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Research Communication

Elevated Circulating IL-1β and TNF-Alpha, and Unaltered IL-6 in First-Trimester Pregnancies Complicated by Threatened Abortion With an Adverse Outcome

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The purpose of the present study was to examine the profile of selected proinflammatory cytokines in maternal serum of first-trimester pregnancies complicated by threatened abortion (TACP) and its relevance to obstetric outcome. Serum levels of Th1-type cytokines interleukin-1β (IL-1β), tumor necrosis factor alpha (TNF-alpha), and Th2-type cytokine interleukin 6 (IL-6) were measured, by ELISA, in 22 women with TACP and adverse outcome at admission (group A) and compared with the corresponding levels of 31 gestational age-matched women with TACP and successful outcome at admission (group B1) and discharge (group B2) and 22 gestational age-matched women with first-trimester uncomplicated pregnancy (group C) who served as controls. Mann-Whitney U or Wilcoxon test was applied as appropriate to compare differences between groups. IL-1β and TNF-alpha were detected with significantly higher levels in group A, compared to all other groups. On the contrary, IL-6 levels were detected with no significant difference among all the other groups studied. It is concluded that in first-trimester TACP with adverse outcome, a distinct immune response, as reflected by elevated maternal IL-1β, TNF-alpha, and unaltered IL-6 levels, is relevant to a negative obstetric outcome.

INTRODUCTION

Spontaneous abortion is the loss of an intrauterine pregnancy without outside intervention before 20 weeks' gestation and can be subdivided into threatened abortion, inevitable abortion, incomplete abortion, missed abortion, septic abortion, complete abortion, and recurrent spontaneous abortion [1]. Threatened abortion refers to an intrauterine viable clinical pregnancy accompanied by an intrauterine source of painless vaginal bleeding and successful or adverse pregnancy outcome [2].

The cytokine network has been suggested to be involved with positive or negative evolution of the ongoing pregnancies [3]. Prevalence of Th2-type cytokines (secreted by T-helper 2 cells and certain antigen-presenting cells (APCs)) may be associated with successful pregnancy; whereas the dominance of Th1-type cytokines (derived from T-helper 1 cells and APCs) may be indicative of a pathological pregnancy both in experimental animals and in humans [4, 5].

IL-1β is an essential proinflammatory, Th1-type cytokine, produced by monocytes, macrophages, and epithelial cells. Its secretion leads to production of tumor necrosis factor (TNF-alpha), interferon (IFN-γ), IL-2, and IL-12 [6], uterotonic prostaglandin E_2 and/or matrix metalloproteinases by fetal membrane cells, as well as to promotion of apoptotic cell death in fetal membrane tissues [7]. Due to these properties, IL-1β may express abortogenic action. On the other side, elevated IL-1β levels may increase the likelihood of successful and complete implantation, and, during the first trimester, may also offer the fetus increased protection against microbial pathogens that were present in the uterus before the conception period, during the conception period, or in the early postconception period [8].

TNF-alpha, a Th1-type cytokine, is mainly produced by mononuclear phagocytes, natural killer cells, and antigen-stimulated T-cells. Similarly to IL-1β, TNF-alpha promotes apoptotic cell death in fetal membrane tissues [7] and activates coagulation via upregulating the novel prothrombinase, fgl2 [9]. The proinflammatory, proapoptotic, and procoagulant properties of TNF-alpha probably contribute to the widely accepted abortogenic profile of this cytokine.

IL-6 is a multifunctional Th2-type cytokine produced by immune cells, fibroblasts, endothelial cells, adipocytes, and myocytes [10]. The role of IL-6 expression during pregnancy, as well as its predictive value for pregnancy outcome, is unclear, although it generally seems to favor pregnancy success [10]. IL-6 is considered to be a proinflammatory cytokine. Elevated levels of IL-6 in the placenta, amniotic cells, and deciduas have been demonstrated in pregnancies complicated by preterm premature rupture of the membranes, intrauterine infection, and prematurity [11]. However, IL-6 also has antiinflammatory properties. In this context, IL-6 has been shown to induce the release of human chorionic gonadotrophin from trophoblasts, leading to a subsequent cascade of progesterone production, release of Th2 cytokines, for example, IL-4, and suppression of Th1 cytokines [12].

