

Detection of interleukin-6 and interleukin-8 in serum from women with recurrent spontaneous abortion

*Rana S. Aboud**

Received 19, February, 2009

Acceptance 3, June, 2009

Abstract:

To investigate the role of IL-6 and IL-8 in the immune-regulatory mechanisms involved in the recurrent spontaneous abortion of the first trimester of pregnancy. Serum level of IL-6 and IL-8 were determined in 25 women of age (20-35) years who had a spontaneous abortion of unknown aetiology during the first trimester of pregnancy. They were compared with the corresponding levels of 20 pregnant and non-pregnant women as control groups. Cytokine levels were measured by (ELISA) technique. The women with spontaneous abortion had highly significant ($P < 0.01$) increased serum level of IL-8 and highly significant ($P < 0.01$) decreased level of IL-6 compared to those with normal pregnant and non-pregnant women. The results of this study may be related to the underlying aetiopathogenetic mechanisms, however, there is sufficient evidence for their use as predictive markers of pregnancy outcome.

Key words: cytokines, interleukin -6, interleukin-8, recurrent spontaneous abortion

Introduction:

Cellular immune effector mechanisms have been proposed as being responsible for at least a proportion of "unexplained recurrent spontaneous abortion" (RSA). Unexplained RSA accounts for about 40-60% of all cases of RSA [1]. Recent attention has focused on elucidating the immunobiological roles of cytokines in normal human pregnancy following the accumulated reports of complex cytokine activity within uteroplacental tissue [2]. Cytokines are important mediators in the bi-directional interaction between the maternal immune system and the reproductive system during pregnancy [3]. Interleukin-6 (IL-6) and interleukin-8 (IL-8) are pro-inflammatory cytokines produced by several tissues upon the stimulus of a number of factors, among which are

membrane LPS from gram negative bacteria, viruses and several cytokines. Their action is directed towards either myeloid or non-myeloid cellular targets [4]. IL-6 produced by murine fetoplacental tissues and this cytokine comprise the profile of cytokines produced by the T-helper (Th-2) subset of T-helper cells [3]. During pregnancy these cytokines are involved in different ways in the regulation of the mechanisms of implantation and placentation, fetal maturation and uterine contraction [5,6]. These cytokines help to maintain the trophoblast in early pregnancy. They also play a major role in intrauterine infection, especially after premature rupture of membranes and in preterm and term labor irrespective of infection [7,8]. Successful pregnancy may depend, at least in part

*University Of Baghdad, College Of Sciences, Department Of Biology

, on the bias of the maternal immune response shifting away from Th-1 type response towards a Th-2 phenotype , both in murine model and human [9]. Normal pregnancy was accompanied by a decrease in Th-1 productive capacity together with an increase in Th-2 production , most notably in the third trimester , The cause of recurrent pregnancy loss (Three or more consecutive spontaneous miscarriages) are unexplained in the majority of women and it is thought that abnormalities in the immune system may have a role in idiopathic recurrent abortion [10].

The aim of this study was determine the role of IL-6 and IL-8 in the immuno-regulatory pathways involved in recurrent spontaneous abortion in women with first trimester of pregnancy .

Materials and methods:

The study include twenty five(25) women who had recurrent spontaneous abortion in first trimester of pregnancy of age 20-35 years and 20 apparently healthy control groups (10 pregnant women and 10 non-pregnant women) . The level of IL-6 and IL-8 was measured in serum samples (patient

and control groups) by using Enzyme linked immunosorbent assay (ELISA) technique , This was performed as described in the leflet of kit .

Statistical analysis:

Comparison of paired data from the three groups of subjects was done using T-test (t) and least significant difference (LSD) between groups analyzed using pearson correlation coefficient (r) formula . Statisticals tables including observed frequencies with their percentage .SPSS and Microsoft excel programmes were used for T-test and correlation coefficient calculations respectively .[11]

Results and discussion:

The results of this study showed there was a highly significant increased ($P < 0.01$) concentration of IL-8 (44.371 ± 8.772) pg/ml in serum of aborted women in first trimester of pregnancy , compared with control groups (pregnant and non-pregnant women) which were (7.423 ± 2.152) pg/ml , (6.908 ± 3.859) pg/ml respectively as shown in table (1) and figure(1) .

