Role of IL-37 and Dectin-1 during Toxoplasmosis

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Abstract:

Toxoplasmosis is a parasitic infection that triggers immune cells to produce cytokines and inflammatory mediators that are responsible for abnormal or aborted immune responses. This study highlights the evaluation of the Dectin-1 receptor and cytokine IL-37 in the serum of 80 patients who had miscarried in the first trimester and were infected with toxoplasmosis, as well as 40 pregnant women in the first trimester who had a successful pregnancy (control groups). The serum was first screened for the T. gondii IgM and IgG antibodies by an enzyme-linked immunosorbent assay (ELISA) and then the serum levels of IL-37 and Dectin-1 were determined. The results showed that the serum level of Dectin-1 was significantly increased in anti-Toxoplasma IgM and IgG patients as compared with the controls 155.702 ± 26.356 ng/L, while the serum level of IL-37 was convergent in anti-Toxoplasma IgM and IgG patients and controls 52.666 ± 4.272, 66.808 ± 9.132, and 51.984 ± 3.619 ng/L respectively, with no significant differences. In conclusion, Dectin-1 receptor may play a role in pregnancy loss, especially in T. gondii infection.

Keywords: C-type lectin receptor, Cytokine, Dectin-1, IL-37, Miscarriage, Toxoplasmosis.

Introduction:

Toxoplasmosis is a global disease caused by an intracellular protozoan called Toxoplasma gondii. The parasite is mainly spread through the ingestion of contaminated water and food tainted with cat feces that contain mature oocysts, as well as through the ingestion of undercooked meat that contains tissue cysts. It is also possible for T. gondii to be transmitted congenitally from mothers to their fetuses. In healthy people, toxoplasmosis infection is usually asymptomatic or occasionally causes mild flu-like symptoms, followed by lymphadenopathy and hepatosplenomegaly. However, reactivated infection, which is more common in immunocompromised individuals, can result in significant morbidity and mortality, including pneumonia, hepatitis, splenomegaly, and blindness, as well as in pregnancy loss. The immune response to T. gondii varies according to genetic history, immune status, and virulence of the parasite, and a group of immune-modulatory proteins known as cytokines controls a number of immune responses. Several cytokines are important in the formation of effective immune responses against infection. The causes of recurrent miscarriages are many and varied, and some are unknown. Surprisingly, cytokines are one of the variables associated with miscarriage, and those responsible for aberrant immunological reactions. Various inflammatory cytokines are released by activated T-helper cells as a result of T. gondii infection. It is known that protective immunity against T. gondii is dependent mostly on cellular immunity mediated by both CD4+ and CD8+ T cells. T. gondii was one of the first pathogens found to cause a crucially polarizing Th1 response, which is essential for immune protection.

The innate immune system's cells are equipped with a number of receptors referred to as pattern recognition receptors (PRRs), which they use to trigger innate responses. PRRs recognize antigens, which make cells active and produce various responses, such as phagocytosis or the release of inflammatory mediators. C-type lectin receptors (CLRs) are a specific type of PRR and are essential for pathogen responses. Dectin-1 is a type II transmembrane lectin that is a member of the CLR family. In the myeloid lineage, which includes...
macrophages, dendritic cells (DCs), and neutrophils. Dectin-1 is abundantly expressed. It has been demonstrated that Dectin-1 can recognize a variety of ligands, both endogenous and generated by microbes. The effects of this recognition can take many different forms, such as pro-inflammatory reactions like the release of cytokines, reactive oxygen species (ROS), and phagocytosis. In response to Dectin-1 ligands, mitochondrial metabolism also contributes to ROS production. Dectin-1-triggered signaling pathways will provide potential therapeutic or prophylactic targets. It is important to point out that Dectin-1 can trigger immune responses against a wide range of dangerous pathogens, such as fungus, bacteria, and parasites. T. gondii infection stimulates the production of inflammatory cytokines IFNα, IFNβ, IL-1β, IL-6, IL-12, IL-15, and IL-18, which results in the production of IFN by natural killer (NK) cells. This leads to the early control of parasite infection by targeting intracellular parasites. The cytotoxic T cell response is also induced by NK cells. Additionally, NK cells can produce IL-10 and regulate innate responses by down-regulating IL-12 and possibly other cytokines. NK cells can be involved in adaptive immunity as memory-like cells and may be important with regard to secondary infections. According to Mévélec et al., cytokines have been considered a critical mediator in protection against both acute and chronic T. gondii infection.