To our knowledge, there are no reports on maternal serum IL-1β levels and a limited number of reports exploring the profile of TNF-alpha [13] and IL-6 [2] levels in first-trimester threatened abortion-complicated pregnancies (TACP), and, moreover, there are no reports evaluating the relevance of maternal serum levels of all these three cytokines to adverse obstetric outcome. By investigating the profile of IL-1β, TNF-alpha, and IL-6 levels in maternal serum of first-trimester TACP with adverse and successful outcome and of uncomplicated pregnancies, the present study intended to clarify whether an imbalance between selected Th1-type and Th2-type cytokines reflecting a Th1/Th2 ratio shift in first-trimester TACP has any relevance to the subsequent obstetric outcome.

MATERIAL AND METHODS

Study design

This is a prospective, nonrandomized, case-control clinical study conducted in a university-based maternity hospital and a university-based hormone and research laboratory.

Participants

Seventy-five nulliparous women in first-trimester pregnancy, who presented in the 2nd Academic Department of Obstetrics and Gynecology, Aretaieion Hospital, were enrolled in the study. The gestational age of all women, based on ultrasound measurements, ranged between the 7th and 10th weeks. Fifty-three of them were admitted with the indication of threatened abortion, as defined by the presence of vaginal bleeding [light (defined as spotting only) or heavy], closed cervical os, and viable fetus with positive heart activity detected by ultrasonographic examination. All these women were hospitalized, and their management included bed rest and pregnancy well-being evaluation with β-human chorionic gonadotropin evaluation and ultrasound assessment. Thirty-one out of 53 women with threatened abortion (group A) were discharged after resolution of vaginal bleeding and U/S confirmation of a viable fetus. Twenty-two (group B) out of 53 women with threatened abortion subsequently presented profound bleeding and expulsion of the embryos or clinical and ultrasonographic signs of nonviable pregnancy after 7–12 days from admission. The women of this group underwent evacuation of the products of conception under general anesthesia. The rest 22 out of 75 women of the same gestational age range were asymptomatic and recruited as controls (group C). Exclusion criteria were as follows: (1) subjects' age > 35 years, (2) previous history of subfertility, (3) previous abortion, (4) remarkable previous medical, surgical and gynecological history, (5) smoking or alcohol habits and medication intake. The study was approved by the Institutional Ethics Committee of Aretaieion Hospital, and informed consent was obtained from all subjects prior to participation.

Analytical methods

Venous blood samples were collected once (on admission) from women of groups B and C, and twice (on admission and discharge) from women of group A, into 10 mL sterile tubes without anticoagulant before administration of any medication. All sera were stored at -75°C until analysis.

Serum concentrations of IL-1β were assessed, in dublicate, by a high sensitivity ELISA kit (HSLB50, RnD Systems Europe, Ltd, Abingdon, UK). The range of measurement was 0.125–8 pg/mL, the intra- and interassay coefficients of variation were 10.2% and 19.2%, respectively, and the analytical sensitivity was 0.1 pg/mL. Serum concentrations of TNF-alpha were estimated, in dublicate, by a high sensitivity ELISA kit (HSTA00C, RnD Systems Europe, Ltd, Abingdon, UK). The range of measurement was 0.5–32 pg/mL, the inta- and inter-assay coefficients of variation were 8.8% and 12.6%, respectively, and the analytical sensitivity was 0.12 pg/mL. Serum concentrations of IL-6 were assessed, in dublicate, by a high sensitivity ELISA kit (HSLB50, RnD Systems Europe, Ltd, Abingdon, UK). The range of measurement was 0.156–10 pg/mL, the intra- and interassay coefficients of variation were 7.8% and 9.2%, respectively, and the analytical sensitivity was 0.04 pg/mL.

