

Detection of IL-10, IFN- γ and IL-8 in sera of patients with recurrent spontaneous abortion

Nidhal Abdul Mohymen¹PhD, Amal Hussain² PhD.

Abstract

Background: Th1-type cytokines secretion such as IFN- γ , and Th2 cytokines such as IL-10, have been shown to exert deleterious effects on pregnancy, inhibiting fetal growth and development

Objective: Estimation of Interleukin-10 (IL-10), IL-8 and IFN- γ levels in sera of patients with recurrent spontaneous abortion (RSA) using ELISA method.

Method: A total of one hundred and nineteen women, ranged from the mean age (23.9 – 28.5)years, were enrolled in the current study and were further classified into three categories: Group A- Recurrent spontaneous abortion (RSA): n= 62 women, with a mean age of (28.5 + 0.68);Group B- non- recurrent spontaneous abortion (non-RSA): n= 34 women, with a mean age of (26.4 \pm 0.85)and group C- Control (successful pregnancy): n= 23 women, with a mean age of (23.9 \pm 0.88). From each patient and control blood sample was collected and serum was separated. Estimation of Interleukin-10 (IL-10), IL-8 IFN- γ levels in sera of patients was done using ELISA method.

Result: the current study failed to demonstrate a significant difference in circulating levels of IL-8 between RSA and control group ($p > 0.05$) and no significant different between non-RSA and control ($p > 0.05$) . IFN- γ expression is significantly increased ($p < 0.001$) in women

with RSA and non-RSA compared with successful pregnancy. Defective IL-10 expression in women with RSA and non-RSA .The ratio of IFN- γ : IL-10 was found to be highly significant ($p < 0.001$) in aborted women. IL-8 was expressed in high levels in aborted women (RSA and non-RSA) and those with successful pregnancy, but no significant difference ($p > 0.05$) was found when compared between successful pregnancy and RSA or non-RSA, whereas highly significant difference ($p < 0.001$) was found between RSA and non-RSA.

Conclusions: IFN- γ expression is highly significant increased ($p < 0.001$) in women with RSA and non-RSA compared with successful pregnancy, indicating that Th1 cytokines might well be implicated in adversely affecting pregnancy. And defective IL-10 expression in women with RSA and non-RSA might be documentary to the previous studies on the possible defect in Th2 cytokines production in these patients.

Key words: Recurrent spontaneous abortion Interleukin-10 (IL-10), IL-, IFN- γ , and ELISA.

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Introduction

Recurrent spontaneous abortion is one of the important complications in pregnancy, its incidence is 0.5–1%, and the etiology of RSA is varied, and includes maternal or paternal

chromosomal aberrations, uterine anatomical abnormalities, endocrine disorders, infections, and reproductive autoimmune defects. However, the etiology is undetermined in 40–60% of women with recurrent abortion^(1,2).

Successful human pregnancy appears to be an immunological paradox, in that the fetus represents a semi-allograft developing in the potentially hostile environment of the maternal immune system^(3, 4). One important mechanism involves the down-regulation of the cellular immune response, which has been

¹Dept. Medical Microbiology. College of Medicine, Al-Nahrain University.²Dept. Medical Microbiology. College of Medicine, Al- Al- Mustansiriya University.

Address Correspondences to: Dr. Nidhal Abdul Mohymen

Email: doctornidhal@yahoo.com

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shown to be dependent upon the suppression of T-helper (Th) 1 and T-cytotoxic (Tc)1 cells, which produce interleukin (IL)-2, interferon (IFN)- γ , and tumor necrosis factor (TNF)- β , and the up-regulation of Th2 and Tc2 cells, which produce IL-4, IL-6, IL-10 and IL-13⁽⁵⁻⁸⁾.

Previous investigations of Th1/Th2 immune responses during pregnancy were able to show that a distinct shift towards Th2-type reactions occurs, especially at the foeto-maternal interface⁽⁹⁻¹²⁾.

On the other hand, Th1-type cytokine secretion such as IFN- γ and much of the work on spontaneous abortions in humans has focused on the analyses of maternal responses and local changes that occur following abortion. Evidence from studies on murine and human pregnancy points to a strong association between maternal Th2-type (IL-4, IL-6, IL-10) immunity and successful pregnancy on the one hand and between Th1-type (IL-2 and IFN- γ) immune reactivity and pregnancy loss on the other⁽¹³⁾. Moreover, during pregnancy, IL-8 is a CXC chemokine that is produced by a variety of cells, mainly monocytes/macrophages⁽¹⁴⁾. Interleukin (IL-8) has inflammatory and growth-regulating properties^(15,16) but is notable for its selective chemotaxis, degranulation, and activation of neutrophils⁽¹⁷⁾. IL-8 induced activation of neutrophils and elastase activity in the intrauterine environment has been implicated in the mechanisms of rupture of fetal membrane⁽¹⁸⁾, and cervical ripening^(19,20). **Hence in this study we intend to determine the concentrations of IFN- γ , IL-10 and IL-8 in circulation of patients using Enzyme Linked Immuno Sorbent Assay (ELISA) technique.**

Materials and methods

One hundred and nineteen women attending the Obstetrics and

Gynecology department of Al-Kadhimiya Teaching Hospital in Baghdad between December 2004 and August 2005 were the subjects of this study. Included recurrent spontaneous abortion (RSA); non-RSA (first and second abortion) and successful pregnancy (full term) as a control groups.

