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No Considerable Evidence In Cytokines Profile Among Latent Toxoplasmosis Infertile Women. Cytokines In Toxoplasmosis Women

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***Abstract**: Toxoplasma gondii is an obligate intracellular protozoan parasite causes toxoplasmosis, is one of the most prevalent human diseases in many countries [2, 19]. Reports on the prevalence of T. gondii infection among infertile females are limited, No previous reports about the relation between T. gondii infection and female infertility although about 21% of abnormal embryos and 24.2% of miscarriages and stillbirths have been related to T. gondii infection [21]. The present study aimed to survey the infertile females and Toxoplasma infection investigating a possible relationship with infertility. Sera from 18 Toxoplasma infected women and sera from 30 free Toxoplasma infertile women were tested by ELISA IgM and IgG toxoplasma antibodies, and the level of serum IL4, IL-8, IL10 and IL33 was tested using ELISA technique.*

INTRODUCTION

Regardless of its function, Toxoplasma gondii is one of the obligatory intracellular protozoans,, which is responsible for common parasitic infections around the world [1], Toxoplasmosis is caused by infection with the Toxoplasma gondii, this type of infections produces a variety of clinical syndromes both in humans and animals, the main infection during pregnancy can result in disease transmission through the placenta and lead to hazardous consequences such as abortion, stillbirth, different degrees of mental or physical retardation, hydrocephalus, and blindness [2]. The infection stimulates cell mediated immunity and humoral immune response as antibody production, in addition to the role of cytokines, which essential for the host and control of intracellular infection [3].

Several serological methods have detected the immunoglobulin (IgG and IgM) antibodies against T. gondii in the serum and among the assays, ELISA shows high sensitivity and specificity [4]. IL8 has an important role in the innate immune response. It's often associated with inflammation. It has been cited as a pro-inflammatory mediator in Toxoplasmosis [5]. It is well recognized that T cell-mediated immunity plays a central role in the host response to intracellular pathogens [6]. T cell- mediated immunity and activated macrophages have been shown to play important roles in resistance to T cell-mediated immunity T. gondii infection [7].

The anti-inflammatory cytokine IL-10 plays an important role in reducing harmful pathological effects of inflammatory responses in T.gondii infection. IL-10 is a cytokine produced by DCs, macrophages, B-cells, Th2 cells and T-regulatory cells (Levings et al., 2002). IL-10-deficient mice showed elevated IL-12 levels and consequently increased IFN-γ

and TNF-α responses and intense hepatic inflammation and tissue necrosis [8]. During acute toxoplasmosis, IL-10 serves a dual role in the suppression of the host's cellular immune response.

IL-33 is a member of the IL-1 cytokine family , in the nucleus is associated with chromatin, [9, 10] upon cell stress or death, biologically active IL-33 is released and truncated by proteolytic cleavage [11] it may have a dual role in different inflammatory conditions, depending on the specific immune mechanisms underlying disease pathogenesis [12], infections induces proinflammatory cytokine and chemokine responses, which are reduced in absence of IL-33R/ST2 signaling [13].

The humoral immune response to T. gondii is rapid and intense, and forms the basis for useful diagnostic tests for the various forms of the disease, the present study aimed to detection of IL4, IL8, IL10 and IL33 by diagnostic method (ELISA) in serum infected and non-infected infertile women by toxoplasmosis.

MATERIALS AND METHODS

Exclusions Criteria
All exclusion criteria, such as family history, chronic and genetic disease, drinking and smoking, have previous abortions were account to exclude women from control one. After an interviewer managed we used forty eight enrolled infertile women.

Blood Collection
Statistical analysis was done using SPSS (Social Science Statistical Package) version 20 in which we use mean and standard deviation as descriptive statistics and LSD (the least significant difference) analysis of variance (ANOVA) for comparison between groups. The P value was considered significant if below 0.05.

Detection of Toxoplasma gondii Infections
Enzyme linked immunofluorescence assay (ELIFA) technique were used to confirm serum anti-toxoplasma IgG antibodies, the manual procedure accomplished by manufacture Biomerieux Company (France).