Interleukin-37 (IL-37) is a newly discovered member of the IL-1 family and is located on human chromosome 2. The majorities of cytokines belonging to the IL-1 family (such as IL-1, IL-18, and IL-33) have pro-inflammatory functions and are produced in response to pathogen infection and tissue damage. In contrast, IL-37 suppresses the production and function of cytokines that contribute to inflammation. During the inflammatory process, IL-37 synthesis is induced and activated in a manner that is comparable to that of other cytokines belonging to the IL-1 family. According to a number of studies, IL-37 plays a crucial role in the regulation of inflammation and has emerged as a significant inhibitor of innate immunity and inflammation in a number of diseases. IL-37 may mediate the production of other cytokines (such as IL-6) and suppress the process of inflammation. The aim of this study was to evaluate and associate the cytokine IL-37 and Dectin-1 receptor in the serum of pregnant women with toxoplasmosis and first-trimester miscarriage and to further compare this with the response of women with normal pregnancies.

Materials and Methods: Subjects
A total of 120 pregnant women were enrolled in the study. They distributed to 80 patients age range: 15 to 38 years; who had toxoplasmosis and miscarriage in the first trimester, and 40 who had a successful pregnancy in the first trimester (controls) and who matched patients for age (age range: 16 to 35 years) and ethnicity. Samples of blood were taken from the Kamal al-Samarrai Hospital as well as private laboratories in Baghdad, during the period from October 2020 to July 2021. Informed consent was obtained from the University of Baghdad/College of Science for Women and from the Ministry of Health (approval number 6562 on 15/12/2019). After a clinical examination of the patient by the medical staff at the hospitals, toxoplasmosis was confirmed, and miscarriages occurred within the first trimester of pregnancy. The serum was first screened for the anti-toxoplasmosis IgM and IgG by the rapid immune-chromatographic Cassette test (Bio-Medical Co., Ltd. in Beijing, China) and, if the test was positive, the serum was further tested by the Toxoplasma gondii (IgM, IgG) ELISA kit (Foresight.com., Ltd., USA) at the Laboratory Department of Biology, College of Science for Women, University of Baghdad.

Sample Collection
At the time of the patients’ and volunteers’ first visit and after clinical examination, 5 ml of venous blood was collected using a 6 ml disposable syringe, dispensed into a plain tube, and left to clot at room temperature 20-25°C for 15 minutes. It was then centrifuged at 358 x g for 10 minutes to separate the serum, which was divided into aliquots 0.25 ml in tightly closed Eppendorf tubes, and was then stored frozen at -20°C until IgM, IgG, and serum levels of IL-37 and Dectin-1 could be determined.

Assessment of IL-37 and Dectin-1 Serum Level
The sera of the toxoplasmosis patients and the controls were assessed for levels of IL-37 and Dectin-1 using commercially available kits (Bioassay Technology Laboratory; China) and by sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed for quantitative measurement of human IL-37 and Dectin-1 receptors in sera.

Statistical Analysis
SPSS version 14 was used to perform statistical analysis on the data. The data were presented as mean ± standard error (S.E.), and differences between means were assessed using One-way ANOVA, followed by LSD (Least
Results and discussion:
Presentation of Subjects
According to the results of laboratory diagnosis of toxoplasmosis, female patients who had a first-trimester miscarriage were divided into two groups: positive for anti-Toxoplasma IgM (n = 40) and positive for anti-Toxoplasma IgG (n = 40). The mean age (Mean ± Standard Deviation) of patients and controls is illustrated in Fig. 1. Toxoplasmosis is caused by the protozoan parasite *T. gondii*, which causes persistent infection. Toxoplasmosis is not routinely screened in asymptomatic patients in Iraq, and it is reported to have a high rate of prevalence, especially in rural areas and underprivileged communities. This has poor effects on reproductive health, like making it harder to get pregnant and making it more likely that the pregnancy will end in loss. As the host's immune system regulates the limitation of parasite replication during infections by *T. gondii*, activation of innate immune responses is essential to the formation of an efficient immune response. The vital role of cellular and humoral immunity, some of which have been labeled as essential and others detrimental to fetal development, has been attributed to healthy embryonic development. There are several immunological aspects of pregnancy that are unknown. So, in this study, we compared the levels of Dectin-1 receptor and IL-37 in the blood of pregnant women who had toxoplasmosis and a miscarriage in the first trimester to those women experiencing a normal pregnancy.

