ORIGINAL ARTICLE

The Role of Some Cytokines inwomen withRecurrent Abortion in Iraqi Women

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ABSTRACT

Recurrent spontaneous abortion (RSA) in the general populations is very common occurrence and often changes among different communities.This study was designed to measure some cytokines in the case of recurrent spontaneous abortion and healthy pregnant. Blood samples were collected from 210 women (180 women with recurrent abortion (three or more abortions) and 30 women with normal pregnancy to three or more birth and without a previous abortion and without any infection) in the first trimester. Based on clinical examination and diagnostic laboratory findings of rapid test and ELISA for TORCH.Measured levels of immunoglobulin (IgMand IgG) for (*Toxoplasmosis, cytomegalovirus*, *herpes* and *rubella)* in normal pregnant and aborted women. Depending on the results of tests, the patients were divided into four groups: group one included 30 women with recurrent abortion with sero-negative for TORCH testwas (17%), group two recurrent aborted with sero-positive for anti-toxoplasma antibodies was (24.8%), group three included women with a healthy pregnancy promise as a control group was (14.3%) while group four recurrent abortion suffering from infected with different causes such as (*cytomegalovirus*, *Rubella* or *Herpes*) ratio was (43.8%). 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-β1). The results showed that the serum levels of IL-6 was highest in group1 and 2 (474.50 pg/ml and 629.60pg/ml) respectively compared with control group (247.03 pg/ml), there was a significant difference. While TGF-β1 was highest level in group1 (2176.30 pg/ml) followed by group 2 (1710.63 pg/ml) then control group (1099.76 pg/ml) with 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.

Keywords: Abortion, cytokines, pregnancy

INTRODUCTION

Spontaneous abortion (SA) is the most common complication of early pregnancy, refers to one of the most frequent reproductive events is recurrent spontaneous abortion (RSA). It is defined as three or more repeated pregnancy losses before the fetus has reached a viable gestational age[1]. Reasonably accepted etiologic causes include, genetics, immunologic factors, placental abnormality, endocrine disorder, nutritional, environmental factors and infection with microorganisms like *Toxoplasma gondii*, *Cytomegalovirussyphilis*, *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 barrier is traffic of cytotoxic cells to the fetus and cytotoxic antibodies are removed by the placenta before they reach the fetal circulation[5]. T cells may play an important role in pre-implantation and embryo development in implantation process and in the phenomenon of fetal allograft tolerance[6]. Th_1 cytokine inhibit Th_2 cells expansion and Th_2 cytokines block activation of Th_1 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 other 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-inflammatorycytokines (TGF-β) and pro-inflammatory cytokines (IL-6) in women with recurrent abortion.

MATERIAL AND METHODS

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Patients and control: The current study included 180 women with recurrent abortion during the first-trimester and 30 women as acontrol groups were with normal third delivery or more and with no previously recognized miscarriage.The ages of these women wereranged between 20-35 years. The total number of women (210) was referred to Obstetrics and Gynecology Department of AL-Yarmook Teaching Hospital, Baghdad Teaching Hospital and AL-Batool 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 placed in a plain tube with gel clot and left to stand for one hour at room temperature for clot formation, for serum collection, the tube centrifuged for 10 minutes at 3000 rpm. Then the serum aspirated by using a Pasteur pipette and dispensed into sterile eppendorf tube and stored at -20 C° until used.

TORCH rapid Test for IgM and IgG: Practical work was done according to the instructions ofmanufacturers (Ecotest, China).

Enzyme Linked Immunosorbent Assay for the detection of IgG or IgM antibodies in human serum: Thepractical work was done according to the instructions ofmanufacturers (Diagnostic Automation, INC, USA).

Study groups: According to TORCH and ELISA test divided into:

-Group one: women with repeated aborted three or more during the first trimester withrecurrent abortion with sero-negative for TORCH test

-Group two: women with repeated aborted three or more during the first trimester with sero-positive for anti-toxoplasma antibodies.

-Group three: women with normal third delivery or more and with no previously recognized miscarriage.