The gestational age was calculated for each patient from data of the last menstrual period.

These one hundred and nineteen women were grouped into three groups:

Group A: the study group included 62 pregnant ladies all of whom gave a history of previous 3-6 consecutive abortions. History was taken from the patients taking into consideration their hospital records in addition to their previous medical reports (all of them had no family history of genetic disease).

Group B: included 34 pregnant ladies with incomplete abortion for the first time or second time.

Group C: included 23 pregnant ladies had at least two previous normal pregnancies taken as comparison group. All this was done under the supervision of a senior gynecologist

Sample collection: Five ml of venous blood was collected from each patient and control group. The blood was placed in a plain tube and left to stand for one hour at room temperature for clot formation. The tube was centrifuged for 10 minutes at 4 °C at 450 x g for serum collection. The serum was then aspirated by using a Pasteur pipette and dispensed into sterile glass tubes (1 ml in each) and stored at -20 °C until used. The repetitive freezing and thawing of serum sample was avoided.

Enzyme Linked Immuno Sorbent Assay (ELISA) for the detection of IL-10, IFN- γ and IL-8 in serum:

ELISA was used for the estimation of Interleukin-10 (IL-10), IL-8 and IFN- γ level in the sera. This ELISA is a two immunological step sandwich type assay. In the first step the cytokine is captured by a monoclonal antibody bound to the wells of amicrotiter plate. In the second step a monoclonal antibody linked to abiotinylated monoclonal antibody is added together with streptavidine.

Monoclonal antibody to IL-10 (mAb9D7, Biotinylated monoclonal antibody 12G8.), IFN- γ (monoclonal antibody 1-D1K and Biotinylated monoclonal antibody 7-B6-1) and IL-8 (monoclonal antibody IM2237) were used in this study; the procedure was

according to Cell Com (cellular communication investigations) kit. France.

Statistical Analysis

The ANOVA analysis program was used to calculate the values, Mean, Median, Standard deviation and standard error were all used in the analysis.

The chi-square used for the qualitative data.

Results

As shown in table 1 a significant correlation between gestational age and IFN- γ (in circulation detected by ELISA) in group A women. There were no significant correlation among the other combination between gestational age and cytokines.

Table 1: Correlation between gestational age and cytokine tested in this study in sera of group A.

^aG.A= gestational age

Variables	Correlation Coefficient r =	P value
^a G.A – IL-8	0.027	>0.05
G.A – IL-10	0.297	>0.05
G.A – IFN- γ	0.228	<0.05

Based on ANOVA test analysis (Table 2) shows, the mean value of serum levels of these cytokines .The results revealed that there was a highly significantly difference ($p<0.001$) in the mean percentage of IL-8; IFN- γ and IL-10 between group A and group B (865.7 \pm 81.3 versus 190.5 \pm 19.5 ; 1850.3 \pm 311.4 versus186.3 \pm 14.7 and 9.4 \pm 1.4 versus 70.1 \pm 5.1, respectively) . But the difference was not significant ($p>0.05$) when we compared the mean

value of IL-8 between group A and B with group C; and it was found highly significant difference ($p<0.001$) in the mean value of IL-10 in sera of women in group C (553 \pm 58.9) compared with that of group A (9.4 \pm 1.4), and highly significant difference between group C and group B (70.1 \pm 5.1). In addition, highly significant difference ($p<0.001$) was found in the mean value of IFN- γ in sera of women in group A and group C. as shown in (Figure 1).

Table 2: Comparison between the mean values of concentration (pg/ml) of IL-8, IL-10 and IFN- γ (ELISA assay) in sera of studied groups.

