens [21]. Additionally IL-10 injection prevents fetal loss in abortion-prone mice [22]. As alterations in IL-10 production became evident to correlate with pregnancy outcome it has been suggested that certain *IL10* genotypes may be associated with higher risk of RPL. Impact on expression is studied to be the best for polymorphic variants -592C/A and -1082A/G. Alleles -592A and -1082A are associated with lower IL-10 production [12]. Both these polymorphic variants were studied for association with RPL in individual studies and meta-analysis but the results turned out to be controversial [13]. Partly this ambiguity may result from heterogeneity of case groups. The results of this study are consistent with multiple evidence of low-production phenotype negative impact on pregnancy outcome. Women with *IL10* gene -592AA, -1082AG and -1082AA genotypes may have fewer chances for pregnancy maintenance during early stages of gestation.

5. Conclusions

Despite the significant achievements in understanding the immunological features of pregnancy development many controversial issues are yet to be clarified. However, on the basis of statistically significant differences in genotype distribution established in this study, *IL10* gene -592AA genotype and -1082A allele may be considered the markers of increased RPL risk.

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