-Group four: women with repeated aborted three or more during the first trimester with different causes.

Selected thirty samples were taken from each three groups for determined levels of cytokines.

Measurement of cytokines:

Human IL-6 and TGF-β1 ELISA Kits: The practical work was done according to the instructions of manufacturers (Komabiotechinc / korea).

Statistical analysis: Statistical analysis was performed with SPSS22. Numerical data were described as mean, standard deviation and least significant difference (LSD) test was used to significant compare between groups. Results were analyzed by comparison between groups by one way ANOVA.

RESULTS

Table 1 shows the distribution of studied groups according-to TORCH results by lateral flow chromatographic immunoassay and ELISA TORCH as following divided into group1: recurrent abortion with Unknown causes the percentage was 36(17.1%), group2: recurrent abortion with Toxoplasma only was 52(24.8%), group3: healthy women with normal pregnancy (control group) was 30 (14.3%) and group 4: recurrent abortion with different causes was 92(43.8%).

Seroprevalence of IgM and IgG among study groups: Table (2) Illustrates seroprevalence of IgM and IgG antibody for pathological causesamongstudy groups according-to ELISA test. The seropositive Antibodies of IgM and IgG for *Toxoplasma gondii* were 18(8.5%), 30(14.3%) followed by *Cytomegalovirus* were 17(8.09%), 28 (13.3%), *Rubella*were 9(4.3%), 15(7.1%), HSV were 3(1.5%), 8(3.8%), while for healthy pregnant were 0(0%), 0(0%) respectively. The seroprevalence of (IgM and IgG) antibody for *Toxoplasma gondii* was 4(1.9%), *Cytomegalovirus* was 5(2.4%), *Rubella* was 6(2.9%), *Herpes Simplex Virus* was 1(0.4%) and Healthy pregnant was 0 (0%).

The mean of IL-6 in the group1 was (474.50±158.86) pg/ml, group 2 was (629.60±115.45) pg/ml and in group 3 was (247.03±46.00) pg/ml as shows in the table 3. The serum levels of IL-6 among groups of the present study were with highest level in group 2 with *Toxoplasma* while the level of IL-6 in group 3 was with lower level compared with other groups.

Concentration of TGF-β1 in the study groups: The value for TGF-β1in group1 was (2176.30±474.74) pg/ml, in group 2 was (1710.63±149.56) pg/ml and in group 3 was (1099.76±289.01) pg/ml as shows in the table 4. The highest level found in group 1 and the lowest level in group 3 and there was significant difference at ($p<0.001$) between different groups

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Table 1: Distribution of studied groups according-to TORCH by rapid test and by ELISA test.

Study groups	TORCH by rapid test	TORCH by ELISA test
Recurrent abortion with sero-negative for TORCH test (group1)	39(18.6%)	36(17.1%)
Recurrent abortion withsero+ve for anti-*Toxoplasma*antibody (group2)	50(23.8%)	52(24.8%)
Pregnant normal without any abortion as a control group (group3)	30(14.3%)	30(14.3%)
Recurrent abortion with different cause (group4)	91(43.3%)	92(43.8%)
Total	210(100%)	210(100%)

Table (2): Seroprevalence of IgM and IgG amongstudy groups according-to TORCH of ELISA test.

Pathogens	IgM %	IgG %	(IgM&IgG) %	Total
Toxoplasma gondii	18 (8.5%)	30 (14.3%)	4(1.9%)	52
Cytomegalovirus	17 (8.09%)	28 (13.3%)	5(2.4%)	50
Rubella	9 (4.3%)	15 (7.1%)	6(2.9%)	30
Herpes Simplex Virus	3 (1.5%)	8 (3.8%)	1(0.4%)	12
Healthy pregnant	0 (0%)	0 (0%)	0 (0%)	0
Total	47 (22.3%)	81(38.5%)	16(7.6%)	144

Concentration of IL-6 in the study groups.

Table 3: Serum level of IL-6 in the study groups.

