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Study the Correlation between Cytokines (IL-6, IL-27, TGF-Beta and IFN-Gamma) Levels and Microbial Abortion among Woman in Thi-Qar Province

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ABSTRACT

The study included 150 women (100 women with recurrent abortion (one or more abortion) and 50 women with normal pregnancies for three or more births without prior abortion and no infection. In the first three months (control group) samples were collected from Bint Al Huda Teaching Hospital in Dhi-Qar province for the period from September 2018 to March 2019. The current study included a number of immunological tests, including the determination of levels of immunoglobin IgG and immunoglobin IgM for (Toxoplasmosis, CMV, Rubella and HSV) and measuring some levels of cytokines (IL-6, interleukin-27 ,TGF-Beta and IFN-Gamma) in the case of recurrent abortion and healthy pregnant women were all performed using ELISA. Depending on the results of tests, the patients were divided into four groups: group one included 28 women recurrent aborted with sero-positive for anti-Toxoplasma antibodies IgG was (28%) and only two women with recurrent abortion sero-positive for anti-Toxoplasma antibodies IgM was (2%), group two included 26 women recurrent aborted with sero-positive for anti-CMV antibodies was (26%), group three included 20 women recurrent aborted with sero-positive for anti-Rubella antibodies was (20%), an group four included 24 women recurrent aborted with sero-positive for anti-HSV antibodies IgG was (24%), women with a healthy pregnancy promise as a control group was (50.0%), then measured serum levels of pro-inflammatory cytokines Interleukin-6 anti-inflammatory 16 Marker Tumor growth factor-beta (TGF-β),and Interleukin-27, IFN-Gamma for both women with recurrent abortion and healthy pregnant women (control group). The results showed that serum levels of IL-6 were low in the first and third groups, respectively, compared to the control group. There was a significant difference, whereas IL-27 was high in the first group compared to other groups 2, 3, 4 and the control group, Beta-TGF showed a significant increase in group 1,2,3,4 compared with the control group with significant differences between the two groups. IFN-Gamma showed a significant increase in group 4, 1 compared to control group with significant difference between groups. The results of this study showed a significant increase in the average concentration of (interleukin-27, TGF-Beta and IFN-Gamma) and levels interleukin-6 were low in women with recurrent abortion compared with healthy pregnant women.

Keywords: TORCH, Cytokines, Recurrent abortion, Interleukin-27

INTRODUCTION

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Receiving Date: April 10, 2020
Acceptance Date: April 30, 2020
Publication Date: May 04, 2020

Miscarriage is very common, with nearly one in four women experiencing an early pregnancy loss in her lifetime. Approximately 30% of all pregnancies end in abortion, the rate of pregnancy loss among clinically diagnosed pregnancies is 8 to 15 percent. As many as 80 % of abortion occur before 12 weeks' gestation, with abortion rates decreasing sharply after the first trimester [1]. Causes of RA

ISSN 2047-9093

are related to genetic factors, anatomical abnormalities, infections, and endocrine disorders [2]. However, the causes of the majority of RA cases are unknown, and may be related to autoimmunity [3]. T cells may play an important role in pre-implantation and embryo development in implantation process and in the phenomenon of fetal allograft tolerance [4]. Th1 cytokine inhibit Th2 cells expansion and Th2 cytokines block activation of Th1 cells [5]. 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 [6]. TGF-β in the presence of IL-6 can promote inflammation and autoimmune conditions [7].

AIMS OF THE STUDY

Measuring serum level of some cytokines, including (IL-6, IL-27, TGF-Beta and IFN-Gamma) in aborted women and investigating the correlation between the cytokines levels and microorganism in women with recurrent abortion 27 Li ve in Thi-Qar province.

MATERIAL AND METHODS

Blood samples were collected from 150 women (100 women with recurrent abortion (one or more abortions) and 50 women with normal pregnancy to three or more birth and without a previous abortion and without any infection) in the first trimester (control group) and then centrifuged the blood to separate the serum, the collected serum put in refrigerator (deep freezing) until used.

