Evaluation of IL-2, IL-8, IL-10 Expression in Trophoplastic Tissue of Women with Spontaneous Miscarriage Infected by *Toxoplasma Gondii*

Ahmed Majid[1], Nidhal Abdul Mohaimen[2]

Abstract

Background: For normal pregnancy to be established, a Th2 type immune response must be induced by the maternal immune system at the maternal-fetal interface .The induction of a strong type 1 cytokine response at the fetal maternal interface may result in rejection of the fetus. Thus, such response could contribute to spontaneous abortion during acute toxoplasmosis in pregnant women.

Aim: To investigate the level of IL-10, IL-2 and IL-8 proteins expression within trophoplastic tissue in patients complaining spontaneous miscarriage, and they were *T.gondii* positive.

Materials and Methods: A total of fifty women, aged between (16 − 42) years, were involved in this study. Enzyme Linked Immunosorbent Assay test was used for the detection of specific IgM(using serum samples) and immunohistochemistry(using . trophoblastic tissue) method was used for the detection of antigen in trophoplastic tissue as diagnostic methods for *T.gondii* and evaluation of IL-10, IL-2, IL-8 proteins .Samples were classified into three groups: Group A- patients with spontaneous miscarriage and *Toxoplasma gondii* positive (n= 20 women) , with a mean age of (23.8± 1.631);Group B- patients with spontaneous miscarriage and *Toxoplasma gondii* negative(n= 20 women), with a mean age of (25.5± 1.60);Group C- Control group ,women with induced abortion for medical causes(n=10 women), with a mean age of (26.4± 1.628).

Results: The highest percent of IL-2(65.25±1.599)% was within group(A) then lower than this(63.50±2.335)% was within group (B) and the lowest percent (11.60±1.522)% was within the control group. While the highest percent of IL-10 (70.60±2.272) % was within the induced abortion group, and the lowest was found within (A) and (B) groups (20.80±1.268) %and (22.25±1.859) % respectively, The highest percent of IL-8 (55.80±3.427)% was within group (A) , lower than this(43.65±3.224)% was within group (B) and the lowest percent(14.40±1.327)% was within the control group,

Conclusions: These results highlighted the possible protective role of IL-10 during successful pregnancies, and that IL-2 increased in expression in cases of spontaneous miscarriage .

Key words: trophoplastic tissue, Spontaneous miscarriage, *Toxoplasma gondii*, IL-2, IL-8, IL-10.

[1] Al-Nahrain Medical College
[2] Al-Nahrin Medical College, Department of Microbiology

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Introduction:

Spontaneous miscarriage is the term used for a pregnancy that ends on its own, within the first 20 weeks of gestation, a baby that dies after 20 weeks gestation (or who weighs more than500 grams) is classified as a stillborn[1]. *Toxoplasma gondii*, a common protozoan parasite responsible for both severe congenital birth defects and fatal toxoplasmic encephalitis in immunocompromised people. Congenital fetal toxoplasmosis may result in abortion, stillbirth, or severe mental retardation; infections in late pregnancy may be asymptomatic but present with retinal or neurological damage later in life[2]. For normal pregnancy to be established, a Th2 type immune response must be induced by the maternal immune system at the maternal-fetal interface[3], which leads to up- regulation of Th2 and Tc2 cells, which produce IL-4, IL-6, IL-10 and IL-13[4].

Women who undergo spontaneous abortion may have a stronger Th1-response which produces interleukin IL-2[5], interferon IFN-γ, and tumor necrosis factor TNF-β. So, infection of a pregnant mother by *T.gondii* may induce an aggressive Th1 CMI and hence leads to abortion[6].

In addition, IL-8 may be indirectly stimulated via endotoxin-induced inflammatory cytokine, such as IFN-γ, TNF-α and IL-1-α, these cytokines are known to up regulateIL-8 expression in hemopoietic cells[7]. IL-8 displays both inflammatory and growth –regulating properties, but is notable for its selective chemotaxis, degranulation, and activation of neutrophils [8,3],which lead to up-regulation of Th2 and Tc2 cells, which produce IL-4, IL-6, IL-10 and IL-13[4].

