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Study of IL-8 and IL-17 levels among certain group of Repeated Spontaneous Abortion Women with or without Toxoplasmosis, Iraq

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Abstract

Toxoplasmosis is one of the most important zoonotic diseases worldwide caused by Toxoplasma gondii that leads to abortion or hydrocephalus during pregnancy. Thirty four female patients, age range from (14) years to (44) years with mean (29.45), with repeated spontaneous abortion suspected with toxoplasmosis were subjected for this study. Serum samples were collected from women undergoes more than two consecutive spontaneous abortion visit Babylon hospital for Maternity and children, Babylon-Iraq. All samples were investigated for specific IgG and IgM anti-toxoplasma antibodies to check toxoplasmosis in those women by using ELISA technique. This study also included measuring the level of IL-8 and IL-17 cytokine by (ELISA) to investigate their role in the immune-regulatory mechanisms involved in the repeated abortion. The results revealed that 13(38.2%) women were positive for anti-toxoplasma antibodies, 9(26.5%) women were positive for specific IgG anti-toxoplasma antibodies, while 4(11.8%) women were positive for both (specific IgG and IgM anti-toxoplasma antibodies). The results revealed that the toxoplasmosis were more prevalent in the women with age groups ranged from 14-24 years. Also there is substantial rise in the levels of IL-8 (1261.45±704.97) of Repeated abortion women with toxoplasmosis the when compared with Repeated abortion without toxoplasmosis (703.41±199.15) and control group (487.7 ± 134.6). The same results were observed with IL-17 levels which is significantly increased in the Repeated abortion women with toxoplasmosis (1491.6±500.98) when compared with Repeated abortion without toxoplasmosis (915.41±115.23) and control group (532.6 ± 127.8). Our study conclude that IL-17 and IL-8 involved in the induction of inflammation and occurring of repeated abortion.

Keywords: Toxoplasmosis, Abortion, IL-8, IL-17.

Introduction

Toxoplasmosis is a widespread parasitic zoonosis that caused by *Toxoplasma gondii* and occurs throughout the world. It can infect almost all the warm blooded animals, including human beings[1]. *Toxoplasma gondii* is an obligate intracellular protozoan parasite that can infect an extremely wide host range, from birds to mammals, including humans. The disease is of economic significance with regard to animal production, and it has become a public health concern since it leads to abortions and neonatal complications in humans. The definitive host for *T. gondii* is cat and the intermediate hosts are mammals and birds. The infection is acquired mainly by eating food or drinking water contaminated with oocysts or tissue cysts of *T. gondii*[2].

Many organisms, like *T. gondii*, *Chlamydia trachomatis*, *Niesseriagonorrhoeae* and others can cause recurrent pregnancy wastage due to maternal infections during pregnancy[3]. Perinatal infections account for 2% to 3% of all congenital anomalies. Toxoplasmosis is the most common infections associated with unfavorable outcome of pregnancy[4]. Primary infection with *Toxoplasma gondii* during pregnancy can lead to contrary outcomes, which are initially unobvious or asymptomatic and thus problematic to diagnose on clinical grounds[5].

This parasite can survive in all nucleated cells, including blood cells in acute stage, forms a specific vacuole that protect the parasite from host cell immune system. In the chronic stage, the parasite can form a cyst in the central nervous system, skeletal muscle and eye tissue and can exist for the lifetime of its host. The cysts can rupture and release highly invasive trophozoite, which may cause a recurrent infection and potentially fatal if the host is in a state of immune deficiency[6].

Primary maternal *T. gondii* infection through pregnancy is regularly associated with its transmission to the fetus[7]. The transmission rate of maternal infection to the fetus is estimated to be about 45%; of these, 60% are sub-clinical infections, 9% resulting in death of the fetus and 30% have severe damages such as hydrocephalus, intracerebral calcification, chorioretinitis and mental retardation[8].

Women who have acquisate T. *gondii* infection during pregnancy are treated with spiramycin to avoid transmission of *T. gondii* from the placenta to the fetus and with sulfadiazine and pyrimethamine to prevent fetal grievance if the fetus is found to be infected[9]. Successful pregnancy may dependon the bias of the immune response of mother shifting away from Th-1 type response towards a Th-2 phenotype, both in murine model

and human[10]. Normal pregnancy was accompanied by a reduction in Th-1 productive capacity together with an rising in Th-2 production, most markedly in the third trimester, The cause of repeated pregnancy loss (three or more successive spontaneous miscarriages) are unsolved in the majority of women and it is thought that anomalies in the immune system may have a role in idiopathic recurrent abortion[11].

Cellular immune effector mechanisms have been suggested as being responsible for at least a percentage of repeated spontaneous abortion (RSA). Recent attention has focused on clarifying the immunobiological roles of cytokines in normal human pregnancy following the collected reports of complex cytokine activity within uteroplacental tissue[12]. Cytokines are important mediators in the bi-directional interaction between the maternal immune system and the reproductive system during pregnancy[13,14].

Interleukin-8 (IL-8) and interleukin-17 (IL-17) are pro-inflammatory cytokines produced by several tissues upon the inducement 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[15].

