

Research Article

Effects of early spontaneous abortions (ESA) and latent toxoplasmosis on Interleukine-23 in women

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Abstract

Cytokines play an important role in intercellular communications, cell growth, differentiation, and immune system modulations. Some of these functions have crucial roles in pregnancy gaining or losing, especially those correlated with T helper 1, T helper 2, and T helper 17 cells. Several studies showed significant variations in Interleukine-23 (IL-23) with Toxoplasma infection. However, little is known about the regulations of this interleukin in the case of early spontaneous abortion (ESA) patients. The present study aimed to evaluate IL-23 to explain the effect of this cytokine on pregnancy gaining or losing with and without toxoplasma infections. Eighty-nine subjects were registered in the current study, 16 Toxoplasma infected ESA women, 41 unknown ESA aborted women, 16 healthy pregnant women, and 16 healthy non-pregnant women served as control negative. Detections of anti-Toxoplasma IgG and IL-23 by immunoenzymatic assay (IFA and ELISA, respectively). A significant difference ($P > 0.05$) was found in all subjected groups except between Toxoplasmosis ESA and unknown EAS women. The data showed a substantial increase in sera IL-23 in unknown ESA with mean and standard deviation (SD) (13.679 ± 2.461) and Toxoplasma infected women with mean and SD (14.279 ± 4.757) as compared with non-Pregnant women having mean and SD (5.824 ± 1.040) and healthy pregnant women with mean and SD (17.273 ± 6.418). Therefore, this considerable evidence may indicate a role of IL-23 in the development of pregnancy gaining or losing, especially those ESA women.

Keywords: Aborted, Interleukine-23, Spontaneous, *Toxoplasma gondii*, Women

INTRODUCTION

Toxoplasmosis is a parasitic disease that is caused by *Toxoplasma gondii*. This obligate opportunistic organism infects almost one-third of the world's population, and it is the most prevalent infection in humans (Afifi and Al-rabia, 2015). In the first trimester of gestation, spontaneous abortion is the most complications during pregnancy; therefore, more than 80% of women's spontaneous abortions could be occurred, especially those related to infections (Abbas and Al-hassnawi, 2020). Although toxoplasmosis has significant asymptomatic diseases in women having a pregnancy, this infection has been associated with dangerous consequences like abortions (Saki *et al.*, 2015).

Most protozoan parasites such as *Leishmania spp*, *Trypanosoma cruzi*, and *Malaria Spp* can modulate the host immune system orchestrated via cytokines that

activate different immune cells such as T-cell and NK cells (Mammari *et al.*, 2019).

The cytokines have been proposed to be associated with positive or negative development of continuous pregnancies related to downregulation of TH1 activity and enhancement of TH2 activity (Jain *et al.*, 2015). *Toxoplasma gondii* induces a type-1 immune response by producing interferon-gamma, NK-cells, and TH-1. Also, the previous study indicates that IL-12 and IL-23 play a dominant role, although the limited mechanism of resistance, in toxoplasmosis resistance (Ismail *et al.*, 2017).

Moreover, abortion may be occurred by six several factors: hereditary, anatomical, metabolic, infective agents, hormonal or immunological, so about 80% of abortions suggested may be immunological. In addition, all five remaining factors are also affected by cytokine expression. Therefore, these cytokine expression changes may create a microenvironment leading to

spontaneous abortions (Cai *et al.*, 2016).

The heterodimeric cytokine, Interleukine-23 (IL-23), constitutes the p40 and p19 subunit, which is similar to the IL-12 configuration in the p40 subunit (Cai and Li, 2016). IL-23 is an IL-6 family secreted from macrophages and activated dendritic cells and suggested like IL-12. IL-23 plays a vital role in developing inflammation, especially in intracellular infection (Yannam *et al.*, 2012). Thus, IL-23 is considered an essential component in the innate immune system and autoimmune disease characterized by stimulations of IL-17A (Chackerian *et al.*, 2006). It was reported that IL-23 up regulations in the ileum of infected mice with *Toxoplasma gondii* (Muñoz *et al.*, 2009). Another data suggested that IL-23 can promote and activate TH1 and TH2 immune cells (Aggarwal *et al.*, 2003). Also, it was recently reported the role of IL-23 in spontaneous abortions (Russo *et al.*, 2021), whereas another study (Ivanova *et al.*, 2019) showed that IL-23 or IL-12 blockade can reduce *Toxoplasma* reinfection.