Study groups	Number	Mean ± SD (pg/ml)
Recurrent abortion with sero-negative for TORCH test (group1)	30	474.50 ± 158.86 *
Recurrent abortion withsero-positive for anti-*Toxoplasma*antibody (group2)	30	629.60 ± 115.45 *
Pregnant normal without any abortion as a control group (group3)	30	247.03 ± 46.00 *
Total	90	---
LSD value	---	85.5
P-value	---	0.001

*= mean significant difference at (p≤ 0.001) by a nova.

Table 4: Serum level forTGF-β1 in difference groups.

Study groups	Number	Mean ± SD (pg/ml)
Recurrent abortion with sero-negative for TORCH test (group1)	30	2176.30 ± 474.74 *
Recurrent abortion withsero-positive for anti-*Toxoplasma*antibody (group2)	30	1710.63 ± 149.56 *
Pregnant normal without any abortion as a control group (group3)	30	1099.76 ± 289.0 *
Total	90	---
LSD value	---	244.19
P-value	---	0.001

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

the rate expanded with age from the (15-19) years the rate was (5.6%) and from (40-44) years the rate was (16.7%)[17].

The National Health and Nutrition Examination Survey (NHANES), found that the prevalence of toxoplasmosis has declined in the past decade[18]. Also, Acharya in 2014 showed that in Nepal the women with spontaneous abortions had IgG for *T. gondii* in rate (77.9%)[19]. Other Study was done in Egypt on pregnant women found the prevalence of*T. gondii* in the age (21-30) years was (50.8%)[20]. Study done in Mexico on pregnant women infected with *T. gondii* recorded that IgG and IgM antibodies level in rate (6.1%), (9.5%) respectively[21]. Result was recorded by Hadi in 2016 in Iraq showed that in aborted womenthe percentage rate for IgG was (35.4 %) and for IgM was (3.2 %)[22]. Also, in Iraq the women with spontaneous abortions had IgG and IgM for *T. gondii* in rate (31.5%), (7.6%) respectively[23]. The percentage of IgG and IgM for *T. gondii* in aborted women its (22.8%) and (35.1%) respectively[24].

The role of some infection in recurrent abortion has been intensely investigated during the past decades by viruses, especially CMV have been more consideration since they can produce chronic/recurrent intrauterine infections. CMV causes both primary and recurrent (reactivation or

DISCUSSION

Seroprevalence of IgM and IgG among study groups: The rate of IgM antibody refers to acute infection, while IgG antibodies may increase in the 2-4 weeks after infection and gradually rises for many weeks then remain in low level for the rest time, while repeated exposure to the infection may cause high antibody rate for longer time[12].

Toxoplasma, *herpes, Rubella* and *Cytomegalovirus* are known to cause infection in uterus often responsible for abortion, still birth, premature delivery and congenital malformation, detection and treatment of such infections can prevent morbidity and mortality of the infants born to such mothers[13].

The seroprevalence of *T. gondii*infections ranges between 7.7 and 76.7% in different countries like in United Kingdom (7.7- 9.1%); Norway (10.9%); India, (45%); Brazil(50-76%) and Nigeria (75.4%)[14].

Turbadlkar in2003 reveled that in pregnant women which have history of bad pregnancies the percentage of IgG *T. gondii* antibody was (42.10%) and IgM was (10.5%)[15]. Study was done in India the rate of toxoplasmosis in pregnant women for IgG was (45%) and only seven women were found to have IgM in rate (3.3%)[16]. Nash (2005) reported that in United Kingdom the average of *Toxoplasma* in pregnant women for IgG was (9.1%), also obviously,

positive for HSV IgG antibodies was (28.9%), and for IgM antibodies was (2.2%)[43], Hasan in 2013 found that in pregnant women the rate of *herpes simplex virus* of IgG and IgM antibody were (2.19%), (2.19%) respectively[44]. Study done in Nepal on the women with spontaneous abortions found that the HSV IgG antibody in rate (36.4%)[19]. While in Nigeria the titer of HSV IgM in pregnant women was (2.8%)[45]. Other study in India showed that in aborted women the titer of HSV IgM was (30.10%)[46].