Variable	Group	Mean \pm SE	F test <i>p</i> value	Sig. between groups	
				groups	<i>P</i> value
IL-8	A	865.7 \pm 81.3	<0.01	A – B	0.000**
	B	190.5 \pm 19.5		A – C	0.996
	C	820 \pm 202.8		B – C	0.067 ^a
IL-10	A	9.4 \pm 1.4	<0.01	A – B	0.000**
	B	70.1 \pm 5.1		A – C	0.000**
	C	553 \pm 58.9		B– C	0.000**
IFN- γ	A	1850.3 \pm 311.4	<0.01	A – B	0.000**
	B	186.3 \pm 14.7		A – C	0.000**
	C	7.6 \pm 0.4		B – C	0.000**

**= highly significant difference ($p < 0.01$); ^a= marginally significant difference

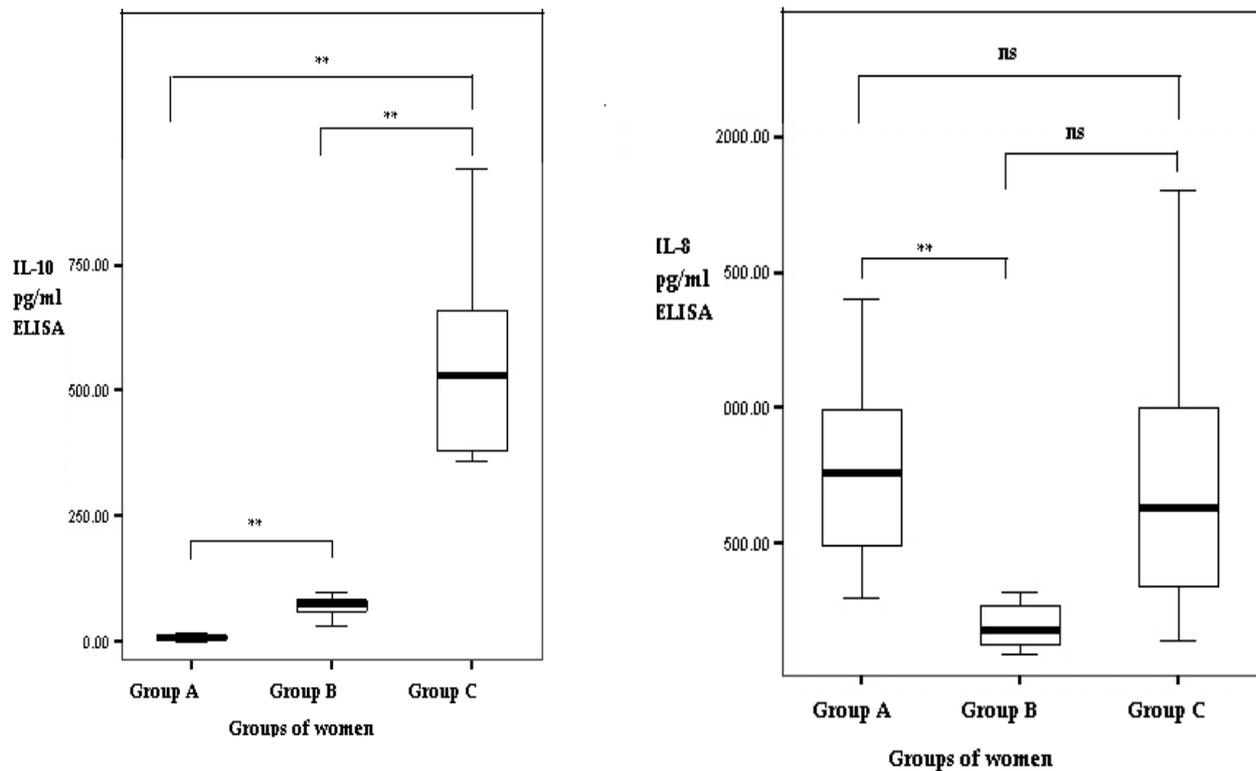


Figure 1: Concentration of IL-8, IL-10 and IFN- γ in sera of investigated women. (ns=not significant $p > 0.05$; **=highly significant $p < 0.001$).

Based on ANOVA test analysis, the results showed a marginally significantly different ($0.05 < p < 0.1$) when we compared between group A

and group C and between group A and B, and a highly significant difference ($p < 0.001$) between group B and C, as shown in (Table3).

Table 3: Comparison between IFN γ /IL-10 ratio of the three groups (ANOVA test analysis).

Variable	Group	Mean \pm SE	F test P value	Sig. between Groups	
				groups	P value
IFN γ /IL-10 (ELISA)	A	630.8 \pm 263.8	<0.05	A–B	0.068 ^a
	B	2.9 \pm 0.3		A –C	0.066 ^a
	C	0.02 \pm 0.002		B –C	0.000 ^{**}

^a =marginally significance (0.05<*p*<0.1);^{**} =highly significance difference (*p*<0.001)

IFN- γ and IL-8 expression, showed a significant correlation (*p*<0.05) between them in group A and group B by using ELISA technique, whereas the result revealed that there was no significant

correlation (*p*>0.05) between IFN- γ and IL-8 expression in control (group C), (Table 4) .

Table 4: Relation between the mean percent of IFN- γ and IL-8 in serum in group A, B and C.

