

Effects of Interleukin-2 (IL-2) and Interleukin-6 (IL-6) in Recurrent Spontaneous Abortion (RSA).

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Abstract

Recurrent Spontaneous Abortion (RSA) is the most painful experience for couples expecting a child. This study aimed to determine the relevance of IL-2 and IL-6 in recurrent spontaneous abortion (RSA). Serum samples were collected from 90 women attend Al Kadhmiya teaching hospital in Baghdad. 60 women (first group) had recurrent abortion the women were negative for rubella virus, herpes simplex virus and toxoplasma gondii. And they were negative from bacterial infection eg. Niesseria gonorrhoea and Chlamydia trachomatis. The histopathological tests for fetus abnormalities were negative in this group, and 30 women (second group) with successful pregnancy (normal delivery). All samples were analyzed for IL-2 and IL-6 by commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits. The data showed highly significant increase in the serum level of IL-2 in group 1 compared with group 2 ($P < 0.001$). However, IL-6 showed highly significant increase level in group 2 compared with group 1. In addition, there was no significant correlation between these two markers in studied groups. The data of this study strengthen the possibility that high level of IL-2 and low level of IL-6 may explain the role of type-1 cytokines in the pathogenicity of recurrent spontaneous abortion.

Key words: - interleukin 2, interleukin 6 and recurrent spontaneous abortion (RSA)

الخلاصة

بعد الإسقاط الذاتي المتكرر من التجارب الأليمية في حياة الزوجين الراغبين في إنجاب الأطفال. أجريت هذه الدراسة للتحري عن دور الانترلوكين 2 و 6 في إحداث الإسقاط الذاتي المتكرر حيث جمعت تسعون عينة مصل من مجموعتين من نساء ادخلن إلى مستشفى الكاظمية التعليمي المجموعة الأولى ستون عينة كانت من نساء عانوا من الإجهاض الذاتي المتكرر واللاتي سبق واثبت خلوهن من الإصابة بفيروس الحصبة الألمانية (الحميراء) Rubella virus, وفيروس الحلا التناسلي Herpes simplex virus وداء المقوسات *Toxoplasma gondii* وكذلك عدم إصابتهن ببكتريا المسببة السيلان Niesseria gonorrhoea, والكلاميديا Chlamydia trachomatis وكذلك أثبتت الدراسة النسيجية من عدم وجود تشوهات في أجنة هذه المجموعة. المجموعة الثانية كانت من ثلاثون عينة كانت من نساء وضمن حملهن بصورة طبيعية. جميع العينات فحصت بطريقة الاليزا (ELISA) للتحري عن الانترلوكين 2 و 6, أظهرت النتائج فروق معنوية عالية في مستويات الانترلوكين 2 في مصول المجموعة الأولى مقارنة مع المجموعة الثانية ($p < 0.001$) , وكذلك كانت معدلات الانترلوكين 6 في مصول المجموعة الثانية مرتفعة مقارنة مع المجموعة الأولى. بالإضافة إلى ذلك لم تظهر النتائج ارتباط معنوي بين هذين الواصمين في المجاميع قيد الدراسة. نتائج هذه الدراسة دعمت احتمالية دور السابتوكاين I (في أمراضية الإسقاط التلقائي المتكرر في حالات ارتفاع الانترلوكين 2 وانخفاض الانترلوكين 6

Introduction

Recurrent spontaneous abortion (RSA) is one of the important complications in pregnancy. Half of recurrent miscarriages loss is multifactorial, can be explained by genetic, hormonal, anatomical, metabolic abnormalities infections or autoimmune mechanisms and can be divided into embryological driven causes (mainly due to abnormal embryonic karyotypes) and maternally driven causes which affect the endometrium and/or placental development⁽¹⁾. Known causes of maternal

defects include coagulation disorders, autoimmune defects, endocrine disorders and endometrial defects⁽²⁾. Mammalian pregnancy is thought to be a state of immunological tolerance. The mechanisms underlying this phenomenon are still poorly understood, Successful mammalian pregnancy depends upon tolerance of a genetically incompatible fetus by the maternal immune system. When tolerance is not achieved pregnancies fail⁽³⁾.