Level of interleukins: The concentration of IFN-γ serum; IL-6; IL-27 and TGF-Beta levels in the sera of patients was measured by sandwich Enzyme Linked Immunosorbent Assay (ELISA) according to the manufacturer's instructions.

RESULTS

The results of current study found cytokines level (IL-6, IL-27, TGF-Beta, IFN-Gamma) as 6.16 ; 35.28; 18.19 ; 14.68) respectively in serum for study group (women with recurrent abortion) compare to control (healthy pregnant women) as 7.67 ; 33.18 ; 9.43 ; 11.12) respectively in control group with high p.value (0.000) for TGF-Beta, the following table(1) below discuss the results above.

Table 1: The mean concentration of some immunological parameters among patients and healthy women

Immunological Parameter	Healthy (control group 50) Mean±SD	Study group (Patient 100) Mean±SD	t.value	p.value
IL-6	7.67 ± 6.16	6.16 ± 5.54	-1.33	**0.183**
IL-27	33.18 ± 11.10	35.28 ± 19.21	0.39	**0.608**
TGF-Beta	9.43 ± 2.18	18.19 ± 12.21	4.13	**0.000***
IFN-Gamma	11.12 ± 6.67	14.68 ± 10.11	1.21	**0.151**

df = 148, *Value ≤ 0.05 Significant

ISSN 2047-9093

The present study also shows the relation between IL-6, IL-27, TGF-Beta, IFN-Gamma and microorganisms and reveals it were (37.61) for IL-27, (19.66) for TGF-Beta, (19.72) for IFN-Gamma and (6.16) for IL-6 for aborted women infected with Toxoplasmosis with p. value (0.023), while aborted women with CMV found that 7.00 for IL-6, 27.47 for IL-27, 20.72 for TGF-Beta, and 11.07 for IFN-Gamma. Interleukins levels for those infected with Rubella found as 3.46 for IL-6, 21.58 for IL-27, 14.52 for TGF-Beta and 6.55 for IFN-Gamma. Aborted women infected with HSV also checked for interleukin levels it reported as 7.57, 30.37, 18.58, and 15.00 for IL-6, IL27, TGF-Beta, and IFN-Gamma respectively. All results above illustrated in the table (2) below.

Table 2: Correlation between the cytokines levels and microorganisms among women with recurrent abortion

Microorganisms		IL-6	IL-27	TGF-Beta	IFN-Gamma
Toxoplasmosis		6.16 ±5.94	37.61 ±22.81	19.66 ±11.17	**19.72 ±13.90**
CMV		7.00 ±5.20	27.47 ±14.84	20.72 ±9.59	**11.07±7.94**
Rubella		3.46± 2.54	21.58 ±11.68	14.52 ±8.52	**6.55 ±5.10**
HSV		7.57 ± 6.51	30.37 ± 21.44	18.58 ± 14.96	**15.00 ± 12.48**
P.Value		0.023*	0.016*	0.525	**0.008***
LSD	**1,3**	———	10.80*	———	**7.97***
	3,4	2.97*	———	———	———

The current study were also checked the levels of interleukins among ages groups and reported as 5.64 for IL-6, 28.70 for IL-27, 16.62 for TGF-Beta and 10.70 for IFN-Gamma among aborted women with age group (15-24) followed by those with age groups (25-29) it found as (6.30), (39.01),(20.60), (16.11) for IL-6, IL-27, TGF-Beta, IFN-Gamma respectively .finally for whom with age group (30-34) it represented as (6.77), (38.77), (14.61) ,(118.99) for IL-6, IL-27, TGF-Beta, IFN-Gamma respectively .All results above discussed in the table (3) below.