The mother's immune system tolerance for the various pathogens is essential for a successful pregnancy. Thus, the present study aimed to measure IL-23 imbalanced with and without toxoplasma infection to improve our understanding of unexplained causes of early spontaneous abortion (ESA) women.

MATERIALS AND METHODS

Subjects

All exclusion criteria, such as family history, chronic and genetic disease, drinking, and smoking, were excluded from this study. The completed clinical data of eighty-nine enrolled women for bio-investigation was subdivided into four groups, i.e., 41 patients unknown aborted (toxoplasma-free) women, 16 aborted (toxoplasma-infected women), 16 healthy Pregnant women, and 16 non-pregnant and free-toxoplasma women served as control negative.

Blood collection

Five ml of blood was obtained from each volunteer woman, and the collected blood was transferred into sterilized gel tubes. Serum was separated from whole blood by centrifugation (4000 rpm for 5 min.) and then immediately was stored at -20 °C for 30 days until all samples collected to ELISA worked.

Detection of Toxoplasmosis

The immunofluorescence test was used to identify anti-toxoplasma IgG antibodies in serum. In brief, this test concept combines an enzyme immunoassay technique based on immunocapture with final fluorescence detection (ELFA). The current study employed the immunoserological analyzer VIDAS with a set of reagents (bioMérieux, France).

Detection of serum Interleukin-23

Interleukin-23 was determined in blood serum using the Enzyme-Linked Immunosorbent Assay (ELISA). In brief, 100 mL of serum was added into appropriate wells and then incubated for 90 minutes at 37°C. Serum was discarded from each wells, and 100 mL of biotinylated antibody was added immediately and then incubated for 1 hour (37°C). The unconjugated antibody was removed by washing (3 times by 1X wash buffer). The enzyme (HRP) was added to all wells and then incubated for 30 minutes. The unconjugated enzyme was also removed by the same washing using the previous step. 100 mL of Specific enzyme-substrate (TMP) was added to wells, then incubated for 15 minutes in an incubator. 50 mL of diluted H₂SO₄ was added immediately to stop reactions. Finally, the yellow color was measured at 450 nm by the Elisa reader. All steps of this work were accomplished by a commercial kit known as Elabscience company produced in China.

Study protocol and ethics

The local ethical committee approved the current study protocol in the College of Science/ Babylon University/ Babylon /Iraq, and all declaration Helsinki standards were adopted in this study to carry out the present work (World Medical Association, 2013).

Statistical analysis

The Sigma-plot software program (12.5) was used to perform statistical analysis. Normality was checked before analyzing data for this purpose. Mann-Whitney test was used for inconsistency with this distribution. The T-Student test was used for comparisons between groups. Variations were considered significant when P-value ≤ 0.05.

RESULTS AND DISCUSSION

Several studies have been carried out to investigate the role of IL-23 in spontaneous abortions (Russo *et al.*, 2021), as well as the ability of IL-23 or IL-12 blockade to reduce *Toxoplasma* reinfection (Ivanova *et al.*, 2019). Furthermore, other studies found significant differences in IL-23 with *Toxoplasma* infection (Abbas and Al-hassnawi, 2020). However, little is known about the regulation of this interleukin in the case of patients undergoing Early Spontaneous Abortion (ESA).

The present study aimed to evaluate IL-23 to explain the effect of this cytokine on pregnancy gaining or losing with and without toxoplasma infections

Results of interleukin-23 among all groups, pregnant, non-pregnant, *Toxoplasma*-free, infected, and unknown ESA women, are shown in Fig. 1-6. Firstly, pregnant women showed a significant difference (P = <0.001, as shown in Fig.1) in a concentration of sera IL-23 when compared with healthy non-pregnant women. Among

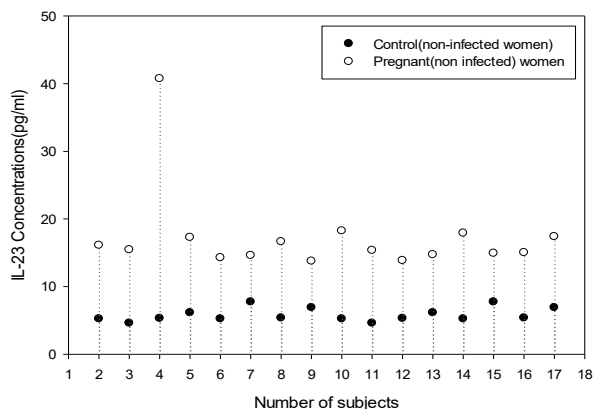


Fig. 1. Serum IL-23 concentration in pregnant and non-pregnant (*Toxoplasma*-free) women ($P = <0.001$)