Differences between studies results can be due to the several laboratories utilize different techniques used in these aspects, type of test that had been used and the number or types of sample tested for infection and it is well-known that epidemiology of infection is different among different populations. Also, patients' characteristics have been different among studies.

Measurement of cytokines levels in the serum: Pregnancy requires physiological adaptations in all maternal systems, including the immune system[47]. Cytokines, as critical immunoregulatory molecules, responsible for determining the nature of an immune response, have been shown to influence on all steps of reproduction and playing a fundamental role in pregnancy outcome[48].

Interleukin-6 is an early and sensitive marker of inflammation, the major function of it was the contribution to immune response through the action of lymphocytes and consider a mediator responsible for producing acute phase proteins and increased cytotoxic activity of NK cells[49].

In this study, group 1 appeared high serum level of IL-6 compared with group 3, that indicate peripheral blood lymphocytes of women with recurrent spontaneous abortion secrete high level of IL-6 may explain the role of this cytokines in the pathogenicity of recurrent spontaneous abortion[50]. Study done by Bakir in 2010 found that IL-6 increased in recurrent spontaneous abortion more thanin healthy pregnant[51].

Also, Hua in 2013 recorded that in the rat the concentration of IL-6 was significantly higher in recurrent miscarriage than normal pregnant[52]. On the other hand, Makhseed in 2000 showed that the serum level of IL-6 was significantly higher in pregnant women than in spontaneous abortion[53]. Also, Koumantaki in 2001 recorded that the reduced plasma levels of IL-6 in women with spontaneous abortion may be related to the underlying etiopathogenetic mechanisms[54]. Other study done by Ahmed in 2008 demonstrated that IL-6 level was lower in women with RSA than in those undergoing normal delivery[55]. In sporadic miscarriage found that the increased in plasma levels of (IL-6)[56].

reinfection), viral strain variation may contribute to reinfection, and low maternal IgG avidity may unexpectedly promote transmission of virus across placenta and cause production of toxic metabolites, fetal loss, placental disorder, and chronic endometrial infection[25,26]. Uyar in 2008 reportedthat in Turkey the pregnant women infected with CMV were had IgG and IgM in rate (97.3%) and (1.0%) respectively[27]. Other studydone in Sudan revealed that the prevalence of CMV IgG in pregnant women was 97.5%, while IgM was (6%)[28]. Sherkatin 2014 recorded that in Iran CMV was significantly higher in recurrent pregnant loss (RPL) than the women without history of abortion and the titer of CMV IgG was (90.6%)[29]. Study in Iraq on women with bad obstetric history (BOH) found that IgM and IgG antibodies to *Cytomegalovirus*in rate (7.2%), (96.6%) respectively[30]. Other study in India, also on women with (BOH) the percentage of infection with CMV was (14.6%)[31]. Al-Baiatiin2014 showedthatin aborted women the percentages of CMV for both IgG and IgM were (85%), (10%) respectively[32]. Also, other study in Iraq found that 90 pregnant women with an average age of 23 years had CMV IgG in rate (98.9%), while CMV IgM was (1.1%)[33].

Rubella is caused by RNA virus of paramyxovirus group. The pregnant women if contact with *Rubella* during the first 20 weeks of pregnancy will be harmfully affected by the virus. Its readily attacks the placenta and fetus then lead to miscarriage or stillbirth[34].Study was done in Iraq inaborted women with *Rubella* antibodies was positive for IgG in rate of thirty-four percent[35]. Other study in Turkey the seropositive of the pregnant women for *Rubella* IgG, IgM were (96.1%) and (0.2%) respectively[36]. Sadik in 2012 observed in India the seropositive of IgG for *Rubella* in pregnant women was (29.06%)[37]. Acharya in 2014 showed that in Nepal the women with spontaneous abortions had *Rubella* IgG in rate (11.7%)[19]. Khudhair and Ahmed in 2015 recorded that the women in Iraq who had a one abortion the titer of *Rubella* IgG was (40%)[38].