Table (1): Mean levels of IL-8 (pg/ml) in serum of women with recurrent spontaneous abortion and control groups

	N	Mean	Std. Deviation	Std. Error	Range		P-value	C.S (ANOVA)
					Minimum	Maximum		
Pregnant control	10	7.4237	2.1528	.6808	5.51	12.28	.00	Highly Sig. (P<0.01)
Non pregnant control	10	6.9087	3.8597	1.2205	2.11	13.24		
Patients	25	44.3718	8.7724	1.7545	30.20	61.48		
Total	45							

Dependent Variable: IL-8 (Pg/ml)

LSD

(I) Studied groups	(J) Studied groups	LSD	
		P-value	Sig.
Pregnant control	Non pregnant control	.869	NS
	Patients	.000	HS
Non pregnant control	Patients	.000	HS

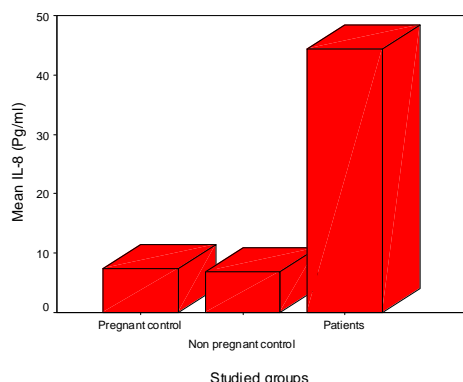


Fig. (1): Mean levels of IL-8 (pg/ml) in serum of women with recurrent spontaneous abortion and control groups

Wherease , There was a highly significant decreased ($P<0.01$) in the concentration of IL-6 (4.999 ± 1.571)pg/ml in serum of aborted women with first trimester of pregnancy compared with control groups pregnant women which were (45.116 ± 12.850)pg/ml and non-pregnant women (32.455 ± 12.382)pg/ml as shown in table (2) and figure (2) .

Table (2): Mean levels of IL-6 (pg/ml) in serum of women with recurrent spontaneous abortion and control groups

	N	Mean	Std. Deviation	Std. Error	Range		P-value	C.S (ANOVA)
					Minimum	Maximum		
Pregnant control	10	45.1163	12.8500	4.0635	29.84	66.69	.00	Highly Sig. ($P<0.01$)
Non pregnant control	10	32.4552	12.3827	3.9158	20.21	54.03		
Patients	25	4.9996	1.5712	.3142	2.52	8.95		
Total	45							

Dependent Variable: IL-6 (Pg/ml)
LSD

(I) Studied groups	(J) Studied groups	LSD	
		P-value	Sig.
Pregnant control	Non pregnant control	.002	HS
	Patients	.000	HS
Non pregnant control	Patients	.000	HS

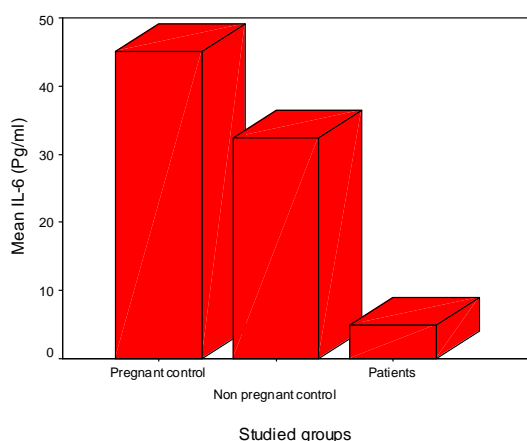


Fig.(2):mean level of IL-6 (pg/ml) in serum of women with recurrent spontaneous abortion and control groups

Spontaneous abortion is the most common of pregnancy failure , with 20% of pregnancies culminating in detectable spontaneous abortions . The risk of a subsequent miscarriage after one spontaneous loss is about 24% and is 26% after two and 32% after three consecutive losses [10].

The current study was prompted by an interest in elucidating the immunological milieu in which habitual aborters have a successful pregnancy and in particular to a scertain whether their cytokine profiles are more conducive to successful pregnancy as opposed to habitual aborters who continue to abort .The results of this study were inagreement with other studies on human pregnancy failure . In many studies , There was decreased level of IL-6 in serum of normal pregnant women compared with level of this cytokine in women undergoing recurrent spontaneous abortion [12,13,14]. Studies in

monkeys have shown that an increase in IL-6 concentration precedes uterine contractions, suggesting that IL-6 may play a role in physiological mechanisms involved in uterine contractions and the propagation of labour [15]. Thus, increased concentration of IL-6 may reflect a systemic reaction in the mother, leading to labour and delivery and IL-6 found in the serum may originate from the trophoblast [16]. The lower concentration of IL-6 in women with spontaneous abortion consider that IL-6 is a Th-2 type cytokine and that normal pregnancy appears to be a Th-2 biased condition and this might reflect a bias away from Th-2 type reactivity and a shift towards Th-1 dominance [10].