Women who undergo spontaneous abortion may have a stronger Th1-response which produces interleukin IL-2, interferon IFN-γ, and tumor necrosis factor TNF-β. So, infection of a pregnant mother by *T.gondii* may induce an aggressive Th1 CMI and hence leads to abortion[9].

In addition, IL-8 may be indirectly stimulated via endotoxin-induced inflammatory cytokine, such as IFN-γ, TNF-α and IL-1-α, these cytokines are known to up regulateIL-8 expression in hemopoietic cells[7]. Hence this study aimed to investigate the level of IL-10, IL-2 and IL-8 proteins expression within trophoplastic tissue in patient complaining spontaneous miscarriage, and were *T.gondii* positive**.**

Materials and Methods:

A Total number of 40 pregnant ladies were included in this study; they were admitted to Al-Kadhmya Teaching Hospital for spontaneous miscarriages for evacuation. Other 10 females were also included with elective termination of pregnancy due to maternal condions that need termination of pregnany (induced abortion), they were considered as a control group. According to the results of the ELISA for the detection of anti *T.gondii* IgM antibodies and immunohistochemical analysis for the detection of *T.gondii* antigen, which was done,the patients were divided into three groups:-

Group A: 20 patients positive for *T.gondii*
Group B: 20 patients negative for *T.gondii*
Group C: 10 negative for *T.gondii* induced abortion) control group.

Blood Collection:

Five mls of venous blood were collected and sera were then aspirated using a Pasteur pipette and dispensed into sterile eppendorf tubes (100 µl in each) and stored at -20 ºC until used.

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Enzyme Linked Immunosorbent Assay for the detection of IgM antibodies for Toxoplasma gondii in serum

Materials that were provided with the kit: (BioCheck, Inc. Foster City, CA, USA; 2004).

a. The mean of duplicate cut-off calibrator values XC was calculated.
b. The mean of duplicate positive control (XP), negative control (Xn) and patient samples (XS) was calculated.
c. The *Toxoplasma* IgM Index of each sample was calculated by dividing the mean values of each sample (X) by calibrator mean value, XC.

Negative: Toxo M Index less than 0.90 is negative for IgM antibody to *T. gondii*.

Immunohistochemical analysis for the detection of *Toxoplasma gondii* antigen and IL-10, IL-8, IL-2.

DakoCytomation LSAB2 System-HRP code K0673 (DakoCytomation , USA), immunohistochemistry detection kit, this kit was used for detection of IL-10,IL-8,IL-2.

Table (1): Monoclonal Antibodies Applied in the Study

Type of antibody	Clone	Iso Type	Company
Rabbit anti –human IL-8.	AHP781	IgG1 to human IL-8.	Serotec,Ltd Oxford, UK.
Mouse-anti-human IL-10.	MCA926	Mouse IgG1	Serotec,Ltd Oxford, UK.
Mouse-anti-human IL-2 conjugated		Mouse IGg1	Biosource,Belgum
Mouse anti-human *T. gondii* antigen		IgG1-kto *T.gondii* RH strains.	Chemichon, USA

Trophoblastic Tissue

Trophoblastic tissue was collected from the evacuation of retained pieces during the procedure of curettage and placed in 10% formaldehyde. Two to three paraffin embedded blocks were prepared for each patient. Staining with haematoxyline and eosin was carried out to decide which block can be used in the study (only sections that contained trophoplastic tissue was included in this study).

Paraffin embedded sections were cut into 5µm thickness, placed on Fisherbrand positively charged slides and left overnight to dry at room temperature.

To determine the signal specificity, negative control slides were included. In the first run, the negative control slides included sequential omission of reactive components in the test; the primary (monoclonal) antibody, the secondary antibody (the biotinylated link), the conjugate and the substrate. Then, in each immunohistochemistry run, the negative control slides were obtained by omitting the primary antibody and applying antibody diluent alone (Lyall *et al.*, 2001)(9). This was under identical test conditions (i.e. on the same slide).

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Placental tissue obtained from women with normal vaginal delivery was considered as a positive control for IL-10, IL-8 and IL-2 (Tamiolakis *et al*., 2005)[10].

