



The Role of IL-6 and TGF- β 1 in Iraqi Women with Recurrent Abortion

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Abstract

The alteration in the cytokines stability synthesis by Thelper1 cells with a rise of interleukin 6 (IL-6) and Thelper2 cells with the increase of TGF- β 1 secretion is measured critical for retaining of usual pregnancy and increased risk of recurrent spontaneous abortion (RSA). This study was designed to measure some cytokines and affect these cytokines in the case of recurrent spontaneous abortion and healthy pregnant. Blood samples were collected from 210 women 180 women with the recurrent pregnancy loss (three or more abortions), and 30 women with normal pregnancy to three or more birth and without a previous abortion in the first trimester Based on clinical examination and diagnostic laboratory findings for TORCH of ELISA examination measured serum levels of immunoglobulin (IgM and IgG) for *Toxoplasmosis*, *cytomegalovirus*, *herpes*, and *rubella* in normal pregnant and in aborted women. Depending on the results of the test, the patients were divided into four groups: group one included 30 women with recurrent abortion with seronegative for TORCH test was (23.8%), group two recurrent aborted with seropositive for anti-*toxoplasma* antibodies was (20.9%), group three included recurrent abortion suffering from infected with different causes such as (*cytomegalovirus*, *Rubella* or *Herpes*) ratio was (41%). while group four women with a healthy pregnancy promise as a control group was (% 14.3). Then taking thirty serum samples of women from the first and second group with recurrent abortion and compared with control group then measured serum levels of pro-inflammatory cytokines Interleukin-6 (IL-6) and anti-inflammatory marker Tumor growth factor-beta1 (TGF- β 1). The results presented that the serum levels of (IL-6) were highest in group1 and 2 (474.50 pg/ml and 629.60 pg/ml) respectively compared with control group (247.03 pg/ml), there was a significant difference. While TGF- β 1 was the highest level in group1 (217.63 pg/ml) followed by group 2 (171.06 pg/ml) then control group (109.97 pg/ml) with the significant difference between three groups. The results indicated that the serum levels of pro-inflammatory and anti-inflammatory marker found with high level in recurrent abortion compared with healthy pregnant.

Introduction

Spontaneous miscarriage is the injury of the intrauterine pregnancy without separate intervention previously 20 weeks of pregnancy.

The recurrent pregnant loss is mostly considered to be the harm of 3 or more gestations [1].

Reasonably accepted etiologic causes include, genetics, immunologic factors, placental abnormality, endocrine disorder, nutritional, environmental factors and infection with microorganisms like *Toxoplasma Gondi*, *Cytomegalovirus* syphilis, rubella, herpes

and maternal disease (such as diabetes mellitus, thyroid disease) [2].

Toxoplasmosis caused abortions usually occur during the first half of gestation and effects on liver and spleen functions [3].

When the congenital *toxoplasmosis* occurs early in pregnancy, it may lead to severe damage or abortion. Embryo acts as an allograft to the mother's body, it is remaining normally in the mother's womb during the entire gestational period in the case of normal successful pregnancy [4].

The placental wall is the transportation of cytotoxic cells to the fetus and cytotoxic antibodies are disinterested by the placenta before they extent to the fetal circulation [5].

T cells could play the essential role in pre-implantation and embryo change in embedding process and in the occurrence of fetal allograft acceptance [6].

Th₁ cytokine inhibits Th₂ cells expansion and Th₂ cytokines block activation of Th₁ cells [7]. IL-6 might have both beneficial effects and detrimental effects on the events of early pregnancy also implicated in the pathophysiology of abnormal pregnancies and another disease such as Rheumatoid Arthritis, Autoimmune disease, preeclampsia and obesity [8,9,10]. TGF- β in the presence of IL-6 can promote inflammation and autoimmune conditions [11]. The aims of the present study were to detect the serum levels of anti-inflammatory cytokines TGF- β and pro-inflammatory cytokines IL-6 in women with recurrent abortion.

Material and Methods

Patients and Control

The current study included 180 women with recurrent abortion during the first-trimester and 30 women as a control group were with normal third delivery or more and with no previously recognized miscarriage.

The ages of these women were ranged between 20-35 years. The total number of women (210) was referred to Obstetrics and Gynecology Section of Baghdad Teaching Hospital, and AL-Yarmook Teaching Hospital during the period from March to December 2016.

Samples Collection

The 5 ml of venous blood was taken at the time of miscarriage by using sterile disposable syringes, (3ml) of blood located in a plain tube with gel clot and left-hand to stand for one hour at room temperature for clot realization, for serum collection, the tube centrifuged for 10 minutes at 3000 rpm. Then the serum extracted by using a Pasteur pipette and distributed into a sterile Eppendorf tube and stored at -20 C° until used.