Distribution of IL-1β, IL-6, and TNF-alpha concentrations were tested for normality with the use of the Shapiro-Wilk test, and the hypothesis that they are normally distributed was rejected. Statistically significant differences of median values between groups were calculated using Mann-Whitney U test (unpaired differences) or Wilcoxon test (paired differences) and P-values for median values less than .05 were considered as significant. The results are expressed as median (range). The statistical package SPSS 8.0 (Statistical Package for the Social Sciences, SPSS Inc., Chicago) was used.

RESULTS

Median age was similar among all the studied groups: 30.4 (range 21–34) years in group A, 27.2 (range 22–34) years in group B, and 27.3 (range 22–34) years in group C, ($P = .34$). Similarly, median gestational age did not differ among all the studied groups: 8.5 (range 8–10) weeks in group A on admission, 8.7 (range 8.1–10) weeks in group B, and 8.2 (range 8–9.8) weeks in group C, ($P = .20$). The median gestational age of group A on discharge was 9.6 (range 8.5–10.5)

Table 1: Maternal serum levels of IL-1β, IL-6, and TNF-alpha in the study population [values are medians (range)].

	GROUP A		GROUP B	GROUP C
	On admission	On discharge		
IL-1β (pg/mL)	0.23(0.14–1.00)	0.25(0.14–0.99)	0.43(0.20–1.13)	0.20(0.14–1.16)
TNF-alpha (pg/mL)	2.93(1.40–24.30)	2.48(1.00–23.70)	7.81(4.34–26.30)	4.27(1.56–22.12)
IL-6 (pg/mL)	1.97(0.13–8.00)	1.76(0.27–8.00)	1.82(0.52–8.00)	2.51(0.24–9.60)

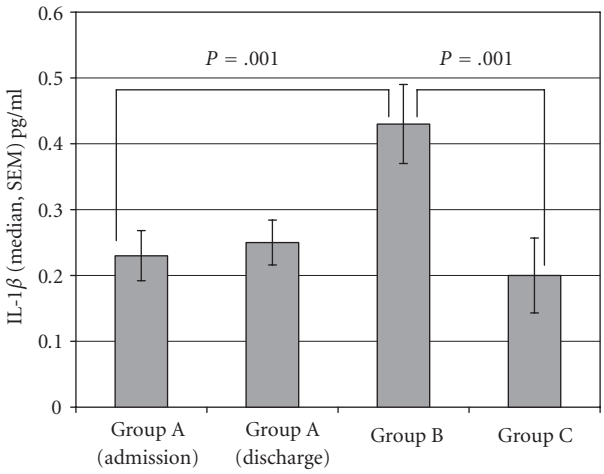

Figure 1: Serum levels (median, SEM) of IL-1β in the three study groups.

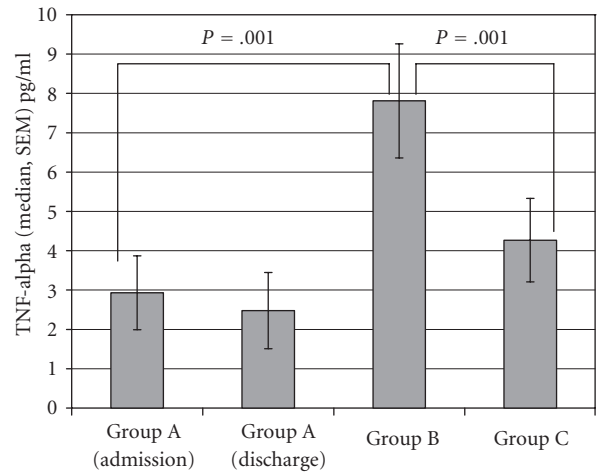

Figure 2: Serum levels (median, SEM) of TNF-alpha in the three study groups.

Table 1 shows the serum levels of IL-1β, IL-6, and TNF-alpha in Group A (on admission and on discharge) as well as in Groups B and C (on admission/presentation).

In Group A, IL-1β, IL-6, and TNF-alpha values on admission were comparable to those on discharge ($P = .9$, .12, and .06, resp) as well as to those found in group C (.55, .73, and .07, resp).

Admission serum values of IL-1β in Group B were significantly higher when compared to those in Group A ($P = .001$) as well as to those in group C ($P = .001$) (Figure 1).