Table 3: Correlation between the cytokines levels and different age groups among women with recurrent abortion

Ages groups	IL-6	IL-27	TGF-Beta	IFN-Gamma
15-24	5.64 ±5.61	28.70 ±20.26	16.62 ±11.27	**10.70 ±9.30**
25-34	6.30 ±5.91	39.01 ±31.72	20.60[a] ±14.0	**16.11 ±15.10**
35-44	6.77 ±6.77	38.77 ±35.40	14.61 ±6.79	**18.99 ±15.90**
P.Value	0.788	0.394	0.278	**0.349**

ISSN 2047-9093

The present study were also checked the levels of interleukins in relation with frequency of abortion and reported as (6.82) for IL-6, (40.67) for IL-27, (13.39) for TGF-Beta and (14.41) for IFN-Gamma among aborted women with once abortion followed by those with two abortion it found as (5.09), (28.30), (20.39), (14.43) for IL-6, IL-27, TGF-Beta, IFN-Gamma respectively. Also for those aborted women with three abortion found that the interleukin levels are (4.53), (21.01), (14.23), (6.69) for IL-6, IL-27, TGF-Beta, IFN-Gamma respectively. Finally, for whom women with four abortion are represented as (8.19), (44.26), (28.69), (19.65) for IL-6, IL-27, TGF-Beta, IFN-Gamma respectively. With high p.value for TGF-Beta (0.004). All results above discussed in the table (4) below.

Table 4: Correlation between the cytokines levels and the frequency of abortion

Frequency of abortion		IL-6	IL-27	TGF-Beta	IFN-Gamma
Once		6.82 ±6.38	40.67 ±30.65	13.39 ±6.15	**14.41 ±12.99**
Twice		5.09 ±5.09	28.30 ±20.65	20.39 ±12.95	**14.43 ±10.19**
Third		4.53 ± 3.54	21.01 ±7.99	14.23 ±6.87	**9.69±8.79**
Fourth		8.19 ± 8.51	44.26 ± 30.06	28.69 ± 19.15	**19.65 ± 13.79**
p.value		0.265	0.187	0.004*	**0.656**
LSD	**1,2**	———	———	5.39*	———
	1,4	———	———	6.90*	———
	3,4	———	———	9.28*	———

The study also explain the levels of interleukins in relation with times of abortion (trimesters) and reported as (5.65) for IL-6, (30.27) for IL-27, (16.27) for TGF-Beta and (13.40) for IFN-Gamma among aborted women with first trimester of abortion followed by those with second trimester it found as (5.04), (27.98), (19.97), (18.61) for IL-6, IL-27, TGF-Beta, IFN-Gamma respectively. Also, for those aborted women with the third trimester found that the interleukin levels are (9.93), (21.37), (11.72), (6.08) for IL-6, IL-27, TGF-Beta, IFN-Gamma respectively. Finally, for whom with first and second trimester of abortion together it represented as (7.74), (21.10), (31.03), (25.28) for IL-6, IL-27, TGF-Beta, IFN-Gamma respectively. With high p.value for TGF-Beta (0.007). All results above discussed in the table (5) below.

Table 5: Correlation between the cytokines levels and abortion times

Time of abortion	IL-6	IL-27	TGF-Beta	IFN-Gamma
First	5.65 ±5.34	30.27 ±17.52	16.27 ±9.23	13.40 ±10.00
Second	5.04 ±3.52	27.98 ±12.39	19.97 ±7.98	18.61 ±12.11
Third	9.93± 9.10	21.37 ±6.26	11.72 ±3.34	6.08 ±3.06
First & second	7.74 ± 7.90	21.10 ± 6.67	31.03 ± 15.89	25.28± 17.20
P.Value	0.240	0.412	0.007*	0.047*