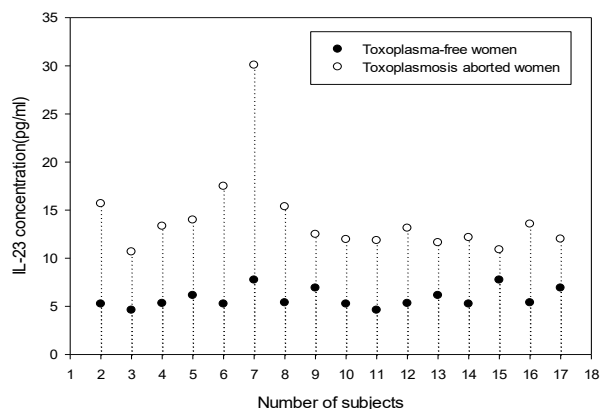


Fig. 2. Serum IL-23 concentration in *Toxoplasma*-free and *Toxoplasmosis* aborted women ($P = <0.001$)

patients, women who had early spontaneous abortions and toxoplasma infection, IL-23 increased significantly compared to healthy ones ($P = <0.001$, as shown in Fig.2). Also, the same increase occurred in IL-23 in women with ESA of unknown cases ($P = <0.001$, as shown in Fig.3). In contrast, variations did not reach significant ($P = <0.059$, as shown in Fig.4) between pregnant women and those with ESA and toxoplasmosis. The current study showed a considerable decrease in sera IL-23 in unknown aborted women groups compared to healthy pregnant women ($P = <0.002$, as shown in Fig.5). Finally, significant differences were not found between women who had an abortion with toxoplasmosis and those unknown ESA ($P = <0.763$, as shown in Fig.6)

The present study investigated sera IL-23 among ESA women in an attempt to understand possible causes of unexplained spontaneous abortions with and without toxoplasma infection. Previous studies reported higher concentrations of IL-1, IL-6, IL-12,17, IL-8, and IL-23 (pro-inflammatory cytokines) in patients with recurrent spontaneous abortion (RSA) patients. Still, little is known about IL-23 levels in ESA women in toxoplasma-free and infected ones. Though IL-12 is essential in toxoplasmosis resistance, some findings suggest that

IL-23 and IL-12 have separate but compensated functions within innate immunity, and also different signaling pathways control their production against *Toxoplasma gondii* (Quan et al., 2015). In the present data, there were significant variations in IL-23 between aborted and healthy groups. However, no significant differences were shown between healthy pregnant and toxoplasma aborted women. Suggesting that IL-23 increases with toxoplasma and during pregnancy. In fact, parasitologist refers to toxoplasma and fetus as one kind of parasitic infection; however, both are considered stressors of the host's immunity. Thus, the impact of stress on the innate immune system may explain why IL-23 concentration increased in pregnant and toxoplasmosis groups compared with healthy women (McCray and Agarwal, 2011).

Moreover, the present study found no significant differences in IL-23 between ESA and toxoplasma-infected patients, but IL-23 is still high in both groups compared with healthy non-pregnant women. Consistent with our data, (Quan et al., 2013) demonstrated elevated IL-23 concentration in cell lines to respond to four types of toxoplasma antigens. There are several mechanisms by which TH1 can develop pregnancy losing and gaining. In addition, the IL-17/IL-23 axes function so at the

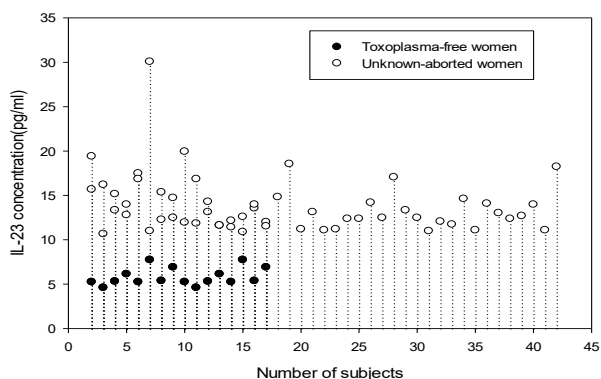


Fig. 3. Serum IL-23 concentration in *Toxoplasma*-free and in unknown-aborted women ($P = <0.001$)

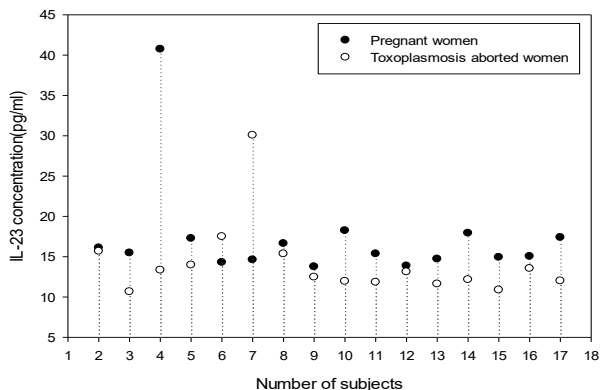


Fig. 4. Serum IL-23 concentration in pregnant and *Toxoplasmosis* aborted women ($P = <0.059$).

