

## Cytokines Level (IL8 and IL17) in Pregnant Women with Toxoplasmosis in Khartoum State

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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. It's a comparative cross-sectional one designed to assess immunoglobulins and cytokines in pregnant women. A total of 300 venous blood samples were collected from each pregnant woman and centrifuged to obtain serum. Patient's information was recorded in a questionnaire previously designed for the purpose of analysis. In addition, 40 uninfected women were enrolled in the study as control group to assess the level of IL8 and IL17 cytokines. The overall seropositive rate of *Toxoplasma gondii* infection was 22.6%. Within the positive cases of study population, only 16 and 13 showed positive results of IL8, IL17 respectively. The results showed highly significant increase in the mean serum level of IL8 (210.25pg/ml) and IL17 (203.15 pg/ml) when compared to the control group who showed 68.9 pg/ml and 54.8 pg/ml respectively. The serum level of proinflammatory cytokines investigated in this study seems to be increased in patients with serological evidence of *Toxoplasma gondii* infection. Our study concludes that IL-17 and IL-8 involved in the induction of inflammation and toxoplasmosis disease.

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## 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<sup>1</sup>. *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*<sup>2</sup>. Toxoplasmosis is the most common infections associated with unfavorable outcome of pregnancy<sup>3</sup>.

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<sup>4</sup>. Primary maternal *T. gondii* infection through pregnancy is regularly associated with its transmission to the fetus<sup>7</sup>. 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<sup>5</sup>.

Women who have acquired *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<sup>6</sup>. Successful pregnancy may depend on 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<sup>7</sup>. 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. Cellular immune effector mechanisms have been suggested as being responsible for at least a percentage of repeated spontaneous abortion (RSA). Cytokines are important mediators in the bi-directional interaction between the maternal immune system and the reproductive system during pregnancy<sup>9,10</sup>. 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<sup>11</sup>. 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. 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. Mast cells are essential for inflammation by liberating several multifunctional cytokines including IL-8. If a pregnant mother has high levels of interleukin-8, there is an increased risk of schizophrenia in her offspring<sup>11</sup>.

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.

The aims of this study were: 1-to assess seroprevalences (IgG and IgM) of *T. gondii* in pregnant women 2-To determine the role of IL-8 and IL-17 in the immuno-regulatory pathways involved in in pregnant women with or without toxoplasmosis.

**Material and Method**

A comparative cross-sectional study conducted during the period of November 2016 – June 2017 in ante natal care unit of Omdurman friendship hospital. The study aimed to assess seroprevalences (IgG and IgM) of T. gondii and determine the role of IL-8 and IL-17 in the immuno-regulatory pathways involved in pregnant women with or without toxoplasmosis. After obtaining the informed consent socio-demographic data (age, residency) and, history of miscarriage were collected by using questionnaire. Then venous blood samples were collected in plain vacutainers and allowed to clot. Then sera were collected in Eppendorf tubes and stored at -20°C and used for ELISA test to measure (IgG, IgM, IL8 and IL17) after completion of the study period. Very briefly, 50µl of diluted serum sample loaded to the antigen-coated well without touching the wall. Then incubated for 30minutes and washed thereafter by using washing buffer. Then 50µl of horseradish peroxidase-labelled anti-IgG enzyme added to the wells. Coloring solution added and finally the absorbance read at 450nm using Microtiter Plate Reader.

**Statistical Analysis**

Data were entered into computer using Statistical Package for Social Sciences (SPSS) version 20 and doubled check. Data was analyzed to determine the biological importance ( $p \leq 0.05$ ) of cytokine level.

**Results**

*Sociodemographic Data*

A total of 300 pregnant women were enrolled in this study with mean (SD) age of 30.28(6.4) years and 40 non infected pregnant women were enrolled as control group. Previous miscarriage was reported in 186 (62%).

**Screening Results**

Out of 300 pregnant women screened for anti T.gondii antibodies by using ELISA, 65(21.6%) and 4 (1.3%) had seropositive T.gondii IgG and IgM, respectively table 1.