Furthermore, admission serum values of TNF-alpha in Group B were significantly higher when compared to those in Group A ($P < .001$) as well as to those in Group C ($P < .001$) (Figure 2).

Finally, the IL-6 levels were comparable among the three groups (Group A versus Group B: $P = .55$, Group A versus Group C: $P = .73$, and Group B versus Group C: $P = .33$) (Figure 3).

DISCUSSION

The data of this study demonstrate that (a) maternal IL-1β and TNF-alpha levels are higher in first-trimester TCAP with adverse outcome than in first-trimester TCAP with successful outcome, both at admission and discharge, and first-trimester uncomplicated pregnancy; (b) maternal IL-6 levels in first-trimester TCAP with adverse outcome are comparable to those in first-trimester TCAP with successful outcome, both at admission and discharge, as well as those in first-trimester uncomplicated pregnancy; (c) maternal all IL-1β, TNF-alpha, and IL-6 levels are also similar in first-trimester TCAP with successful outcome, both at admission and discharge, and in first-trimester uncomplicated pregnancy.

Cytokines, as critical immunoregulatory molecules, responsible for determining the nature of an immune response, have been shown to influence all steps of reproduction, playing a fundamental role in pregnancy outcome.

As a Th1-type proinflammatory cytokine, IL-1β may influence Th1/Th2 immune responsiveness, thus implicating in the establishment of successful pregnancy. Although known to alter IL-1β expression, IL-1β gene functional polymorphisms are not associated to recurrent miscarriage [14]. Similar serum levels of IL-1β have been reported in a large Caucasian population of women with a history of three or more consecutive pregnancy losses before 20 weeks' gestation in comparison to those of healthy women with at least two live births and no history of pregnancy loss [15]. Elevated IL-1β serum levels were reported in first-trimester pregnant women who presented with inevitable abortion and a history of at least 3 prior spontaneous consecutive abortions, this in comparison to first-trimester pregnant women presenting with their first miscarriage due to chromosomal anomalies [16]. IL-1β serum levels in first-trimester TACP have never been evaluated. In the present study, IL-1β levels in maternal serum of first trimester threatened aborters who eventually miscarried were significantly higher than those of women

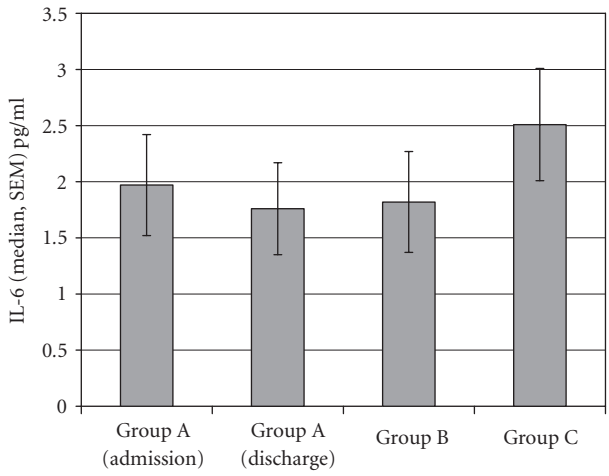

Figure 3: Serum levels (median, SEM) of IL-6 in the three study groups.

with first-trimester uncomplicated pregnancy. Thus, elevated IL-1β levels in first-trimester TACP should not be considered as a compensatory or protective physiological response, but, instead, may probably indicate cytokine-induced detrimental effects on pregnancy continuation. Moreover, maternal IL-1β serum levels in first-trimester TACP with poor outcome were significantly higher than those in first-trimester TACP with good outcome, both at admission and discharge.