Serum Level of Dectin-1
The concentration of Dectin-1 in the serum of women with toxoplasmosis who had miscarried was compared with a control (successful pregnancy). The results showed that the concentration of Dectin-1 in the serum of toxoplasmosis women was significantly higher *P* ≤ 0.05 than that of women undergoing normal pregnancy. The mean serum level of Dectin-1 in patients was 362.382 ± 45.937 ng/L for anti-Toxoplasma IgM and 361.916 ± 71.993 ng/L for anti-Toxoplasma IgG, compared to 155.702 ± 26.356 ng/L in the controls, as shown in Fig. 2.
Figure 2. Dectin-1 serum levels in toxoplasmosis women experiencing miscarriage, and the controls. Results were analyzed with One-way ANOVA, followed by LSD and Duncan post hoc test. ns, non-significant; *P ≤ 0.05.

The localized inflammatory state is essential for implantation and for uterine angiogenesis during a typical pregnancy. During pregnancy, the cytokine secretion profile in the peripheral blood favors the T cell helper 2 (Th2) response over the Th1 response, and any change in this balance could cause miscarriage and other signs of pregnancy loss. The number of Th17 and NK cells is elevated, and the number of regulatory T cells (Tregs) is severely impacted by the excessive activation of Th1 cells. Cytokine production is not limited to T helper cell subtypes; they are also produced and secreted by other immune cell types, including antigen-presenting cells (APCs).

Therefore, it appears that measuring the number of cytokines in peripheral blood serum represents a good way to determine how the body’s immune system is functioning. Invasion of intestinal epithelial cells by *T. gondii* during infection causes the production of inflammatory cytokines and chemokines, which enhance the recruitment of neutrophils and monocytes. Professional APCs that have been trained to recognize parasite antigens produce microbicidal mediators like ROS and nitric oxide (NO), as well as pro-inflammatory cytokines, which help to reduce parasite reproduction. When the innate cells migrate to the lymph nodes and parasite antigens are presented, the adaptive branch of immunity is triggered. The immune system primarily recognizes tachyzoite surface antigens such as glycosyl-phosphatidylinositol (GPI)-anchored proteins and secreted proteins. As a result, the development of an immune response depends on the ability of innate cells to recognize parasite antigens. PRRs that bind to specific extracellular surface proteins produced by microorganisms, pathogen-associated molecular patterns (PAMPs), or damage-associated molecular patterns (DAMPs), enable the recognition of parasites. Innate immunity requires ongoing immune cell surveillance to identify pathogenic threats, thus PRRs are an essential part of this surveillance. Because of this process, the parasite is known to activate various PRRs during infection, such as Toll-like receptors (TLRs) and CLRs. These receptors have been observed to play a role in parasite recognition and elimination by sensing pathogen-associated molecular patterns and stimulating signaling pathways that regulate gene transcription. CLRs can be able to transmit signals independently or in collaboration with TLRs and are able to identify carbohydrates and glycosylated proteins. Dectin-1
or C-type lectin domain family 7 member A (CLEC7A) is part of a family of CLRs, which are expressed in a variety of immune cells, including monocytes, macrophages, DCs, and neutrophils, as well as epithelial cells; it is also expressed by some subsets of T cells and B cells. Dectin-1 detects different ligands and thus acts in a variety of innate immune responses against pathogens as a mediator for recognition. Dectin-1 recognizes glucans, carbohydrates commonly found in the cell walls of plants and fungi, and also recognizes tropomyosin (present in arthropods) and unidentified ligands in mycobacteria and Leishmania. Several endogenous ligands have also been identified, such as galectins, galactosylated immunoglobulins, and vimentin. Therefore, they play an essential role in immune responses to other pathogens such as bacteria, viruses, nematodes, and protozoa like Leishmania. Several endogenous ligands have also been identified, such as galectins, galactosylated immunoglobulins, and vimentin.