In another study there was increased level of IL-8 in serum of women with at least three spontaneous abortion [17]. The inflammatory cytokines, such as IL-8, may play an important role in the mechanism of protease-induced neurogenic inflammation leading to labor or abortions by recruiting neutrophils and lymphocytes in the endometrium [18,19].

Whereas previous study reported that women with spontaneous abortions had significantly decreased plasma level of IL-8, IL-6 and IL-11 compared to those with normal pregnancies [13]. The high level of IL-8 in aborted women may be due to the release of IL-8 from the endometrium [20], as well as from an increased number of resident mast cells that are degranulated in abortions [21].

Uterine mast cells degranulate after stress exposure of pregnant mice, possibly leading to release of IL-8 and TNF- α that could be involved in abortions [19].

References:

1. Rezae, A., and Dabbagh, A. 2002. T-helper (1) cytokines increase

during early pregnancy in women with a history of recurrent spontaneous abortion. *Med. Sci. Monit.* 8:CR 607-CR610.

2. Michimata, T., Sakai, M., Miyazaki, S., Ogasawara, M.S., Suzumori, K., Aoki, K., Nagata, K., and Saito, S. 2003. Decrease of T-helper 2 and T-cytotoxic 2 cells at implantation sites occurs in unexplained recurrent spontaneous abortion with normal chromosomal content. *Hum. Reprod.* 18:1523-1528.
3. Wegmann T.G., Lin, H.; Guilbert, L. and Osmond, T.R. 1993. Bidirectional, cytokine interactions in the Maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol. Today.* 14:353-356
4. Vesce, F.; Scapoli, C., Giovannini, G., Tralli, L., Gotti, G., Valerio, A. and Piffanelli, A. 2002. Cytokine imbalance in pregnancies with fetal chromosomal abnormalities. *Hum. Reprod.* 17:803-808.
5. Denison, F.C., Kelly, R.W., Calder, A.A. and Riley, S.C. 1998. Cytokine secretion by human fetal membranes, deciduas and placenta at term. *Hum. Reprod.* 12:3560.
6. Hebisch, G., Neumaier-Wagner, P.M., Huch, R. and Ursula von, M. 2004. Maternal Serum interleukin-1B, IL-6 and IL-8 levels and potential determinants in pregnancy and peripartum. *J. perinat. Med.* 32:475-486.
7. Romero, R., Gomez, R., Ghezzi, F., Yoon, B.H., Mazon, M., and Edwin, S.S. 1998. A fetal systemic inflammatory response is followed by the spontaneous onset of preterm parturition. *Am. J. Obstet. Gynecol.* 179:186
8. Sennstrom, M.B., Ekman, G., Westergren-Thorsson, G., Malmstrom, A., Bystrom, B. and Endresen, U.