TNF-alpha is a proinflammatory, proapoptotic, and procoagulant and, thus, abortogenic, mainly Th1-type, cytokine. Its production is also partly under genetic control. Contradictory data report that TNF-alpha functional gene polymorphisms are not correlated with recurrent spontaneous abortion (RSA) in Caucasians [17] show a trend to be associated with RSA [18], or associated with an increased risk for RSA [19]. In pregnant women at < 20 weeks of gestational age and with a history of RSA, several reports agree that serum TNF-alpha levels are elevated in comparison to those of healthy pregnant women at the same gestational age [20, 21]. More importantly, two studies report increased or unaltered TNF-alpha production in peripheral blood of nonpregnant women with a history of RSA, when blood was cultivated in the presence of phytohemagglutinine (PHA) [21] or trophoblast cells [4]. Contradictory data report decreased [22] or increased [23] TNF-alpha levels in or at onset of spontaneous abortion, respectively, in comparison to normal pregnancies. TNF-alpha levels similar to those in normal pregnancy were reported in threatened abortion with a good outcome [13]. There are no studies evaluating TNF-alpha levels in first-trimester TACP with an adverse outcome. In the present study, the elevated maternal TNF-alpha levels in first-trimester TACP with a poor outcome, in comparison to those of uncomplicated pregnancy and TACP with a good outcome, further support the abortogenic role of this cytokine. Moreover, resembling maternal IL-1β levels, maternal TNF-alpha levels were higher in first-trimester TACP with poor outcome than in first-trimester TACP with good outcome, both at admission and discharge. This result, together with the corresponding result noticed in IL-1β levels, further supports the concept that first-trimester TACP with adverse outcome may be an immunologically different entity, at least compared to the first-trimester TACP with good outcome. Furthermore, all these combined results indicate the relevance of the elevated Th1-type cytokines IL-1β and TNF-alpha to an adverse obstetric outcome in TACP.

IL-6 is a Th2-type, pro- as well as antiinflammatory cytokine. Because of its properties, IL-6 has been extensively proposed to counterbalance detrimental effects of Th1-type cytokines [2]. IL-6 production is also partially determined by cytokine genotyping. Contradictory data report different functional gene polymorphisms of IL-6 to be well [24] or not [17] associated with the pathogenesis of RSA or even to decrease the risk for RSA [25]. No significant differences in circulating IL-6 levels were reported for pregnant women at < 20 weeks of gestation with RSA, in comparison to nonpregnant women with a past history of habitual abortion as well as healthy nonpregnant women [20]. Moreover, lipopolysaccharide-stimulated IL-6 production in peripheral blood of nonpregnant women with RSA and, at least, three consecutive spontaneous abortions was similar to that of women with a history of successful pregnancies and no miscarriage [26]. Recently, unaltered maternal serum IL-6 levels are reported in women with threatened abortion compared to those of normal pregnant and nonpregnant women; whereas significantly lower values were observed in women with missed abortion [2]. In the present study, maternal serum IL-6 levels in first-trimester TACP with adverse outcome were unaltered in comparison to those of first-trimester healthy pregnant women. This result grossly agrees with that previously reported data [2]; however, for the first time, it distinguishes between positive and negative obstetric outcome of first-trimester TACP. Similarly, maternal serum IL-6 levels in first-trimester TACP with adverse outcome were found to be comparable to those of first-trimester TACP with good outcome, both at admission and discharge. These results together suggest that although there is not any Th2 deficiency in first-trimester TACP, however, an increased Th1-type immune response cannot be counterbalanced by the unaltered Th2-type immune response, thus resulting in negative obstetric outcome. Further more, this result emphasizes that, in first-trimester TACP with adverse outcome, the shift in Th1/Th2 ratio is attributed only to the increase of Th1-type immune response and not to the simultaneous decrease of Th2-type immune response, as it has been proposed for other types of spontaneous abortion [2]. In this context, this shift represents a distinct Th1/Th2 ratio pattern strengthening the concept of the distinct immune response profile in first-trimester TACP.

In the present study, no differences were noticed in IL-1β, TNF-alpha, and IL-6 levels of first-trimester TACP with good outcome between admission and discharge. This result, in accordance with other previously published data for IL-6 levels [2], suggest that among threatened aborters matched for the severity of vaginal bleeding, subsequent obstetric outcome is determined by the type of immune response rather than by

remission of symptoms (vaginal bleeding). Finally, in accordance with previously reported data, at least for TNF-alpha [13], no differences were noticed in all maternal serum IL-1β, TNF-alpha, and IL-6 levels between first-trimester TACP with good outcome, both at admission and discharge, and first-trimester uncomplicated pregnancy. This result further emphasizes that in first-trimester TACP with good outcome, the Th1/Th2 ratio, as reflected by the selected cytokines, resembles this one of healthy pregnancy. Thus, first-trimester TACP with good outcome is immunologically similar to a first-trimester uncomplicated pregnancy.