Enzyme-Linked Immunosorbent Assay for the Recognition of IGG or IGM Antibodies in Human Serum

The practical work was done according to the instructions of manufacturers (Diagnostic Automation, INC, USA).

Study Groups

According to TORCH of ELISA test divided into:

Group One

Women with repeated aborted three or more during the first trimester with recurrent abortion with seronegative for TORCH test

Group Two

Women with repeated aborted three or more during the first trimester with seropositive for anti-*toxoplasma* antibodies

Group Three

Women with repeated aborted three or more during the first trimester with different causes such as (*cytomegalovirus, Rubella or Herpes*)

Control Group

Women with normal third delivery or more and with no previously recognized miscarriage. Selected thirty samples were taken from every two groups for determined levels of cytokines and compared with control group.

Measurement of Cytokines

Human IL-6 and TGF- β 1 ELISA Kits

The practical work was done according to the instructions of manufacturers (Komabiotech inc / Korea).

Statistical Analysis

Statistical analysis was performed with SPSS software package (Version 22 for Windows).

Numerical data were described as mean, standard deviation and least significant difference (LSD) test were used to significant compare between groups. Results were analyzed by comparison between groups by one way ANOVA.

Results

Concentration of IL-6 in the Study Groups

The mean of IL-6 in the group1 was (474.50 \pm 158.86) pg/ml, group 2 was (629.60 \pm 115.45)

pg/ml and in group 3 was (247.03 ± 46.00) pg/ml as shows in the Table (1).The serum levels of IL-6 among groups of the present study were with the highest level in group 2

with *Toxoplasma* while the level of IL-6 in group 3 was with lower level compared with other groups.

Table 1: Serum level of IL-6 in the study groups

Study groups	Number	Mean ± SD (pg/ml)
(group1): Recurrent abortion with seronegative for TORCH test.	30	47.45 ± 15.88 *
(group2): Recurrent abortion with seropositive for anti- <i>Toxoplasma</i> antibody.	30	62.96 ± 11.54 *
(Control group): Pregnant normal without any abortion.	30	24.70 ± 4.60
Total	90	---
LSD value	---	8.55
P-value	---	0.001

*= mean significant difference at (p< 0.001) by ANOVA.

Concentration of TGF-β1 in the Study Groups

The value for TGF-β1 in group1 was (217.63 ± 47.47) pg/ml, in group 2 was (171.06 ± 14.95) pg/ml and in control group was (109.97 ± 28.90) pg/ml as shown in table 2. The high

level found in group 1 with seronegative for TORCH test and the low level in control group in pregnant normal. There was the significant alteration at p < 0.001 among group 1, and 2 with the control group.

Table 2: Serum level of TGF-β1 in the different groups

Study groups	Number	Mean ± SD (pg/ml)
(group1): Recurrent abortion with seronegative for TORCH test.	30	217.63 ± 47.47 *
(group2): Recurrent abortion with seropositive for anti- <i>Toxoplasma</i> antibody.	30	171.06 ± 14.95 *
(Control group): Pregnant normal without any abortion.	30	109.97 ± 28.90
Total	90	---
LSD value	---	24.4
P-value	---	0.001

*= mean significant difference at (p< 0.001).

Discussion

Measurement of Cytokines Levels in the Serum

Pregnancy requires physiological adaptations in all maternal systems, including the immune system [12]. Cytokines, as serious immune-regulatory molecules, accountable for responsible the nature of an immune reaction, have been revealed to influence on all stages of reproduction and playing an essential role in gestation outcome [13].

Interleukin-6 is a premature and sensitive indicator of inflammation, the major function of it was the contribution to immune reaction through the action of lymphocytes and consider a moderator responsible for the making acute phase proteins and amplified cytotoxic activity of NK cells[14].

In this paper showed that the high serum level of IL-6 in group1 compared with control group, which indicate that peripheral blood lymphocytes of women with recurrent abortion secrete the high level of IL-6 may explain the role of this cytokines in the pathogen city of recurrent abortion [15].

The study done by Bakir in 2010 found that IL-6 increased in recurrent spontaneous abortion more than in healthy pregnant [16]. Also, Hua in 2013 recorded that in the rat the concentration of (IL-6) was significantly upper in recurrent miscarriage than in normal pregnancy [17]. In sporadic miscarriage found that the increased in the serum levels of (IL-6) compared with pregnant healthy [18].

On the other hand, Koumantaki in 2001 recorded that the reduced serum levels of (IL-6) in women with spontaneous miscarriage may be related to the causal etiopathogenetic instruments [19]. Other study done by Ahmed in 2008 demonstrated that IL-6 level was lower in women with RSA than in those undergoing normal delivery [20].

Also, Makhseed in 2000 showed that the serum level of (IL-6) was significantly greater in pregnant women than in spontaneous abortion [21]. The high serum level of (IL-6) in group2 with seropositive for anti-*Toxoplasma* antibody compared with pregnant normal in control group which indicated that the presence of inflammatory

status and the role of IL-6 in resistance the infection with *Toxoplasma* and contributes to transformation from acute to chronic inflammation.