Interleukin 8 (IL-8) is a chemokine produced by macrophages and other cell types such as epithelial cells. It is also synthesized by endothelial cells, which store IL-8 in their storage vesicles, the Weibel-Palade bodies. IL-8, also known as neutrophil chemotactic factor, has two primary functions. It induces chemotaxis in target cells, primarily neutrophil but also other granulocytes, causing them to migrate toward the site of infection. Endometrium also produces IL-8 that is abortogenic. The Endometrium, myometrium, and outer decidua contain mast cells that are increased by more than 10-fold in decidua in abortions. Mast cells are essential for inflammation by liberating several multifunctional cytokines including IL-8[16].

The fact that Interleukin-8 secretion is increased by oxidative stress, which in that way causes the enrolment of inflammatory cells, induces a further increase in oxidative stressmediators, making it a key parameter in localized inflammation. If a pregnant mother has high levels of interleukin-8, there is an increased risk of schizophrenia in her offspring[15].

Th17 cells are directly involved in chronic inflammatory processes, by secreting IL-17, which recruits neutrophil to tissue through induction of granulocyte colony stimulating factor and IL-8[17].

The aims of this study were: 1-to assess seroprevalences (IgG and IgM) of *T. gondii* in women repeated spontaneous abortion and to find out if there is any significant relationship between this infection and repeated spontaneous abortion. 2-To determine the role of IL-8 and IL-17 in the immuno-regulatory pathways involved in repeated spontaneous abortion in women with or without toxoplasmosis.

Material and Methods

This study included thirty four female patients with repeated spontaneous abortion suspected with toxoplasmosis. The age assortment from (14 - 44 years) with mean (29.45). Fifteen health women were subjected as control. All patients visit Babylon hospital for Maternity and Children, Babylon-Iraq; with more than two consecutive spontaneous abortion during a period from February 2011- march 2012. Clinicalinformation at demonstration was records, and sera were collected and stored in aliquots at -20°C.

Enzyme linked immunosorbent assay (ELISA) was used to evaluate Toxoplasma IgG, IgM –specific antibody (Biocheck®) level among patients. It is also used to determine the level of IL-8 and IL-17 (Komabiotech).

Statistical analysis: Evaluation of paired data from the three clusters of subjects was done using T-test (t) and least significant difference (LSD) between groups. Statistical tables including observed frequencies with their percentage[18].

Results and Discussion

The results showed that 13(38.2%) of the 34 aborted women have antibodies against T. gondii, in which only 4(11.8%) women give positive results for both specific IgM and IgG anti-toxoplasma antibodies. Only 9(26.5%) women were positive for IgG, while the specific IgM anti-toxoplasma antibodies alone not recorded in the current study table-1.

Regarding the distribution of toxoplasmosis among age groups of women with repeated the current result record that there was no significant differences and it was common among all studied age groups. The mean of IgM index was (4.63) and (0.95) for IgM-seropositive womenand control group respectively, while the mean of IgG level was (53 u/ml) for IgG- seropositive women and (10 u/ml) for control group (table-2, table-3).

Table-1
Distribution of T. gondii IgG and IgM antibodies using ELISA test in women with repeated abortion compare with healthy controls

Case	Both IgM+IgG		IgM		IgG	
	Positive	Negative	Positive	Negative	Positive	Negative
Repeated abortion (n=34)	4(11.8%)	30(88.2%)	0.0	34(100%)	9(26.5%)	25(73.5%)
Control	0.0	15 (100%)	0.0	15 (100%)	0.00	15 (100%)

The results gathered from table-4 shows significant increase in both cytokine (IL-8 and IL-17) in aborted women infected with acute Toxoplasmosis in comparison with control samples and there is a significant increase in both cytokines in aborted women having no toxoplasmosis IgM antibodies in comparison with control. Also table-5 reveal significant increase in result of both cytokines (IL-8 and IL-17) in both groups, infected and non-infected with toxoplasmosis among aborted women, in comparison with control sample. The result also shows that increased cytokine levels in non-infected women compared with infected aborted women. In comparison between, patients with IgG and IgM, the cytokine levels are variable for both groups at both cytokine according to IgM and IgG antibodies.

Both groups infected and non-infected revealed significant increase in interleukin levels, this result might refer to that acute toxoplasmosis have a role in abortion as causative factor in certain group of patients, while there is another group having causes other than toxoplasma antibody induces this abortion. Presence of anti-Toxoplasma IgM antibody refer to either acute infection, latent or reactivated, this might appear at the beginning of pregnancy because the pregnancy itself lead to immune suppression.

In Infected women, with IgM antibody, elevation of cytokines and reduced with chronicity of infection, while in abortion without Toxoplasma antibodies the cytokine are reduced in IgM association and increased in association with IgG antibody.

Presence of IgG antibody might refer to chronic state of infection, IgM in toxoplasma infection may remain for long time from onset of infection, so presence of both IgM and IgG among same patients need follow up and should be repeated after different period of pregnancy, from the first month of pregnancy to first trimester.