ISSN 2047-9093

LSD	**1,4**	———	———	7.18*	———
	3,4	———	———	11.53*	15.83

DISCUSSION

Measurement of cytokines levels in the serum: Pregnancy requires physiological adaptations in all material systems, including the immune system [8]. Cytokines, as critical immune regulatory 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 [9]. In the present study, the values of IL-6 were similar to the control group while values of IL-27 was elevated and TGF Beta was high in women infected with toxoplasmosis when compared with non infected women, also IFN-Gamma was high in sera of infected women. The results of this study agree with Aldabagh*et al* (2019) that showed that the serum levels of IL-6 showed significant dropping in infected women with toxoplasmosis compare to control [10]. Mohammed *et al* (2012) demonstrate a highly significant increase in serum levels of IL-17 and IL-10 in patients with acute toxoplasmosis [11]. Mohamed (2017) indicating increasing levels of IL-8 and IL-10 in patents sera infected with toxoplasmosis [12]. Differences in results among these studies may be attributed to the timing of sample collection after abortion and to the immune-competent of volunteer's subjects as well as to the size of data included in the study. Cytokines have been shown to play an important role in the pathogenesis of toxoplasmosis. The induction of type 1 inflammatory cytokine (IL-12, TNF-α, and IFN-γ) response is a key event in the initiation of immunity to T. gondii [13].

IL-6 is an important cytokine produced by a variety of cells such as macrophage, endothe27 Li al cells, and Th2 cells. This cytokine is responsible for the production of acute-phase proteins, increase cytotoxicity of NK cells and cytotoxic T-lymphocytes, and it enhances differentiation of B-cells to plasma cells and increases antibody production. In the current study, IL-6 levels showed a significant decrease in the case than controls. This result may occur after abortion in women with toxoplasmosis. Shifting toward Th1 immune response in aborted women leads to dropping of IL-6 levels, which elevated during pregnancy. Makhseed*et al., 2001*. reported that after abortion the immune system will begin to shift toward Th1 immune response after Th2 immune response during pregnancy [14]. On the other hand, Mousa and Bakhiet (2013) mentioned that IL-6 levels have increased during the course of infection by T. gondii in aborted women [15]. Differences in results among these studies may be attributed to the timing of sample collection after abortion and to the immune-competent of volunteer's subjects as well as to the size of data included in the study.

Many cytokines appear to be important in immune control of CMV infection, although defining cytokines that either may correlate with protection or increased susceptibi27 Li ty to infection in the context of congenital or prenatal CMV infection has been difficult in studies reported to date [16]. The values of IL-6 and IL-27 in women infected with CMV was less than of non-infected women while TGF-Beta was higher from non-infected women. The values of IFN-Gamma were similar for the values of the healthy group. The results of this study were confirmed by Banja*et al* (2018) who showed that no significant difference was noted in levels of IgM anti – CMV, IL-17, and TGF-Beta among healthy groups and women infected with cytomegalovirus in Baghdad [17]. Abdul Hussain*et al* (2018) refers to the levels of the cytokines IL-1, IL-6; IL-12 was highly significantly increased in sera of women infected with CMV compared to control groups [18]. MaBgorzata*et al* (2007), who noted that the concentration of IL-6 was statistically higher ($p = 0.05$) in patients with fetal defects and viral infection with CMV in comparison to patients with fetal defects but without infection [19]. IL-6 level was not significantly higher in women infected with CMV [20]. There is a shortage in levels of IL-6, IL-27 and IFN-Gamma in women infected with rubella while TGF-Beta was higher than women not infected with rubella. The present study showed that the level of IL-6 was similar between infected

ISSN 2047-9093

and non-infected with HSV while IL-27 was less than from non-infected women and TGF-Beta and IFN-Gamma were higher than non-infected women. Magdoud*et al.,* (2013) showed that the higher level of TGFβ1 in pregnant women with miscarriage, compared with healthy pregnant women [21]. Abdul kh27 Li q*et al* (2017) showed that the IL-6 and TGF-B1 were highest in cases of recurrent abortion and toxoplasma gondii infected women when compared with the control group [18]**.**