Enzyme linked immunosorbent assay (ELISA)
All cytokine biomarkers test in this study (IL-10,IL-33,IL-8 and IL-4) checked by sandwich ELISA briefly, serum were added in pre-coated micro ELISA plate wells, then a biotinylated detection antibody for each specific cytokine added after one half hour of incubation. HRP detection enzyme then added after 3 washes. TMP Specific substrate were used after 5 washes, blue color terminated by stop solution (diluted H2SO4). Optical density measured by using ELISA reader and result calculated by comparing O.D of sample by O.D of stander.

Study protocol and ethics
Study protocol approved and ethical issues done by local committee in college of science/ Babylon University, already the committee depends on principles of declaration Helsinki.

Statistical Analysis
All statistical analyses were performed according statistical software program (SPSS 10 Inc., Chicago, USA). All statistical comparisons were done by T-test. Variations were considered significant when P-value ≤ 0.05

RESULTS

In the present study the enrolled women (18 Toxoplasma infected and 30 free Toxoplasma infertile women) were checked by ELIFA techniques to confirm the infection of Toxoplasma gondii (positive just for IgG antibodies). The percentage of infection in women was 37%. Seroprevalence of cytokines profile in Toxoplasmosis free and infected women showed no significant variation in interleukin-8, 33 and interleukin-10 concentration (Figures 1, 2 and 3). Our finding showed a significant variation in serum Interlukin-4, were infected infertile women showed higher level as compare with free infected one($P = 0.018$, Figure 4). In additions, personal correlations between anti-toxoplasma IgG antibodies and all serum cytokine profile showed no significant variation see figures 5,6,7,8 were $P > 0.050$.

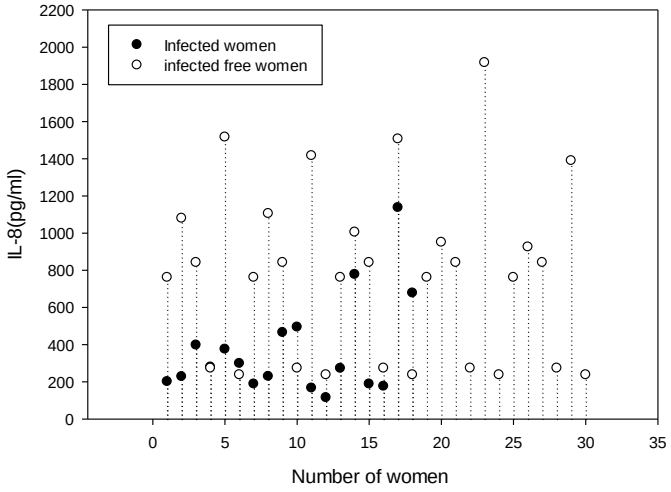

Figure 1. Comparison of mean concentration serum IL-8 Interleukin between patients and control Group.

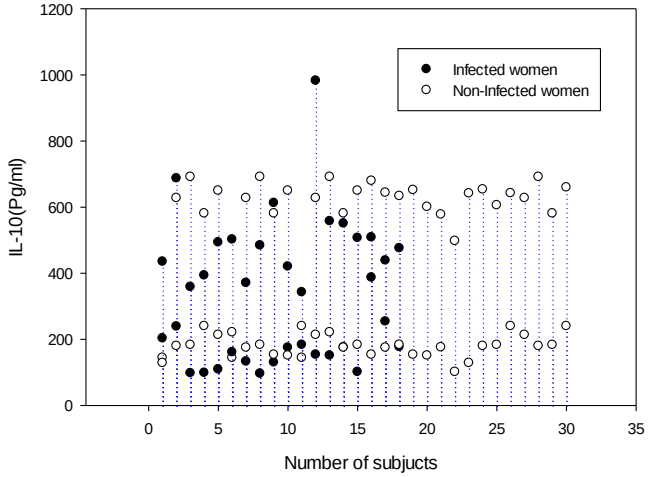

Figure 2. Comparison of mean concentration serum IL-10 Interleukin between patients and control Group.