*The Rate of Cytokines Profile (IL8 and IL17) in Pregnant Women who had Toxoplasmosis*

Within the 65 positive cases of ELISA IgG, 16 (24.6%) and 13 (20%) were had high level of IL8 and IL17 respectively, (P value=0.00) significant. The result showed no high level of IL8 and IL 17(0%) within the 3 positive cases of ELISA IgM. table1.

*Comparison of Mean Level of Cytokines (IL8, IL17) in Pregnant Women and Non-Infected Pregnant Women Control*

In non-infected pregnant women controls, the mean IL8 serum concentration was 68.9pg/ml while in the pregnant it reaches 210.25 pg/ml. This showed statistical difference (p.value=0.00).Also, in non-infected pregnant women controls, the mean IL17 serum concentration was 54.8pg/ml while in the pregnant it reaches 203.15 pg/ml. This showed statistical difference (p.value=0.00) table 2.

**Discussion**

Toxoplasmosis is widely spreading around the world affecting human and animal<sup>12</sup>. Serum testing is still used for diagnosis of toxoplasmosis with IgG antibodies that indicate latent or chronic infection and IgM indicate recent or active infection.

Using enzyme linked immunosorbent assay (ELISA) technique the prevalence in this study was 22.6%. Several studies were done over the world using ELISA IgG, Some showed a high prevalence rate than the results obtained for example in Turkey 77% was

Table 1. The rate of cytokines profile (IL8 and IL17) in pregnant women who had toxoplasmosis.

Type of immunoglobulin	Positive cases	IL8 positive	IL17 positive
IgG	65	16(24.6%)	13(20%)
IgM	3	0(0%)	0(0%)
Total	68	16	13

Table 2. Comparison of mean level of cytokines (IL8, IL17) in pregnant women and non-infected pregnant women controls.

Interleukin	Number examined	Positive number	IgM/IgG	Mean of interleukin	Mean of control	p. value
				In pregnant		
IL8	300	27	16	210.25	68.9	0
IL17	300	24	13	203.15	54.8	0

recorded<sup>13</sup>. And in Togo 75% were reported by<sup>14</sup>. Also the results obtained by<sup>15</sup>. Showed that the prevalence was 38.9% by ELISA IgG in Khartoum state; also<sup>16</sup> was found 73.1% by using ELISA IgG in rural areas in Sudan. It may also disagree with result obtained in<sup>17</sup> who showed that the prevalence using ELISA was 35.1% positive IgG antibodies to *T. gondii* in Sudanese pregnant women. The result however, agreed with<sup>18</sup> who showed that 20.2% of pregnant women were positive for IgG. The role of toxoplasmosis in women with history of miscarriage is still unsettled, Previous miscarriage was reported in 186(62%).

Increased level of IL-8 correlates with early acute inflammation or with a reactive form of toxoplasmosis. IL-8 is responsible for activation and recirculation of neutrophils and neutrophils can phagocytose and kill or inhibit tachyzoites of *T. gondii* and showed that human intestinal epithelial cells infected with *T.gondii* elicit rapid secretion of IL-8<sup>19</sup> so it has an important role in innate immunity in response to *Toxoplasma*.

In this study, a high significant increase in the mean serum level of IL-8 level in pregnant women with serological evidence of with *T.gondii* (210.25 pg/ml) was statistically significantly higher than in non-infected pregnant control women. (68.9 pg/ml p.value =0.00). In this report, the mean level of IL-8 in pregnant women with serological evidence of *T.gondii* infection was higher than other groups. The significance of this finding for the outcome of the pregnancy remains uncertain. Pregnant women produced an endocervical cytokines response that was 2-fold higher than that produced by non-pregnant women, also the proinflammatory cytokines milieu in the cervix is enhanced in pregnant women with bacterial vaginosis compared with that in

non-pregnant women<sup>20</sup>. The present study showed a highly significant increase in the mean serum level of IL-17 in patients with IgM/IgG seropositivity (203.15pg/ml) when compared with serum levels in non-infected pregnant control group (54.8 pg/ml). The early increase in serum level of IL-17 in the present study match the results of several researchers<sup>21</sup> who found that an early increase in IL-17 had been reported in early stage of infection. Also one 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<sup>22</sup>.

### Conclusion

Th17 cells play avital role in the induction of inflammation<sup>23</sup>. Our study concludes that IL-17 and IL-8 involved in the induction of inflammation.

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