IFN- γ – IL-8		Correlation Coefficient r =	P value
groupA	ELISA–ELISA	0.279	<0.05
groupB	ELISA–ELISA	0.501	<0.05
groupC	ELISA–ELISA	-0.629	>0.05

Discussion

The current study, showed a highly significant difference in expression of IL-10 (systemic) (*p*<0.001) between first; second trimester abortion and control groups (successful pregnancy).In addition, no significant difference in expression of IL-10 (systemic) (*p*>0.05) between first trimester and second trimester abortion. These results agreed with other studies that found higher concentrations of IL-10 at delivery than during any other stage of gestation tested, though the reasons for this and its significance were not readily understood⁽²¹⁾. Moreover, this study, showed that the expression of

IL-10 proteins in circulation of women with successful pregnancy (group C) was significantly higher (*p*<0.001) than that of women with RSA (group A) and higher than that in women with non-RSA (group B).this result indicated the systemic immune response might be associated with local cytokine milieu at the fetomaternal interface. This significantly higher level of IL-10 with successful pregnancy in this study, could be explained by previous study that showed that IL-10 production was significantly lower in patients with recurrent miscarriage as compared with normal pregnancy or spontaneous

abortion cases⁽²¹⁾. IL-10 plays a positive role in the prevention of spontaneous pregnancy failure in a mouse model; the injection of IL-10 into abortion-prone mice resulted in the prevention of fetal wastage⁽²²⁾. Results of previous studies⁽²³⁻²⁵⁾ showed that, IL-10 was produced at higher concentrations by PBMC of women with normal pregnancy than those with a history of unexplained RSA. Thus, IL-10 has emerged as an important Th2-type cytokine in the maintenance of normal pregnancy⁽²⁶⁾. Since it is directly involved in down-regulating Th1-type activity by inhibiting IFN- γ production, IL-10 has been proposed to play an important immunoregulatory role in pregnancy by maintaining a bias away from the detrimental Th1-type of reactivity^(22, 27). Other study found a lack of cytokine shift in aborted women (RSA or non-RSA) as compared with normal pregnant women at the same time of gestational age⁽²⁸⁾.

There are many confounding studies held the notion on the balance of Th1 and Th2 cells at the circulation and implantation site, expressing them as a ratio of Th1/Th2 cytokines, so that, another dimension was added to the results of this study when it examined the ratio of IFN- γ /IL-10 expression in women with RSA which was significantly higher ($p < 0.001$) than that of successful pregnancy (group C). This significantly high IFN- γ /IL-10 ratio lends further support to the findings in this study as it was in consistence with the previous studies^(23, 21, 29, 30).

Hanna and colleagues (2000)⁽³¹⁾ examined the expression of IL-10 and its receptor in placental explants or freshly isolated cytotrophoblasts from different gestational ages and compared it with the expression profiles of other cytokines. First and second trimester placental tissues from

normal pregnancies predominantly expressed IL-10, whereas the levels of IL-2, IL-4, and IFN- γ were mostly below detection throughout pregnancy.

In the current study, results showed a highly significant difference in expression of IFN- γ (systemic) ($p < 0.001$) between first; second trimester abortion compared with control groups (successful pregnancy). In addition, no significant difference in expression of IFN- γ ($p > 0.05$) between first trimester abortion and second trimester abortion. This result might be explained that IFN- γ associated with pregnancy loss. Other studies showed that the expression of IFN- γ will increase with progress of pregnancy till late first trimester⁽³²⁾. Furthermore, this study, showed that the expression of IFN- γ proteins in circulation of women with RSA was significantly higher ($p < 0.001$) than that of successful pregnancy (group C) and higher than that in women with non-RSA (group B). This results was in agreement with other study that mentioned the elevated maternal serum levels of interleukin-2 soluble receptor-(IL-2 sR), tumour necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) have been associated with pregnancy loss⁽³³⁾.

Results showed no significant difference in expression of IL-8 (systemic) ($p > 0.05$) between first; second trimester abortion and control groups (successful pregnancy). In addition, no significant difference in expression of IL-8 (systemic) ($p > 0.05$) between first trimester abortion and second trimester abortion. This finding supports the proposal that IL-8 may play a maturational role during pregnancy and/or facilitates the process of labor^(34,35) or it might be due to the role of Interleukin-8 (IL-8), that has inflammatory and growth-regulating properties during pregnancy^(15,16). Much of the work on

spontaneous abortions in humans has focused on the analyses of maternal responses and local changes that occur following abortion. Evidence from studies on murine and human pregnancy points to a strong association between maternal Th2-type (IL-4, IL-6,IL-10) immunity and successful pregnancy on the one hand and between Th1-type (IL-2 and IFN- γ) immune reactivity and pregnancy loss on the other^(13,20,36).

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