Other report in Iraq revealed that in pregnant women with previous abortion the rate of *rubella* IgG was (57.8%) and *rubella* IgM was (26.6%), while the rate of *rubella* IgM in pregnant women without history of abortion was (3.8%)[39]. Lamichhane in 2016 found that in spontaneous miscarriage the percentage rate for IgG was (43.68%) and for IgM was (7.76%)[40].

Also, pregnant women infected with *herpes simplex virus* can result neonatal, premature labor and abortion[41]. Biswas in 2011 reported that the pregnant woman in Indian the rate of IgG antibodies for HSV was (8.7%)[42]. Also, some studies in Iraq like Al-Marzoqi in 2012recorded in pregnant women the

dosages of TGF-β1, TGF-β2, TGF-β3 and its receptors, have been found in placentas after miscarriage, often with conflicting findings, highlighted by either decreased expression[76,77] or unmodified protein levels[72]. The higher level of TGF-β1 in pregnant women with miscarriage, compared with healthy pregnant women[78].

In this study, TGF- β1 was higher in group 2 compared with group3. Also, previous studies showed that *T. gondii*induced TGF-β secretion by immune cells as an anti-inflammatory agent to decrease the autoimmune responses and the serum levels of TGF-β increased at an early phase of infection with *toxoplasma*[79]. Another investigation also demonstrated that*T. gondii*induced macro-phages apoptosis through autocrine TGF-β signaling[80].

CONCLUSION

The current study ofIL-6 serum levels were highest in women with recurrent abortion who had positive Anti-Toxoplasma Ab as compared with other groups while TGF-β1serum levels were highest among recurrent abortion women with undefined causes as compared with other groups.

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While Rageb in 2015 showed that the IL-6 level was higher in *toxoplasma* group than control group[57], this may be critical role of IL-6 to resistancethe infection with *T. gondii*[58] and contributing totransformationfrom acute to chronic inflammation[59].

El-Hashimi in 2014 showed that IL-6 in aborted women infected withtoxoplasmosis was significantly higher compared to aborted women without toxoplasmosis[60]. Hafedh in 2015 recorded that in aborted women the concentration of IL-6 was more than in healthy womenbecause IL-6 play a vital role during the infection with *T. gondii* in aborted women[61]. 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 patient, this may occur early in infection before the immune response modified from Th2 into Th1 that responsible for the several cases of abortion[49,62].

Tumor growth factor-βeta may contribute to the regulation of maternal immune responses against the fetal allograft, and thereby prevent immunological rejection of the fetus[63].TGF-β1 is secreted by many cell types, including peripheral blood mononuclear cells and T regulatory lymphocytes[64].

Tumor growth factor-βeta1, exerting a predominantly anti-inflammatory effect and regulate several aspects of pregnancy, thereby contributes to maintaining immune tolerance[65,66,67]. 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 be a risk factor for the pregnancy outcome[68].

Several studies suggested that TGF-β1 may be involved in reproductive related disorders, such as preeclampsia and recurrent spontaneous abortion, although data were controversial[69,70]. An imbalance between the effector and regulator cells would lead to reproductive failure and related pregnancy disorders[71].

Other studies found that the levels of TGF-β1, TGF-β2 and TGF-β3 were unaltered in the plasma of spontaneous abortion[72]. TGF-β1 has both endocrine and paracrine actions, so in situ placental expression are more relevant than analysis of plasma levels[67]. Gutcher in 2011 reported that TGFβ1 was highly expressed by Th17 cells and acted in a predominantly autocrine manner to maintain Th17 cells *in vivo*, the role of TGFβ for activated T cell-produced in promoting the differentiation of Th17 cells and controlling inflammatory diseases[73]. The capacity of TGF-β1 to regulate the cytokine network that controls trophoblastgrowth&uterine invasion[74,75].

Other studies have examined TGF- β1 expression in the placenta of RPL cases, reduced

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