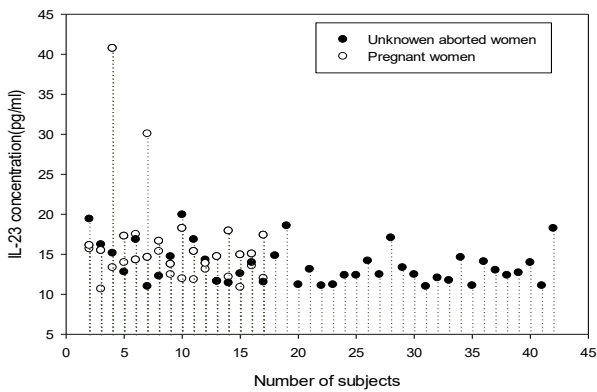


Fig. 5. Serum IL-23 concentration in pregnant and unknown aborted women ($P = <0.002$)

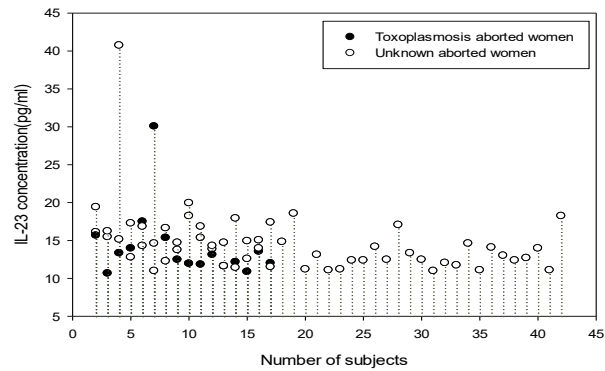


Fig.6. Serum IL-23 concentration in Toxoplasmosis and unknown aborted women ($P = <0.763$)

maternal-fetal interface may be essential towards the immunopathology of RSA women. The study done by Li *et al.*; (2017) revealed that IL-23 and IL-17 decidual tissue decreased in RSA women. Another mechanism showed that the balance between Th17 and T-reg cells is necessary to preserve the implantation, hence a successful pregnancy. Also, increased levels of Th17 cells versus a decreased percentage of T-reg cells indicate an immunological imbalance, which leads to pregnancy failure (Roomandeh *et al.*, 2018). When IL-23 levels rise, it may have an adverse impact, such as raising the proportion of Th17 cells, causing an imbalance in the Treg–Th17 cell ratio, and ultimately leading to the fetus' rejection (Saifi *et al.*, 2014).

In line with previous findings, a study conducted by Darmochwal-kolarz *et al.*; (2017) found that in patients with unexplained recurrent spontaneous abortion, there is an increasing currency ubiquity distribution of TH17 cells as in peripherally and an increase in IL-23, both of which play an important role in TH17 cell expansion. Another study (Saito *et al.*, 2010) discovered that IL-23 expression decreased due to its important function in early pregnancy whenever dendritic cells were exposed to antigens of paternal origin conditioning. According to the findings of the present study and previous research (Darmochwal-kolarz *et al.*, 2017), aborted women had higher levels of appropriate implant and invading of trophoblast and Th17 cytokines.

In addition, Lieberman *et al.*; (2004) showed that the frequency of single nucleotide polymorphism in the receptor of IL-23 in patients with RSA decreased compared with normal control women. Therefore, in the present study, IL-23 will be suggested as a possible RSA therapy target. By present findings, it could be understood the candidate mechanism by which IL-23 drives pregnant women to abortion.

Conclusion

According to the present data and results, it can be

concluded that IL23 concentration increased significantly among patients women who had early spontaneous abortion and Toxoplasma infection compared to healthy ones and in women with ESA of unknown causes. This increase may indicate a role in spontaneous abortion complications in pregnant women. Therefore, future treatment options may be considered depending on this considerable evidence.

Conflict of interest

The authors declare that they have no conflict of interest.

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