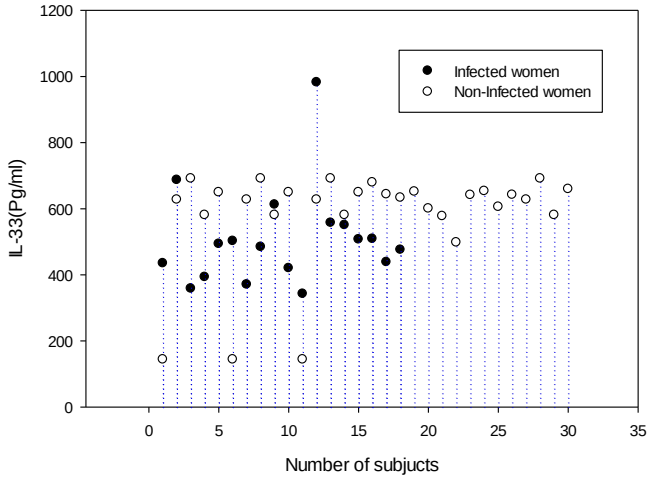

Figure 3. Comparison of mean concentration serum IL-33 Interleukin between patients and control Group.

The mean IL-4 were significantly higher in women patients as compared to control groups, (435.973+307.291pg/ml) versus (291.433+80.203pg/ml) respectively (p=0.0179). Figure (4).

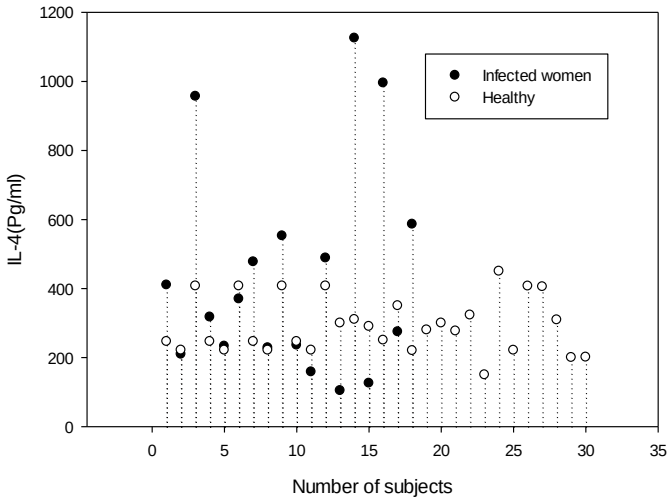

Figure 4. Comparison of mean concentration serum IL-4 Interleukin between patients and control Group

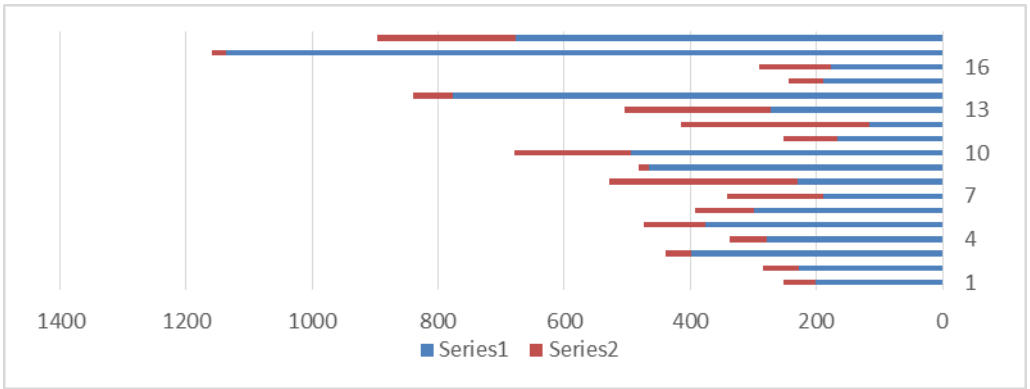

Figure 5. Serum IL-8 level among different levels of anti-Toxoplasma antibody (series 1=IL-8 series2= Anti-toxoplasma IgG antibodies). P-value= 0.101