The expression of IL-8, IL-10, and IL-2 was measured by the same scoring system, by counting the number of positive trophoblastic cells which gave brown cytoplasmic staining system under light microscope.

The extent of the IHC signal in the villi was determined in 10 fields (X100magnification). In each field the total number of villi was counted and the extent of cytoplasmic staining of the trophoblast cells in a given villous was determined as a percent. The total staining score was divided by the number of whole villi per field in 10 fields[11], so the percentage of positively stained villi in the 10 fields was calculated for each case by taking the mean of the percentage of the positivity stained villi in the 10 fields. For detection of *Toxoplasma gondii*, immunostaining was scored according to the cut-off value. This cut-off for positivity was: any trophoblast villous containing *Toxoplasma gondii* Ag (presence of a red reaction) is considered to be positive (according to manufacturer instruction).

Statistical Analysis

Data processing was done by using Statistical Package of Social Science (SPSS) version 16.

The independent sample t-test of significance was used for the comparison between two groups .The lowest level of significance was chosen when the probability (p) was less than or equal to 0.05 ($p \leq 0.05$).

Results:

The expression of IL-2, IL-8 and IL-10 was detected by immunohistochemistry (IHC) technique. Scoring system was used to express the level of expression of these cytokines. Among the three groups (A, B, and induced abortion C), the highest percent of IL-10 was found in induced abortion group(C) (70.60±2.272)% , which is negative for *T.gondii* Ag, while the lowest percent was found in the group(A) with spontaneous abortion(20.80±1.268)%, figure(1) . IL-2 highest percent was found in the group(A) (65.25±1.599)%,the lowest percent was found in induced abortion group(C) (11.60±1.522)% ,figure (2). And the highest percent of IL-8 was found in the group(A) with spontaneous abortion (55.80±3.427)% , while the lowest percent was found in the induced abortion group(C) negative for *T.gondii* Ag(14.40±1.327)% ,Figure (3).

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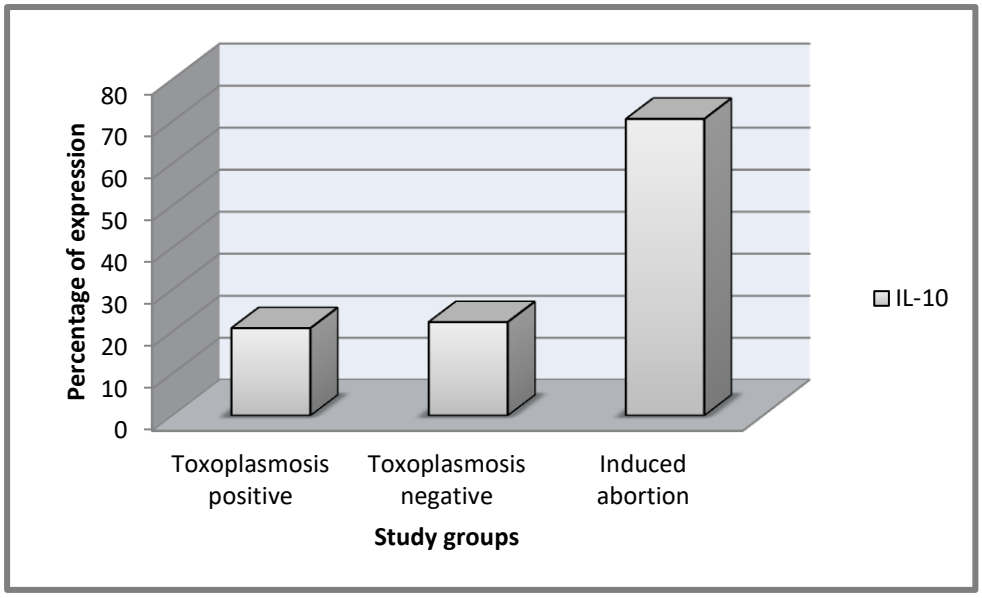

Figure (1): IL-10 percentage in the three groups of cases (A, B and C).