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Immunological rejection of the fetus due to recognition of paternal antigens by the maternal immune system, resulting in abnormal immune cells and cytokine production, is postulated to be one cause of unexplained pregnancy loss⁽⁴⁾. Cytokines have traditionally been divided into families dependent upon the immune cell of origin and the immunological effects that they bring about. CD4+ T-helper cells are the major immune cells involved in cytokine production, and these can be divided into functional subsets based on their cytokine production, T-helper 1 (Th1) cells produce interferon gamma (IFN γ), IL-2 and tumor necrosis factor beta (TNF β) are the main effectors of cell mediated immune response⁽⁵⁾ T-helper 2 (Th2) cells produce IL-4, IL-5, IL-6 and IL-10, which are the main effectors of antibody-mediated humoral responses⁽⁶⁾. Local mechanisms may play an important role in evading immune attack because maternal alloreactive lymphocytes are not systemically depleted. The specialized fetal tissue in contact with maternal uterine tissue might contribute to tolerance by several mechanisms, such as depleting tryptophan,⁽⁷⁾ by inactivating natural killer cells through HLA-G expression,⁽⁸⁾ or by provoking apoptosis of activated maternal lymphocytes⁽⁹⁾. Incomplete tolerance might therefore result in disturbed pregnancy such as spontaneous abortion and pre-eclampsia. Further, Th1/Th2 cytokine balance has been seen as a very important mechanism determining the survival of the fetus in the maternal uterus. Recent evidence suggests that maternal tolerance is established at the fetomaternal interface, by factors deriving from the decidualized endometrium and from the trophoblast itself, is maintained throughout gestation in physiological pregnancy⁽¹⁰⁾. Cytokines released at the fetomaternal interface have been proposed to play an important role in regulating embryo survival controlling not only the maternal immune response but also angiogenesis and vascular remodeling^(10; 11). Th-1 cytokines are considered to be detrimental to pregnancy, via direct embryo toxic activity, or via damage to the placental trophoblast, or possibly by activating cells that are deleterious to the conceptus, whereas Th-2 cytokines may directly or indirectly contribute

to the success of pregnancy by down regulating potential Th-1 reactivity^(12; 13). Protect the fetus and placenta from being rejected and to aid in the maintenance of normal pregnancy. In humans an important role for the T-helper 2 immune response has also been reported during normal pregnancy^(14; 15).

Methods : Studied group

This study included ninety (90) women from the Obstetrics and Gynecology Department of Al-Kadhmiya teaching hospital in Baghdad. Patients' ages ranged between (18-36) years with a mean of (27.5 – 30.1) year. The patients were divided into two groups:

Group 1: sixty (60) women were admitted to the hospital for recurrent spontaneous abortion (3-6 numbers of abortions) for evacuation.

Group 2: thirty (30) women with successful pregnancy (normal delivery) as control group.

Sample collection

From each women included in the study blood samples were collected to obtain the serum.

Procedure

* Enzyme Linked Immunosorbent Assay (ELISA) for the detection of IL-2, IL-6 in **serum**: IL-2, IL-6: ELISA Test Kits provided by (Mabtech Australia Pty Ltd). Product cod: (3460-IA-6) IL-6, (3430-IA-6) IL-2. Estimation of IL-2, IL-6 level in serum or plasma by ELISA method. This method has two immunological steps. In the first step, the cytokine is captured by monoclonal antibody bound to the wells of a micro titer plate. In the second step a monoclonal antibody linked to a biotinylated monoclonal antibody is add together with streptavidine-peroxidase conjugate. The solid phase antibody-antigen complex and in turn, binds the conjugate. After incubation, the wells are washed and the antigen complex bound to the well detected by addition of a chromogenic substrate. The intensity of the color developed is directly related to the specific monoclonal antibodies concentration of the sample⁽¹⁶⁾.

Statistical analysis

The Student test (t-test) analysis program was used to calculate the values, Mean, and standard error were all used in the analysis and the relationship between the indicators was measured qualitatively by using the correlation coefficient

(r). Values of $P < 0.05$ were considered as statistically significant ⁽¹⁷⁾.

Results

The expression of IL-2 and IL-6 was detected by ELISA technique. Table (1) shows the mean value of concentration (pg/ml) of IL-2 in sera of studied group which show highly significant ($p < 0.01$) increased expression of IL-2 in aborted women compared with control. Table (2) shows the mean value of concentration (pg/ml) of IL-6 in sera of studied group which show highly significant ($p < 0.01$) increased expression of IL-6 in aborted women compared with control. In addition, the study failed to found a significant correlation ($P > 0.05$) between IL-2 and IL-6 in aborted women and control groups, as shown in Table(3).

Table (1): The mean value of concentration (pg/ml) of IL-2 in sera of studied group.

Studied group	N	Mean± Std. Error	Comparison of significant	
			P-value	Sig.
Control	20	97.45± 0.90	0.00	Highly Sig.
Aborted women	20	259.32± 82.6		
Total	50			

Table (2): The mean value of concentration (pg/ml) of IL-6 among studied group.