2000. Human cervical ripening an inflammatory process mediated by cytokines .Mol.Hum.Reprod.6:375.
9. Bates,M.D.,Quenby,S., Takakuwa, K.,Johnson,P.M. and Vicne,G.S. 2002.Aberrant cytokine production by peripheral blood mononuclear cells in recurrent pregnancy loss.Hum.Reprod.17:2439-2444.
10. Makhseed,M.,Raghupathy,R.,Azizieh,F.,Omu,A.,AL-Shamali,E. and Ashkanani, L.2001.Th1 and Th2 cytokine profiles in recurrent aborters with Successful pregnancy and with Sabsequent abortions. Hum. Reprod.16:2219-2226.
11. Sorlie,D.E. 1995. Medical biostatistics and epidemiology: Examination and board review.First ed.Norwalk, Connecticut, Appleton and Lange:47-88 .
12. Austgulen ,R.,Lien,E. and Liabakk,N.B.1994.Increased levels of cytokines and cytokine activity modifiers in normal pregnancy. Eur. J.obstel. Gynecol. Reprod. Biol. 57:149-155 .
13. Koumantaki,Y., Matalliotakis, I.,Ssifakis, kyriakou,D., Neonaki, M.,Goymenon ,A.and Koumantakis, E.2001. Detection of interleukin-6, interleukin-8 and interleukin-11 in plasma from women with spontaneous abortion. Eur.J.Obstet. Gynecol.Reprod. Biol.98:66-71.
14. Carp,H.2004.cytokines in recurrent miscarriage.Lupus.13:630-634 .
15. Rosario, G.x., Sachdeva, G., Manjramkar, D.D., Modi, D. N., Meherji, P.K., and Puri, C.P.2005. Endometrial expression of immumodulatory cytokines and their regulator during early pregnancy in bonnet monkeys (*Macaca radiata*). Hum. Reprod 11:3039-3040.
16. Kameda,T., Matsuzaki,N. and Sawai,K.1990.production of interleukin-6 by normal human trophoblast.placenta.11:205-213 .
17. Madhappan,B. ,Kempuraj,D., Christodoulou ,S., Tsapikidis ,S., Boucher, W., Karagannis, U., Athanassiou, A. and Theoharides, T.C.2003.High levels of Intrauterine Corticotropin-releasing hormone, Urocortin, Tryptase, and Interleukin-8 in spontaneous abortions. Endocrinology. 144:2285-2290
18. Arici,A.,Seli,E.,Zeyneloglu,H.B.,Senturk,L.M., Oral,E.and Olive, D.L.1998. Interleukin-8 induces proliferation of endometrial stromal cells: a potential autocrine growth factor.J.Clin.Endocrinol.Metab.83:1201-1205 .
19. Zicari, A., Ticconi C.,Realacci,M., Cela, O.,Santangelo, C., Pietropolli, A.,Russo,M.A. and Piccione,E. 2002.Hormonal regulation of cytokine release by human fetal membranes at term gestation:effects of oxytocin, hydrocortisone and progesterone on tumor necrosis factor- α and transforming growth factor- β 1 output.J.Reprod. Immunol. 56:123-136 .
20. Shimoya, K., Matsuzaki, N., Taniguchi, T.Jo.T., Saji ,F., Kitajima, H.,Fujimura,M., Nakayama,M. and Tanizawa,O.1992.Interleukin-8 in Cord Sera:a Sensitive and specific marker for the detection of preterm chorioamnionitis.J.Infect.Dis.165:957-960 .
21. Marx,L.,Arch,P., Kieslich,C., Mitterlechner, S.,Kapp,M., and Dietl,J.1999.Decidual mast cells might be involved in the onset of human first-trimester abortion. Am.J. Reprod.Immunol.41:34-40 .
22. Singh,L.K.,Boucher, W.,Pang ,X., Letourneau,R., Seretakis, D., Green, M. and Theoharides,T.C. 1999. Potent mast cell degranulation and vascular permeability triggered by urocortin through actiration of CRH receptors.J.pharmacol.Exp.Ther.288: 1356-1356 .

التحري عن الحركيات الخلوية (8،6) في مصول النساء ذوات الاجهاضات العفوية المتكرره

رنا سعدي عبود*

*جامعة بغداد/ كلية العلوم / قسم علوم الحياة

الخلاصة:

لغرض التحري عن دور الحركيات الخلوية (8،6) في آليات التنظيم المناعي المتضمنة لدى النساء ذوات الاجهاضات العفوية المتكررة في الاشهر الاولى من الحمل . تم التحري عن مستوى تلك الحركيات الخلوية (8،6) في مصول خمس وعشرون امرأة بأعمار (20-35) سنة يعانين من اجهاضات عفوية غير معروفة السبب في الاشهر الاولى للحمل وتم مقارنة ذلك مع عشرون امرأة كمجاميع سيطرة (عشرة نساء حوامل وعشرة نساء غير حوامل) . تم قياس مستوى الحركيات باستخدام تقنية الامتزاز المناعي المرتبط بالانطيم (ELISA) . أظهرت نتائج الدراسة وجود ارتفاع معنوي عالي في مستوى الساييتوكين الخلوي (8) وانخفاض معنوي عالي في مستوى الساييتوكين الخلوي-6 ($P<0.01$) مقارنة بالنساء الحوامل والنساء غير الحوامل . ان نتائج تلك الدراسة ذات علاقة بالاليات المرضية لدى النساء ذوات الاجهاضات العفوية ويمكن استخدام تلك الحركيات الخلوية كمؤشرات تخمينية لنتائج الحمل .