Table-2
IgM index and distribution of *T. gondii* IgM antibodies according to age of women with repeated abortion

Age group (years)	No. of Samples	IgM positive		The mean IgM index
		No.	%	
14-24	12	2	16%	4.5
25-34	13	1	7%	5.8
35-44	9	1	11%	3.6
Control	15	0.0	0.0	0.95

Table-3
The Level of IgG and distribution of *T. gondii* IgG antibodies according to age of women with repeated abortion

Age group (years)	No. of Samples	IgG positive		Mean of IgG level (u/ml)
		No.	%	
14-24	12	4	33%	60
25-34	13	5	38%	43
35-44	9	4	44%	56
Control	15	0.0	0.0	10

Table-4
The relationship between interleukin (IL-8 and IL-17) levels and toxoplasmosis (IgM)

Samples	No.	IL-8 level	IL-17 level	IgM level	Statistic
Repeated abortion with toxoplasmosis	4	1261.45±704.97	1491.6±500.98	4.3	LSD = 44.16
Repeated abortion without toxoplasmosis	30	703.41±199.15	915.41±115.23	0.95	
Control	15	487.7 ± 134.6	532.6 ± 127.8	0.95	

Table-5
The relationship between interleukin (IL-8 and IL-17) levels and toxoplasmosis (IgG)

Samples	No.	IL-8 level	IL-17 level	IgG level (u/ml)	Statistic
Repeated abortion with toxoplasmosis	11	700.93±93	931.37±269.35	57	LSD= 46.24
Repeated abortion without toxoplasmosis	23	801.64±238.11	1004.8±128.87	10	
Control	15	487.7 ± 134.6	532.6 ± 127.8	10	

Elevation of IL-8 in acute infection refers to chemotactic activation of macrophages and antigen presenting cells , due to the presence of infective agent and continuous presentation of its, while reduced in chronic because the criteria of chronic immune response differ from acute. IL-17 is a cytokine can act as cross road between humoral and cellular immunity, mostly enhancement production at acute infection especially in viral or intracellular infection reduced with time after maintenance or regulation of immune reaction against such agents.

Discussion: About one third of world`s people is infected by Toxoplasma gondii. In contrary to the immunocompetnet individuals, acute infection during pregnancy constitutes a great maternal challenge due to the threat of congenital toxoplasmosis[19]. Serum testing is still used for diagnosis of toxoplasmosis, antibodies which are increasingly used, most cases with positive IgG titer is enough to establish that the patient has been infected with Toxoplasma gondii, and indicate chronic infection, while positive IgM indicate recent infection, also negative IgM result may indicate so early that antibody response has not yet developed or is undetectable[20].

In acute infections of toxoplasmosis, IgG and IgM antibodies levels generally rise within one to two weeks of infection[21]. The presence of raised levels of T. gondii specific IgG antibodies specifies that infection has arose but does not discriminate between recent infection and infection learned in the distant past. Detection of T. gondii specific IgM has been used as an aid in determining the time of infection: a negative IgM test result with a positive IgG result usually indicates infection at least six months previously. However, the interpretation of T. gondii specific IgM positive result is complicated by the persistence of IgM antibodies up to 18 months after infection[22].

The elevated level of IL-8 can be detected in serum of women with at least three spontaneous abortion[23]. The inflammatory cytokines, such as IL-8, may play avital role in the mechanism of protease-induced neurogenic inflammation leading to labor or abortions by enrolling neutrophils and lymphocytes in the endometrium[24]. Whereas previous study reported that women with spontaneous abortions had pointedly decreased plasma level of IL-8 compared to those with normal pregnancies[25].

The high level of IL-8 in aborted women may be due to the release of IL-8 from the endometrium as well as from an increased number of resident mast cells that are degranulated in abortions[26]. 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[24].

Furthermore, during pregnancy, IL-8 is produced by a variety of cells, mainly monocytes / macrophages[27]. IL-8 induced activation of neutrophils and elastase activity in the intrauterine environment has been implicated in the mechanisms of rupture of fetal membrane and cervical ripening[28]. The role T-helper-17 in the immune response against toxoplasmosis was studied in the current infection, it was found that there was a concomitant increase of IL-17 with the increase of T-helper-1 cytokine[29].

The early increase in serum level of IL-17 in the present study matches the results of several researchers who found that an early increase in IL-17 had been reported in early stage of infection[30]. Ye et al., [31]found that IL-17 was involved in the development and early recruitment of neutrophils, which are essential to clear the parasites during initial stages of infection.

Conclusion

Th17 cells play avital role in the induction of inflammation[32]. In the obstetrics and gynecologic field, it has been stated that IL-17 stimulates IL-8 production in endometriotic stromal cellsand amniotic mesenchymal cells in chorioamnionitis. IL-17 also improves (TNF)-alpha-induced IL-8 secretion by amniotic mesenchymalcells[33]. Thus, (TNF)-alpha and IL-17 might accommodating lyenhance IL-8 secretion, ensuing neutrophil accumulation at the decidua in inevitable abortion[34]. Our study concludes that IL-17 and IL-8 involved in the induction of inflammation and occurring of abortion.

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