Koumantaki*et al* (2001) indicated that women with spontaneous abortion had significantly decreased plasma levels of IL-6, IL-8, and IL-11 compared to those with normal pregnancies [22]**.** Tumor growth factor-βeta may contribute to the regulation of maternal immune responses against the fetal allograft, and thereby prevent the immunological rejection of the fetus [23]**.** Differences between studies results can be because several laboratories different techniques used in these aspects, type of test that had been used and the number or types of the sample tested for infection and it is well-known that the epidemiology of infection is different among different populations. Also, patients' characteristics have been different among studies. The present study demonstrates that the IL-6 was high in healthy pregnant women compared to the women with recurrent abortion while the values of IL-27, TGF-Beta, IFN-Gamma were elevated in women with recurrent abortion more than healthy pregnant women. The result of this study agrees with the study done by Ahmed*et al* (2008) who show that the serum level of IL-6 was low in women with recurrent spontaneous abortion than those undergoing normal level [24] and disagree with Bakiret *al* (2010) found that the IL-6 increased in recurrent spontaneous abortion more than in healthy pregnant women. Also, the concentration of Il-6 was significantly high in recurrent abortion than normal pregnant [25-26]**.**

Tumor growth factor-βeta may contribute to the regulation of maternal immune responses against the fetal allograft, and thereby prevent the immunological rejection of the fetus. Power*et al*., 2002).TGF-β1 is secreted by many cell types, including peripheral blood mononuclear cells and T regulatory lymphocytes [27]**.** Several studies suggested that TGF-β may be involved in reproductive related disorders, such as preeclampsia and recurrent spontaneous abortion, although data were controversial [28]**.** Other studies found that the levels of TGF-β1, TGF-β2, and TGF-β3 were unaltered in the plasma of spontaneous abortion [29]**.** TGF-β1 has both endocrine and paracrine actions, so in situ placental expression are more relevant than analysis of plasma levels [30]**.** Ingman *etal*., (2011) reported that TGFβ1 was highly expressed by Th17 cells and acted in a predominantly autocrine manner to maintain Th17 cells in vivo [31]**.** The present study demonstrates that the IL-6 was elevated among women with age group (35-44) and dropping to a low level among women with age group (15-24). The value IL-27 was similar in the infected women with age group (25-34), (35-44) and low in age group (15-24). The high level of TGF-Beta was recorded in the infected women with age group (25-34) and the low level in the age group (35-44). The age group (35-44) record high level of IFN-Gamma and low level in age group (15-24), Furthermore the age group of 25-34 years old representing the highest number of aborted women ,who shown reduced level of both cytokines (IL-6 and IFN-Gamma) this might be due to the fact that this age representing optimum age group for reproduction, whereas aborted women group of 15- 24 years old reflected the lower IL-6, IFN-Gamma level and elevation in IL-27. The present study demonstrates that the IL-6 was highest in aborted women with fourth abortion and low in women with third abortion. Infected women with a fourth abortion show the high level of IL-27 and low level in the third abortion. TGF-Beta was elevated in women with forth abortion and drop to the low level in women with once abortion with high significant different p.value (0.004). In addition, IFN-Gamma was elevated in women with fourth abortion, similar in level in women with once, twice abortion and low in women with third abortion.

The present study demonstrates that the IL-6 was elevated in women who have an abortion in the third trimester more than those women who have an abortion in the first and second trimester. Women with abortion in the first trimester show that the IL-27 was high and low in women with third-trimester abortion. TGF-Beta was elevated in women who have an abortion in first & second

ISSN 2047-9093

trimesters together and low in women with third-trimester abortion. IFN-Gamma also shows elevated in women who have an abortion in the first &second trimester together and low in those women who have an abortion in the third trimester. The results show no significant difference in IL-6, IL-27, and highly significant in TGF-Beta, IFN-Gamma among women with different time of the abortion. This result revealed to the difference in viral activity at both second and third abortion and the infection might be prolonged for a long time and may lead to chronic state rather than acute phase disease or suggesting that a latent infection persistency at theses group aborted women and the reduction in interleukins among women group with third trimester abortion, this might be due to insufficiency of Cell-mediated immunity especially macrophage and T helper cell.

CONCLUSION

Serum level of IL-6 was decreased and IL-27, TGF-Beta, IFN-Gamma elevated in women with recurrent abortion compared to healthy pregnant women. The role of cytokines in recurrent abortion has become unclear over recent years. However, more research still needs to be done before one can devise treatment options for patients with recurrent abortion, based on the information available so far.

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ISSN 2047-9093