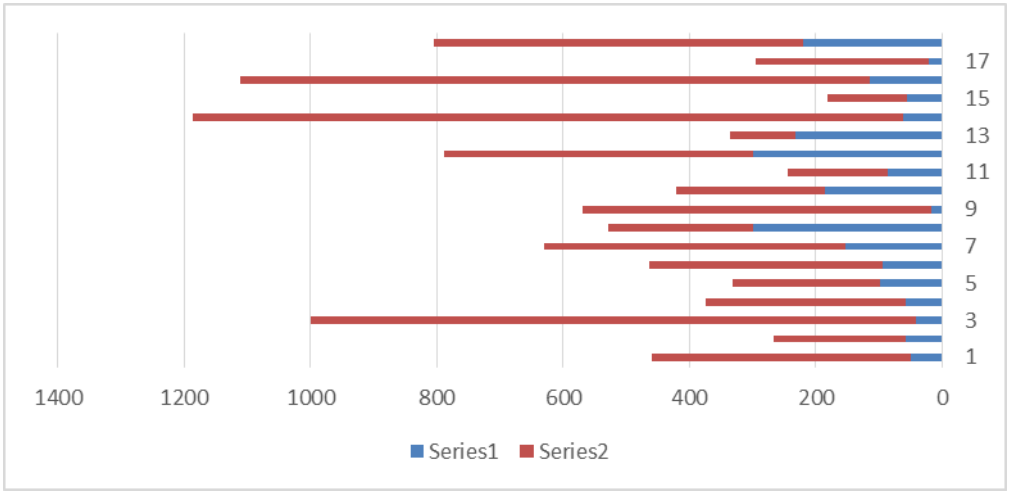

Figure 6. Serum IL-4 level among different levels of anti-Toxoplasma antibody (series 1=IL-4 series2= Anti-toxoplasma IgG antibodies). P-value= 0.797

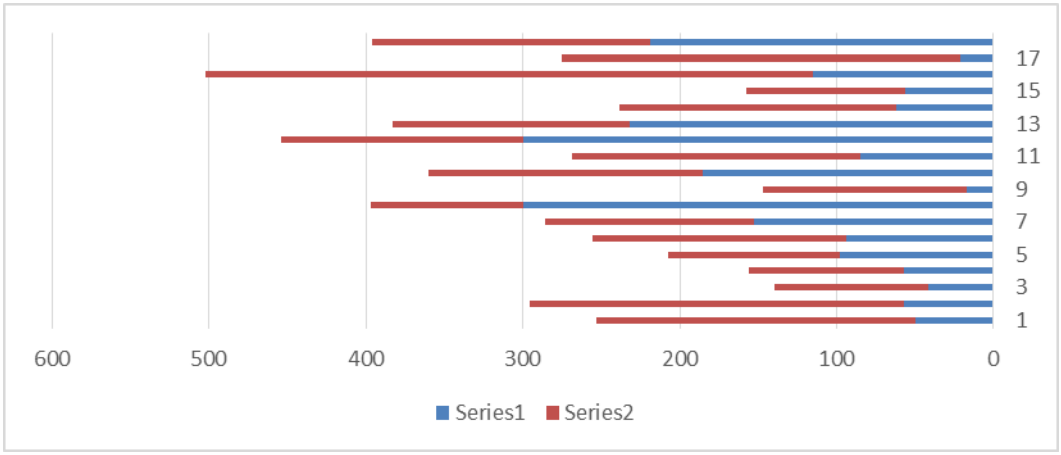

Figure 7. Serum IL-10 level among different levels of anti-Toxoplasma antibody (series 1=IL-4 series2= Anti-toxoplasma IgG antibodies). P-value= 0.742