Studied group	N	Mean± Std. Error	Comparison of significant	
			P-value	Sig.
Control	20	375.30±73.3	0.00	Highly Sig.
Aborted women	20	82.41±0.86		
Total	50			

Table (3): Pearson correlation (r) between IL-2 and IL-6 levels in serum of studied group

Pearson Correlation		IL-2 IL-6
Abortion	r	0.532
	P-value	>0.05
	Sig.	NS
	r	0.421
	P-value	>0.05
	Sig.	NS

NS= Non Significant difference

Discussion

The level of IL-2 increased in women with abortion in comparison with that successful pregnancy as shown in table (1). Evidence supporting this result showed that the administration of one of the Th-1 cytokines like Interferon- γ (IFN- γ), Tumor necrosis factor- α (TNF- α) or interleukin-2 (IL-2) to normal pregnant mice causes abortion ⁽¹⁸⁾. During a normal pregnancy, allogenic fetal tissues are exposed to the maternal immune system. Rejection reactions normally develop after allogenic recognition following the principle of transplantation immunology ⁽¹⁹⁾. Although the immune system is functional in the uterus and the embryo expresses paternal major histocompatibility complex (MHC) molecules, the conceptus nevertheless escapes the deleterious effect of maternal rejection. It may be due to local tolerance or even perhaps immune suppression after interactions between fetal antigens and the maternal immune system ⁽¹⁹⁾. T cells must change from a resting to an activated state during immune responses and lead to de-novo synthesis of interleukin IL-2 and expression of IL-2 receptors {IL-2Rs} ⁽²⁰⁾. Interaction of IL-2 and its receptors triggers cellular proliferation, culminating in the emergence of effectors T cells that are required for the full expression of immune responses. ⁽²¹⁾ Spontaneous abortions in humans have been shown to be associated with increased production of interleukin (IL)-2 and IFN- γ by peripheral blood mononuclear cells (PBMC) and with decreased production of IL-10, as compared to normal pregnancy ⁽²²⁾. Studies by Hill and colleagues have shown that

trophoblast antigens activate the PBMC of women with a history of unexplained recurrent spontaneous abortion (RSA) to produce the embryotoxic cytokines IFN- γ and TNF- β ^(22,23). Interleukin-2, tumor necrosis factor- α , and interferon- γ are deleterious and used to stimulate the apoptosis of human primary villous trophoblast cells. In addition this study reveals in table 2 that the expression of IL-6 proteins in circulation of women with successful pregnancy was significantly higher ($p < 0.001$) than that of women with abortion. Further studies showed that there is a greater increase in IL-6 secretion during pregnant compared to not pregnant state that may be detected by ELISA of endometrial biopsy samples⁽²⁴⁾. human trophoblasts express IL-6 receptor and produce IL-6, which induces the production of hCG in an autocrine manner, suggesting a role of IL-6 in early implantation and its continuation in early pregnancy⁽²⁵⁾. IL-6 may play a role in physiological mechanisms involved in uterine contractions and the propagation of labour. Thus, increased concentrations of IL-6 may reflect a systemic reaction in the mother, leading to labour and delivery⁽²⁶⁾. This study showed that IL-6 concentrations were lower in women with RSA than in those undergoing normal delivery. Considering that IL-6 is a Th2-type cytokine and that normal pregnancy appears to be a Th2-biased condition. These cytokines encourage vigorous antibody production, and are therefore commonly associated with strong antibody responses that are important in combating infections with extracellular organisms. IL-6 may also induce prostaglandin synthesis by intrauterine tissues; thus, it seems to play a physiological role in labour development. High levels of IL-6 have been detected in pregnant women at term and in preterm at labour⁽²⁷⁾. Such human studies do not clearly indicate a cause-and-consequence between increased IL-6 concentrations and events associated with labour, studies in monkeys have shown that an increase in IL-6 concentrations precedes uterine contractions that may play a role in physiological mechanisms involved in uterine contractions and the propagation of labour⁽²⁸⁾. The IL-6 found in the serum may originate from the trophoblast⁽²⁹⁾. Thus the study by

Makhseed and colleagues, demonstrated that IL-6 concentrations were lower in women with RSA than in those undergoing normal delivery, considering that IL-6 is a Th2-type cytokine and that normal pregnancy appears to be a Th2-biased condition⁽¹³⁾. Th1 and Th2 cells are mutually inhibitory to each other when Th1 reactivity is high, Th2 reactivity is usually low and vice versa⁽³⁰⁾. The current study shows in table (3) no significant correlation ($p > 0.05$) between IL-2 and IL-6 in women with recurrent spontaneous abortion (RSA) and successful pregnancy. This un relation might be associated with different role of these two cytokine during pregnancy thus we suggested further study focusing on the role of IL-2 and IL-6 in pregnancy and RSA in placental tissue. In conclusion, IL-2 might play a pathological role in pregnancy in contrast IL-6 might play a role in successful pregnancy.

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