Interleukin 10 gene polymorphisms and recurrent pregnancy loss in Romanian population

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Abstract

Objective. *The study objective was to establish an association between interleukin 10 polymorphisms and etiology of recurrent spontaneous abortion (RSA).* ***Methods.*** *The genetic polymorphism of interleukin 10 genes were studied by PCR – RFLP in the DNA of 69 women with recurrent pregnancy loss and 64 control women with at least one successful pregnancy and without known pregnancy loss.* ***Results.*** *Our results demonstrated a role for - 819 C/T but not for - 592 C/A and - 1082 A/G IL10 promoter polymorphisms in idiopathic RSA in studied group. Frequency of genotype - 592 CC/ - 819 CC was higher in the control group than in experimental group (p = 0,005). In contrast, genotype - 592 AC /- 819 CT was more frequent in the experimental group (p= 0,05).* ***Conclusions.*** *This study is the first one that demonstrated an association between IL-10 -819 C/T polymorphism in idiopathic recurrent spontaneous abortion among Romanian patients.*

Keywords: *interleukin, pregnancy loss, polymorphisms*

Introduction

Spontaneous abortions are very common medical events and may account during the first trimester for one fifth of all intended pregnancies. The mechanisms mediating the survival of the fetus and those leading to pregnancy loss are far from being understood.

Underlying maternal, genetics, endocrine, anatomical and autoimmune disorders are known causes, found in approximately 60% of cases of pregnancy loss (PL), the remaining 40% being unexplained. It has been suggested that these unexplained early pregnancy loss may be due to immunologic factors.

There is active maternal immune recognition of pregnancy that leads to cellular, antibody or cytokine responses, which protect the fetal allograft[(1)].

Recurrent pregnancy loss (RPL) is defined as 3 or more pregnancy loss before 20 weeks of gestation and has been estimated 1 in 300 pregnancies. The etiology of RPL remains unknown in at least 31% of cases[(2)]. In humans, placental and decidual tissues from normal pregnancies express an array of pro- and anti-inflammatory cytokines. This implies that a potent anti-inflammatory cytokine is produced locally to control fetal-ablating immune responses[(3)]. The debate on the role of Th2 cytokines in human pregnancy is likely to focus on interleukin 10 (IL10). IL10 can be produced by both Th1 and Th2 cells, as well as non-T cells.

During pregnancy, IL-10 is produced locally in the feto-placental unit by cytotrophoblasts, and it up-regulates the human leukocyte antigen (HLA)-G expression of cytotrophoblasts at the feto-maternal barrier. It has been suggested that this protects the fetus from rejection[(4)]. IL-10 has important modulatory effects against pro-inflammatory cytokines, especially interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), both of which have been shown to be detrimental to the feto-placental unit in a murine model[(5)], while high serum TNF-α values have been reported in women undergoing recurrent spontaneous abortions[(4,6)].

One major role of IL10 is the down-regulation of chemokine's and cytokines production by Th1 cells and macrophages. Peripheral blood mononuclear cells (PBMC) from women with history of successful pregnancies, when are stimulated with trophoblast antigens, secrete IL10[(7)]. The stimulation of PBMC with autologous placental cells results in the production of Th2 cytokines by women in labor, and of Th1 cytokines by patients with spontaneous miscarriage[(3)]. Higher serum levels of IL10 were detected in women having normal delivery than in patients with recurrent pregnancy loss (RPL) at the time of abortion, and higher concentrations of TNFA (TNF-α) are detected in women with RPL than in women with successful pregnancy[(7)]. Cytotrophoblasts and decidual T cells from normal pregnancies expressed IL10 and participate to the T helper type 2, predominance at the feto-maternal interface during the crucial stage of early pregnancy and the peri-implantation period. Further studies[(3,7)] elucidated the crucial role of IL-10 at the maternal-fetal interface as placental and decidual tissue from

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first trimester missed abortions showed decreased IL-10 production when compared to control tissues obtained from first trimester elective terminations[8]. Similarly, a comparison of placental tissue from elective cesarean (pre-labor) and placental tissue obtained post-labor showed higher IL-10 production in pre-labor tissues. It is noteworthy that extravillous trophoblasts from first trimester exhibit poor IL-10 production while expressing high levels of message for matrix metalloproteinase-9 (MMP9), implying that invading trophoblasts may temporally down regulate IL-10 expression to maintain their invasive, not necessarily endovascular, potential[7,3].

Located on human chromosome 1 (1q31–q32), many single-nucleotide polymorphisms (SNPs) were reported in the proximal (at position -1082A/ G, -819T/C and -592A/C)[9] and distal[10,4] regions of the IL-10 gene and were reportedly involved in IL-10 transcription rate, thereby directly affecting its production level[4,11]. Many studies from different countries and regions have been published, but not any from Eastern Europe.