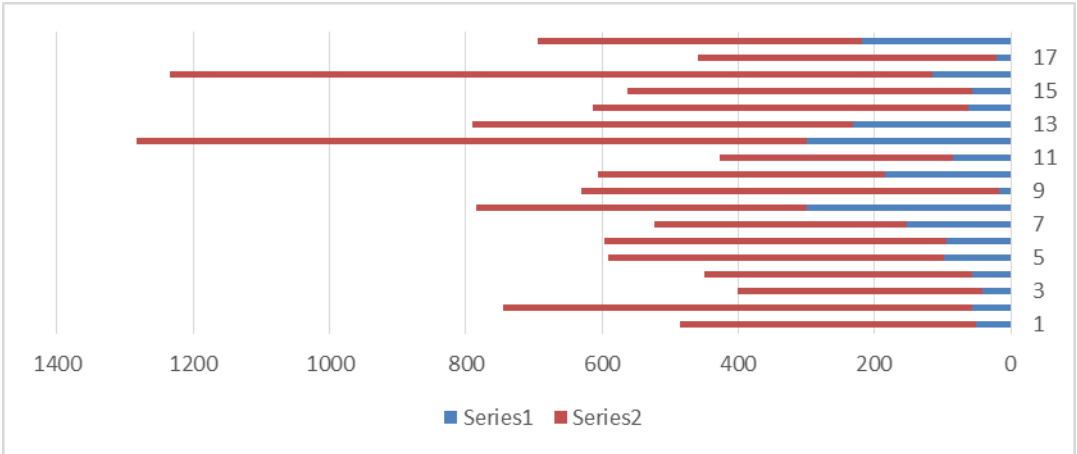

Figure 8. Serum IL-33 level among different levels of anti-Toxoplasma antibody (series 1=IL-4 series2= Anti-toxoplasma IgG antibodies). P-value= 0.497

DISCUSSION

It is known that toxoplasmosis has some unfavorable effects on the reproductive capacity of both men and women [14].

The present study is the first in Iraq, which showed there is no an association between toxoplasmosis and infertility women cytokines IL8, IL10 and IL33 concentration and showed no significant variation in the serum of infertility women infected with toxoplasmosis in comparison with infertility women uninfected (healthy control)

The statistical results indicated no significant variation of IL-8, IL-10 and IL-33 in infertile women patients' comparison with infertile women control, and may be due to the parasite alter or fail to stimulate secretion of the pro-inflammatory chemokines. The decrease level of IL-10 in patients in comparison with healthy control (Table 1) may be due to fail ability of the parasite to enhance TH2 cytokines among these was IL-10. However, IL-10 is strong enemy to macrophages capability in order to kill bacteria inside the cells microbes as well, examples are infections and T. gondii via numbers of pathogens, the presence of T. gondii, will lead to increasing in the IL-10 expression [15].

This results disagree with previous study done by El-Tantawy et al. [16] they found an association between toxoplasmosis and infertility women with significant higher prevalence ($p < 0.01$) of T. gondii infection in infertile female patients (61.85%) in Dakhalia governorate, Egypt in comparison with the pregnant women control group. Previous study in Iraq (2017) found that Interleukins IL-8 and Il-10 plays an important role in the resolution of Toxoplasma gondii infection, their concentration in women patients' serum of all age groups were increased in comparison to that observed in control groups [17].

The statistical results indicated increase of IL-4 in patients in comparison with healthy control of all ages (Figure 4). This increase is due to T. gondii stimulate secretion of the pro-inflammatory chemokines like IL-4. The chemokine response is dependent on invasion by live tachyzoites and subsequent host cell lysis. IL-4 is responsible for activation and recirculation of neutrophils and neutrophils can phagocyte and kill or inhibit tachyzoites of Toxoplasma and showed that human intestinal epithelial cells infected with T. gondii elicit rapid secretion of IL-4 [18].

CONCLUSION

The present study confirms that in SLE patients the antibodies to the antinucleosome are common. We have also revealed that mean antinucleosome antibodies are high in SLE groups in compared to healthy subjects or in rheumatoid arthritis patients. So, it might be a helpful addition to the laboratory tests that can aid with SLE diagnostics.

RECOMMENDATIONS

Our results found there is no significant variation in cytokines IL8 ,IL10 and IL33 of infertility women serum infected with toxoplasmosis in comparison with infertility women serum uninfected (healthy control)

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