A shortcoming of this study might be that control groups with nonpregnant women with a past history of threatened abortion and adverse or successful outcome and healthy nonpregnant women were not included. Although this does not alter the main finding of the study, comparisons with these groups could add information to the emerging concept of a distinguished immune response in first-trimester TACP with an adverse outcome. In addition, functional polymorphisms of TNF-alpha and IL-6 genes as well as fetal chromosomal karyotype of abortive tissue [27], both proposed to partly influence respective cytokine levels were not evaluated in this study. However, it was impossible to evaluate all these genetic profile parameters in the limits of a single study.

In conclusion, first-trimester TACP with positive obstetric outcome is immunologically similar to a first-trimester uncomplicated pregnancy. On the contrary, a first-trimester TACP with adverse outcome represents an immunologically distinct entity of spontaneous abortion. This distinct immune response, characterized by elevated maternal IL-1β and TNF-alpha and unaltered IL-6 levels, when compared to first-trimester uncomplicated pregnancy and TACP with a good outcome, results in a shift of Th1/Th2 ratio that is relevant to an adverse obstetric outcome. Further prospective studies including a large number of first-trimester threatened aborters with good and bad obstetric outcome and an extensive multivariable logistic regression analysis are required to clarify if this distinct type of immune response can be also rationalized as a negative predictor of the obstetric outcome.

REFERENCES

[1] Griebel CP, Halvorsen J, Golemon TB, Day AA. Management of spontaneous abortion. *American Family Physician.* 2005;72(7):1243–1250.

[2] Paradisi R, Maldini-Casadei M, Boni P, Busacchi P, Porcu E, Venturoli S. T-helper 2-cytokine levels in women with threatened abortion. *European Journal of Obstetrics Gynecology and Reproductive Biology.* 2003;111(1):43–49.

[3] Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunology Today.* 1993;14(7):353–356.

[4] Hill JA, Polgar K, Andersomn DJ. T-helper 1-type immunity to trophoblast in women with recurrent spontaneous abortion. *Journal of the American Medical Association.* 1995;273(24):1933–1936.

[5] Raghupathy R, Makhseed M, Azizieh F, Hassan N, Al-Azemi M, Al-Shamali E. Maternal Th1- and Th2-type reactivity to placental antigens in normal human pregnancy and unexplained recurrent spontaneous abortions. *Cellular Immunology.* 1999;196(2):122–130.

[6] Linjawi S, Tin-Chiu L, Laird S, Blakemore A. Interleukin-1 receptor antagonist and interleukin-1 beta polymorphisms in women with recurrent miscarriage. *Fertility and Sterility.* 2005;83(5):1549–1552.

[7] Fortunato SJ, Menon R. IL-1 β is a better inducer of apoptosis in human fetal membranes than IL-6. *Placenta.* 2003;24(10):922–928.

[8] Perni SC, Vardhana S, Tuttle SL, Kalish RB, Chasen ST, Witkin SS. Fetal interleukin-1 receptor antagonist gene polymorphism, intra-amniotic interleukin-1beta levels, and history of spontaneous abortion. *American Journal of Obstetrics and Gynecology.* 2004;191(4):1318–1323.

[9] Knackstedt MK, Zenclussen AC, Hertwig K, et al. Th1 cytokines and the prothrombinase fgl2 in stress-triggered and inflammatory abortion. *American Journal of Reproductive Immunology.* 2003;49(4):210–220.

[10] Unfried G, Bocskor S, Endler G, Nagele F, Huber JC, Tempfer CB. A polymorphism of the interleukin-6 gene promoter and idiopathic recurrent miscarriage. *Human Reproduction.* 2003;18(2):267–270.

[11] Fukuda H, Masuzaki H, Ishimaru T. Interleukin-6 and interleukin-1 receptor antagonist in amniotic fluid and cord blood in patients with pre-term, premature rupture of the membranes. *International Journal of Gynaecology and Obstetrics.* 2002;77(2):123–129.