Methods

This was a case-control study, performed at Life Memorial Hospital, Bucharest, between 2008 and 2011. Data collection procedures were the same for patients and control subjects. The study group comprised 69 women with 2 or more RPL of unknown etiology with the same partner. Exclusion criteria included anatomical abnormalities, previously known systemic disease, endocrine disorders, previous venous or arterial thrombosis or a family history of thromboembolism. Chromosomal abnormalities of the parents were ruled out (karyotype) before inclusion in the study. As infection was linked with RSA, all subjects included were confirmed to be negative for the TORCH agents *Toxoplasma gondii*, rubella, cytomegalovirus (CMV), *herpes simplex* viruses (HSV-1 and HSV-2), varicella zoster virus (VZV) and human immunodeficiency virus (HIV-1 and HIV-2). Pregnancy was confirmed by transvaginal ultrasound and serum level of human chorionic gonadotropin.

Transvaginal ultrasound was performed to confirm spontaneous abortion (no heartbeat detection). 69 women with at least two consecutive spontaneous abortion and 64 control women with at least one successful pregnancy were included in this study. Age distribution was comparable between RPL cases (mean age 29.6 ± 5.6 years) and normal fertile controls (mean age 31.4 ± 4.8 years) (Table 1). All cases and control subjects signed an informed consent before entering the study. Blood samples were obtained from study participants in EDTA tubes.

IL-10 gene polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes by the Promega Extraction Kit. Cytokine gene polymorphisms were determined by PCR- RFLP. For the -592C/A SNP, DNA was amplified using a common forward primer (5′ GCTCACTATAAAAATAGAGACGG-3′) and specific reverse primers for the C (5′-CTGGCTTCCTACAGG-3′) and the A (5′-GACTGGCTTCCTACAGT-3′) alleles. Similarly, for the -819C/T SNP, DNA was amplified using a common forward primer F 5'GACAACACTACTAAGGCTCCTTTGGGA 3' and reverse primer

R 5'GTG AGCAAACTGAGGCACAGAAAT 3'

Positive controls were selected by amplifying and sequencing two regions of the IL-10 promoter.

Statistical analysis was performed to determine odd ratio (OR) and 95% confidence intervals (95%CI) associated with recurrent pregnancy loss, using SPSS software (version 20.0).

Results

The distribution of IL-10-592C/A, -819C/T genotypes was in Hardy–Weinberg equilibrium among controls. The frequency of IL-10-592A (0.32 versus 0.25; P = 0.06) and C (0.67 versus 0.75; P = 0.50) alleles were similar between patients and controls (Table 2). In contrast, the frequency of the IL- 10-819 (mutant) T allele (0.32 versus 0.18; P = 0.02; OR = 2, 09), was higher among patients (Table

Table 1 Clinical characteristics of patients and controls

		Patients (69)	%	Control (64)	%
Age (years)	22-29	28	40,57%	27	42,18
	30-39	39	56,52%	33	51,56%
	>39	3	4,34%	4	6,25%
Number pregnancy loss	2	31	44,92%	0	
	>3	39	56,52%	0	
BMI	< 18,5	2	2,89%	1	1,56%
	18,5 – 24,9	56	81,15%	58	90,62%
	25 – 29,9	11	15,92%	4	6,25%
	> 30	0		1	1,56%

Table 2 IL-10 polymorphisms analysis

	Genotypes			Alleles (frequency)	
IL10 – 592C/A	CC	CA	AA	C	A
Patients	30(69)	33(69)	6(69)	0,673	0,326
Control	35 (64)	26 (64)	3(64)	0,750	0,250
p	0,22	0,48	0,49		
OR	0,637 (0,321- 1,264)	1,33(0,674- 2,66)	1,93 (0,463-8,0916)	0,688	1,477
IL10 – 819C/T	CC	CT	TT	C	T
Patients	28 (69)	37(69)	4 (69)	0,673	0,326
Control	43 (64)	18(64)	3 (64)	0,812	0,187
p	0,003	0,0046	1,00		
OR	0,33(0,16- 0,67)	2,95 (1,435- 6,080)	1,253(0,269- 5,820)	1.25	2.09

2). With the exception of the -819C/C genotype, which was lower among patients than controls (P = 0.003; OR = 0.33; 95% CI = 0.16–0, 67), and 819C/T genotype which was higher among patients than controls P = 0,004; OR = 2, 95; 95% CI = 1, 43- 6, 08) the frequencies of IL-10-592C/A genotypes and 819 TT genotype were comparable between patients and controls (Table 2).