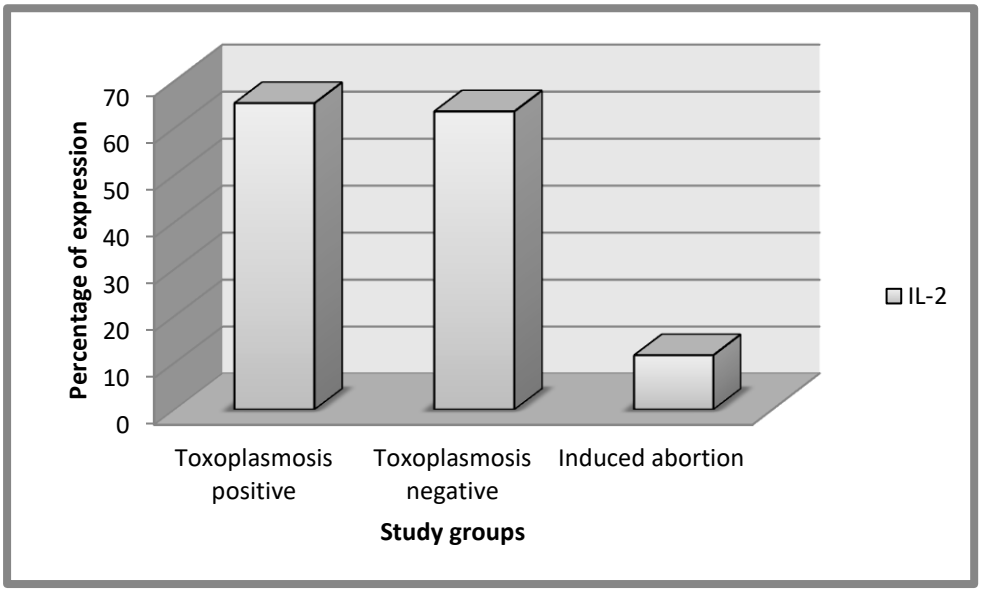

Figure (2): IL-2 percentage in the three groups of cases (A, B and C).

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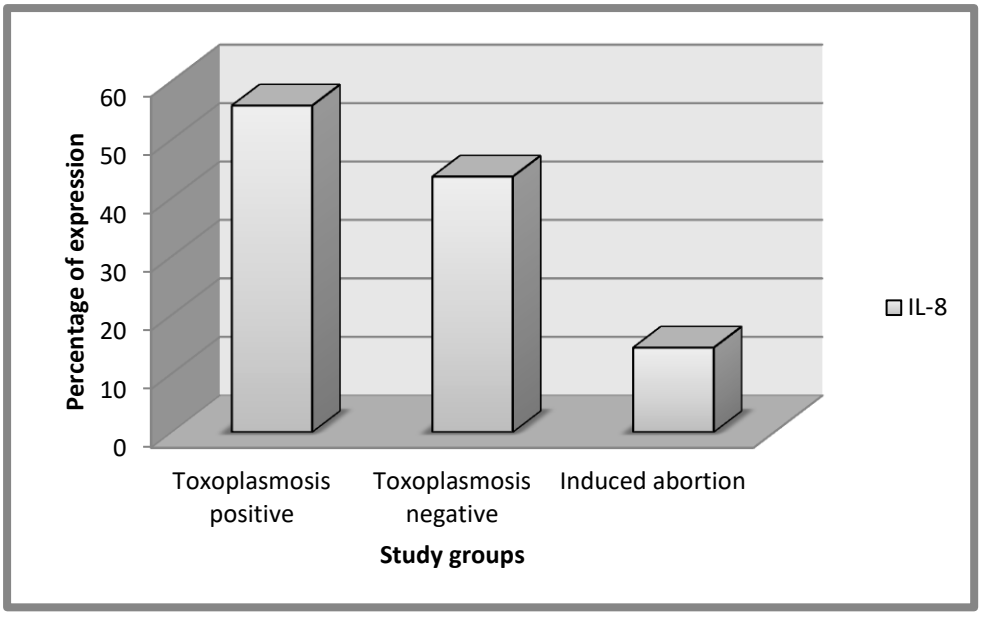

Figure (3): IL-8 percentage in the three groups of cases (A, B and, C).