Rageb in 2015 showed that the IL-6 level was higher in *toxoplasma* group than control group [22], this may be critical role of IL-6 to resistance the infection with *T. gondii* [23], and contributing to transformation from acute to chronic inflammation [24], Matowicka-Karna in 2009 reported that the patients with *T. gondii* had a greater level of (IL-6) as associated with healthy pregnant, which appears to approve the incidence of an inflammatory state and the patients sick with *T. gondii* appearance increased in the making of the cytokines responsible for humoral reaction, while generation of the cell reaction cytokines remainders unchanged [14].

El-Hashimi in 2014 showed that IL-6 in aborted women infected with toxoplasmosis was significantly higher compared to aborted women without toxoplasmosis [25]. Hafedh in 2015 recorded that in aborted women the concentration of IL-6 was more than in healthy women because IL-6 plays a vital role during the infection with *T. gondii* in aborted women [26].

previous studies showed that the high level of IL-6 in the patients with toxoplasmosis may explain the presence of anti- *T. gondii* antibody in serum of the patient, this may occur early in infection before the immune response modified from Th2 into Th1 that responsible for the several cases of abortion [14,27]. Tumor growth factor-beta could contribute to the instruction of maternal immune reactions against the fetal allograft, and thereby avoid immunological rejection of the fetus [28].

TGF-β1 is secreted by several cell types, including peripheral blood mononuclear cells and T regulatory lymphocytes [29]. The high serum level of Tumor growth factor-beta1 with seronegative for TORCH test in group 1 compared with control group with pregnant normal which indicated that TGF-β1 exerting a predominantly anti-inflammatory effect for maintenance of immune tolerance and try to elevate for maintaining of the successful pregnancy and the increased in this molecule might be a danger factor for gestation outcome. TGF-β1 regulate some aspects of

pregnancy, thereby contributes to the keeping of immune acceptance [30, 32].

The plasma level of TGF-β1 in recurrent spontaneous abortion was significantly higher compared with pregnant normal. Also, explained the increased in the production this molecule may possibly be a danger factor for the gestation outcome [33]. Numerous studies recommended that TGF-β1 may be complicated in reproductive associated disorders, such as preeclampsia, and recurrent spontaneous abortion, even though data were provocative [34, 35]. An imbalance among the effector, and regulator cells would lead to reproductive failure and related pregnancy sicknesses [36].

Other studies found that the levels of TGF-β1, TGF-β2, and TGF-β3 were unaltered in the serum of spontaneous pregnant loss [37]. TGF-β1 has both endocrine, and paracrine activities, so in situ placental appearance are added significant than the examination of serum levels [21].

Gutcher in 2011 reported that TGFβ1 was highly communicated by Thelper17 cells and operated in a predominantly autocrine mode to maintain Thelper17 cells *in vivo*, the role of TGFβ for activated T cell-produced in promoting the differentiation of Th17 cells and controlling inflammatory diseases [38]. The capability of TGF-β1 to standardize the cytokine linkage that controls trophoblast growth and uterine attack [39, 40].

Other studies have observed that TGF- β1 appearance in the placenta of RPL cases, reduced amounts of TGF-β1, TGF-β2, TGF-β3 and its receptors, have been found in placentas after the miscarriage, frequently with conflicting results, highlighted by either decreased appearance [41,42], or unchanged protein levels [36]. The higher level of Tumor Growth Factor-β1 in pregnant women with abortion, compared with normal pregnant women [43].

In the current study, TGF-β1 was higher in group 2 with seropositive for anti-*Toxoplasma* antibody compared with control group with pregnant normal which indicated that *T. gondii* induced macrophages, neutrophil and dendritic cell apoptosis and the cell infected with *Toxoplasma* secreted high concentration from TGF-β1.

Through the infection, *T. gondii* multiplies and occurs in the parenchyma tissues and antibody manufacture after *T. gondii* production is unable to prevent the disease and *T. gondii* has numerous mechanisms to escape from the host immune reactions. TGF- β also contributes to tissue alteration which occurs after infections and damages. Also, prior studies showed that *T. gondii* encouraged TGF- β secretion by immune cells as an anti-inflammatory mediator to decrease the autoimmune reactions and the serum levels of TGF- β amplified at a quick phase of infection with

Toxoplasma [44]. Additional investigation also proved that *T. gondii* prompted macrophages apoptosis through autocrine TGF- β signaling [45].

Conclusion

The current study of IL-6 serum levels was highest in women with recurrent abortion who had positive Anti-Toxoplasma Ab as compared with other groups while TGF- β 1 serum levels were highest among recurrent abortion women with undefined causes as compared with other groups.

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