The next step was to analyze the correlation between different combination of studied polymorphisms and increased risk for recurrent miscarriages (Table 3).

Frequency of genotype - 592 CC (homozygous for normal allele)/ - 819 CC (homozygous for normal allele) was higher in control group than experimental group (p = 0,005). In contrast, genotype - 592 AC (heterozygous) /- 819 CT (heterozygous) was more frequent in the experimental group (p= 0,05).

Discussion

There is clear evidence to suggest that the maternal immune system during pregnancy can enhance or inhibit the development of the feto-placental unit. Some cytokines produced by both T cell and non-T cell favor fetal survival and growth. Despite the complexity of the cytokines network, it appears that cytokines favoring the maintenance of fetal survival belong to the Th2 pathway.

Genotype and allele frequencies of cytokine polymorphisms show significant differences among different populations. This differences show that inheritance of certain

Table 3 Genotypic frequencies for polymorphisms - 592 C/A and - 819 C/T

Genotype	Patients(69)	Control (64)	p	OR (95% CI)
CCCC	10	23	0,005	0,30 (0,13- 0,70)
CCCT	20	10	0,095	2,20 (0,94- 5,16)
CACC	16	19	0,43	0,71(0,32-1,55)
CACT	15	6	0,05	2,68 (0,97- 7,42)
CATT	2	1	1	1,88 (0,16- 21,25)
AATT	2	0	0,49	-
CCTT	0	2	0,22	-
AACT	2	2	1,00	0,92 (0,12- 6,77)
AACC	2	1	1,00	1,88 (0,16- 21,25)

cytokine gene polymorphisms is strongly associated with ethnicity. Cytokines production is under genetic control and IL10 promoter polymorphisms were implicated in RSA pathogenesis[12,13].

In this study we investigated the association of the IL10 gene polymorphisms and RPL and established whether these cytokine gene polymorphisms are associated with RPL. Our results demonstrated a role for - 819 C/T but not for - 592 C/A and - 1082 A/G IL10 promoter polymorphisms in idiopathic RPL in studied group.

The lack of association of -1082A/G and - 592 C/A SNPs with RPL were in concordance with previous findings, which did not find any association between this SNP and RPL[12,13,14,15]. This was in disagreement with a study of Iranian women with 3 or more spontaneous abortions where the -592C/A, but not -819C/T, SNP was associated with RPL[13] and in apparent disagreement with other findings that failed to demonstrate any association of these SNPs with RPL[15,16]. Zammiti and all in 2006 demonstrated in a study based on patients and controls that the - 592 C/A and - 819 C/T polymorphisms were associated with RPL in Tunisian population[9]. Accordingly, these discrepancies may be reconciled by differences in ethnicities[13,15,16] and sample size.

It was suggested that Th1, but not Th2, cytokine gene polymorphisms were associated with RPL, an indication that heightened Th1 cytokines (IFN-γ and TNF-α) adversely affected pregnancy outcome. This was exemplified by the findings that the IFN-γ A874T[12,16], TNF-α -308G/A[15] polymorphisms were linked with RPL. In view of the differential effect of the IL-10 SNPs in regulating IL-10 mRNA expression and protein secretion[8,17,18], coupled with ethnic variation in the effect of IL-10 polymorphisms on IL-10 expression[18], this suggests an indirect role for IL-10 in the up-regulation of the expression of pro- inflammatory Th1 cytokines.

Insofar as its production varies as per the specific polymorphism[15,17], the role of IL-10 in RSA pathogenesis remains controversial. It was suggested that increased IL-10 expression was associated with successful pregnancy, whereas low levels were linked with recurrent fetal loss[19]. Others suggested the opposite, that enhanced IL-10 production was seen in RPL cases compared with fertile women[3,20]. Others claimed that IL-10, absent in the serum of healthy pregnant women, was detected during abortion and labor[21]. Although explanation for these discrepancies remains to be seen, the complexity of cytokine balance within the endometrium and decidua[22], coupled with the influence of maternal hormones, dictates the Th1 and Th2 cytokine balance during pregnancy.

Idiopathic RPL is a multifactorial condition with immune and non-immune causes. This study demonstrated an association between IL-10 -819C/T promoter polymorphism in idiopathic RPL among studied patients. Further study investigating IL-10 production as per genotype together with interaction with Th1 cytokines is required to characterize the involvement of IL-10 more precisely, further supporting the notion of Th1–Th2 cytokine imbalance in the pathogenesis of RPL.

Conclusions

This is the first study that has analyzed the association between IL 10 gene polymorphisms and recurrent pregnancy loss among Romanian women. Our results demonstrated a role for - 819 C/T IL10 promoter polymorphism in idiopathic RPL in studied group. ■

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