Table 2 shows that there was no statistical difference (P>0.05) in the mean percent of IL-10 between positive and negative group with spontaneous abortion. While there was a highly significant difference (p<0.001) in the mean percent of IL-10 between group (A) with spontaneous abortion and induced abortion group. And there was a highly significant difference (p<0.001) in the mean percent of IL-10 between group (B) with spontaneous abortion and induced abortion group.

Table2 also shows that there was no statistical difference (P>0.05) in the mean percent of IL-2 between (A) and (B) group with spontaneous abortion. But there was a highly significant difference (p<0.001) in the mean percent of IL-2 between group (A) with spontaneous abortion and induced abortion group. And there was a highly significant difference (p<0.001) in the mean percent of IL-2 between group (B) with spontaneous abortion and induced abortion group.

Regarding IL-8, table 2 also shows that there was significant difference (p<0.05) in the mean percent of IL-8 between (A) and (B) group with spontaneous abortion. While there was a highly significant difference (p<0.001) in the mean percent of IL-8 between (A) group with spontaneous abortion and induced abortion group. Also, there was a highly significant difference (p<0.001) in the mean percent of IL-8 between (B) group with spontaneous abortion and induced abortion group.

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Table (2): IL-10, IL-2 and IL-8 proteins expression in the three study groups.

		Miscarriage groups		Induced Abortion	Sig. between groups		
		A	B	C	A/B	A/C	B/C
IL-10	Mean+ SE	20.80±1.268	22.25±1.859	70.60±2.272	0.523	0.000**	0.000**
IL-2	Mean+ SE	65.25±1.599	63.50±2.335	11.60±1.522	0.540	0.000**	0.000**
IL-8	Mean+ SE	55.80±3.427	43.65±3.224	14.40±1.327	0.014*	0.000**	0.000**

** Highly significant difference ($p<0.001$), SE= Standard Error,*Significant difference ($p<0.05$)

Discussion

This study demonstrated that IL-2 proteins were expressed in lower levels in women with induced abortion which could be explained by previous studies showing that the pro-inflammatory cytokines act physiologically in normal pregnancy and high levels may cause recurrent miscarriage[12]. This significantly higher level of IL-2 in group (A and B) can be explained by the same results of Clark and colleagues (1998)[13] . The association between Thl cytokines and pregnancy failure and the demonstrated deleterious effects of Th1 on the conceptus and pregnancy, suggested that Thl-mediated effects may have been the cause of pregnancy failure in at least a proportion of cases in the study in which the expression of Th1 was significantly higher[14]. The present study demonstrated that induced abortion group (group C) showed high level of IL-10 protein expression detected by immunohistochemical staining, with a highly significant difference ($p<0.001$) from those with spontaneous miscarriage (group A) in whom the expression of IL-10 was in low level, and a highly significant difference ($p<0.001$) from (group B) in whom the expression of IL-10 was also in low level, which is in consistence with a previous studies[15-17].

This significantly lower IL-10 protein expression in group (A and B) could be attributed to defect in Th2 and Tc2 cells at the feto-maternal interface or to the accumulation failure of Th2 cells at the implantation site in women with spontaneous miscarriage[18]. Thus, IL-10 may be critical in normal fetal development and down-regulation of inflammatory responses in the placental microenvironment[19].

Our results come in agreement with those of [16,17] ,concerning the expression of IL-8proteins in trophoplastic tissue of women with recurrent miscarriage which shows high IL-8 level in RM group, but her result regarding the control group was not in agreement with our study, this could be due to many factors depending on the type of antibody, its sensitivity or technical differences.Our results of IL-8 were in agreement with that of[20].

The low expression of IL-8 in induced abortion group could be explained by that IL-8 production seems to be under the control of progesterone[21]. Also, IL-8 may be indirectly stimulated via endotoxin-induced inflammatory cytokines,

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such as IFN-γ, TNF-α and IL-1α, these cytokines are known to up regulate IL-8 expression in hemopoitic cells. IL-8 displays both inflammatory and growth regulation properties [8.22].

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