[12] Hill JA. Cytokines considered critical in pregnancy. *American Journal of Reproductive Immunology.* 1992;28(3-4):123–126.

[13] Gucer F, Balkanli-Kaplan P, Yuksel M, Sayin NC, Yuce MA, Yardim T. Maternal serum levels of tumor necrosis factor-alpha and interleukin-2 receptor in threatened abortion: a comparison with normal and pathologic pregnancies. *Fertility and Sterility.* 2001;76(4):707–711.

[14] Hefler LA, Tempfer CB, Unfried G, et al. A polymorphism of the interleukin-1beta gene and idiopathic recurrent miscarriage. *Fertility and Sterility.* 2001;76(2):377–379.

[15] Linjawi S, Li TC, Laird S, Blakemore A. Interleukin-1 receptor antagonist and interleukin-1 beta polymorphisms in women with recurrent miscarriage. *Fertility and Sterility.* 2005;83(5):1549–1552.

[16] Shaarawy M, Nagui AR. Enhanced expression of cytokines may play a fundamental role in the mechanisms of immunologically mediated recurrent spontaneous abortion. *Acta Obstetricia et Gynecologica Scandinavica.* 1997;76(3):205–211.

[17] Prigoshin N, Tambutti M, Larriba J, Gogorza S, Testa R. Cytokine gene polymorphisms in recurrent pregnancy loss of unknown cause. *American Journal of Reproductive Immunology.* 2004;52(1):36–41.

[18] Daher S, Shulzhenko N, Morgun A, et al. Associations between cytokine gene polymorphisms and recurrent pregnancy loss. *Journal of Reproductive Immunology.* 2003;58(1):69–77.

[19] Reid JG, Simpson NA, Walker RG, et al. The carriage of pro-inflammatory cytokine gene polymorphisms in recurrent pregnancy loss. *American Journal of Reproductive Immunology.* 2001;45(1):35–40.

[20] Arslan E, Colakoglu M, Celik C, et al. Serum TNF-alpha, IL-6, lupus anticoagulant and anticardiolipin antibody in women with and without a past history of recurrent miscarriage. *Archives of Gynecology and Obstetrics.* 2004;270(4):227–229.

[21] Rezaei A, Dabbagh A. T-helper (1) cytokines increase during early pregnancy in women with a history of recurrent spontaneous abortion. *Medical Science Monitor.* 2002;8(8):CR607–CR610.

[22] Matalliotakis I, Sifakis S, Koumantaki Y, et al. Serum soluble CD23 and TNF-alpha in women with spontaneous abortion in the first trimester. *Clinical and Experimental Obstetrics & Gyncology.* 1999;26(2):118–119.
[23] Daher S, Fonseca F, Ribeiro OG, Musatti CC, Gerbase-DeLima M. Tumor necrosis factor during pregnancy and at the onset of labor and spontaneous abortion. *European Journal of Obstetrics, Gynecology, and Reproductive Biology.* 1999;83(1):77–79.
[24] Von Linsingen R, Bompeixe EP, Bicalho Mda G. A case-control study in IL6 and TGFB1 gene polymorphisms and recurrent spontaneous abortion in southern Brazilian patients. *American Journal of Reproductive Immunology.* 2005;53(2):94–99.
[25] Saijo Y, Sata F, Yamada H, Kondo T, Kato EH, Kishi R. Single nucleotide polymorphisms in the promoter region of the interleukin-6 gene and the risk of recurrent pregnancy loss in Japanese women. *Fertility and Sterility.* 2004;81(2):374–378.
[26] Daher S, de Arruda Geraldes Denardi K, Blotta MH, et al. Cytokines in recurrent pregnancy loss. *Journal of Reproductive Immunology.* 2004;62(1-2):151–157.
[27] Yamada H, Morikawa M, Futura I, et al. Circulating cytokines during early pregnancy in women with recurrent spontaneous abortion: decreased TNF-alpha levels in abortion with normal chromosome karyotype. *Hokkaido Igaku Zasshi.* 2004;79(3):237–241.