Dectin-1 ligation leads to the activation and phosphorylation of the protein kinase and can activate the NFκB pathways and can induce or regulate cellular responses, including phagocytosis, autophagy, DC maturation, and antigen presentation. Furthermore, Dectin-1 functions as a dimer with TLR2, where these two receptors can act synergistically for optimal cytokine production, leading to the production of pro-inflammatory cytokines (such as IFN-γ and TNF-α), which causes miscarriage via uterine contraction and necrosis of the implanted embryo via thrombosis, resulting in expulsion, as mentioned in El-Sherbini et al. The second effect is to stimulate Th2 cells, which is a benefit for parasites’ ways of evading the immune response by promoting the down-regulation of IL-12 and ROS, as noted in da Silva et al.

Serum Level of IL-37

The serum level means of IL-37 in women who had suffered a miscarriage with toxoplasmosis was 52.666 ± 4.272 ng/L for anti-Toxoplasma IgM and 66.808 ± 9.132 ng/L for anti-Toxoplasma IgG compared to controls 51.984 ± 3.619 ng/L, but the differences were not significant P > 0.05, Fig. 3.
Among the critical cellular products are cytokines, which are essential to the progression and outcome of infection, both pro-inflammatory and anti-inflammatory types that elicit distinct responses to immunogens at different stages of infection. The way in which cytokines respond to the same stimuli depends on the type of disease, its etiology, and whether it is chronic or acute. IL-37 is a newly discovered member of the IL-1 family of cytokines that binds to the IL-18 receptor (IL-18R) and is an important modulator of both innate and adaptive immunity. IL-37 acts as an anti-inflammatory cytokine, suppressing pro-inflammatory gene expression via inhibiting NF-kB and MAPK signaling. IL-37 has a protective impact on tissue damage and mediates a decrease in the secretion of pro-inflammatory cytokines. IL-37 is expressed in various tissues, but it is specifically expressed in APC as well as the thymus, testis, uterus, and placenta. IL-37 has recently been demonstrated to be a potential therapeutic target for a variety of illnesses, including HIV infection, autoimmune disorders, obesity, cardiovascular diseases, and chronic inflammatory disorders. IL-37 expression decreases pro-inflammatory cytokines (IL-1α, IL-1β, IL-1Ra, IL-6, IL-8, IL-17, IL-23, TNF-α, and IFN-γ), chemokines, GM-CSF, and ICAM-1 while elevating the expression of TGF-β, and IL-10. Moreover, IL-37 inhibits the activation of M1 macrophages, while enhancing the activation of M2 macrophages, as well as tolerant DCs by reducing MHC II, CD40, and CD86. IL-37 has potential immune modulation roles after parasite antigen stimulation by activating Tregs and suppressing Th1/Th2/Th17 cells. Low concentrations of the cytokine TGF-β were effective in stimulating endogenous IL-37.

IL-37 is effective in suppressing inflammatory diseases by blocking TLR signaling through the down-regulation of NF-xB induced by either TLR2 or TLR4. In addition to inhibiting Dectin-1 signaling after pathogenic antigen recognition in APC, it leads to the inhibition of pro-inflammatory cytokines such as IL-1, IL-6, IL-17, TNF-α, and IFN-γ by suppressing mTOR signaling. Thus, increased production of various inflammatory cytokines during pregnancy may be crucial in the development of severe forms of toxoplasmosis, including miscarriage. Therefore, the presence of IL-37 as an anti-inflammatory cytokine is very important for the maintenance of pregnancy.
This study showed that serum levels of IL-37 were slightly higher in toxoplasmosis patients compared to the control group as an important cytokine in the immune regulation and maintenance of pregnancy, while serum levels of Dectin-1 were higher in toxoplasmosis patients as expected because of its importance in the activation of APC and production of cytokines. In this case, we can say that Dectin-1 plays a role in pregnancy loss, especially in relation to diseases caused by parasites.

Conclusions:
This study showed no significant difference in serum levels of IL-37 in toxoplasmosis patients compared to a control group, while serum levels of Dectin-1 were higher in toxoplasmosis patients than expected because of its importance in the activation of APC and production of cytokines. Consequently, the Dectin-1 response has a role in miscarriage because it stimulates the production of pro-inflammatory cytokines.

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Authors' declaration:
- Conflicts of Interest: The authors have no conflicts of interest to disclose.
- We confirm that all the figures and tables in the manuscript are ours.
- Authors sign on ethical consideration’s approval.
- Ethics Approval: The project was approved by the local ethical committee at the University of Baghdad/College of Science for Women and by the Ministry of Health (approval number 6562 on 15/12/2019).

Author’s contributions statement:
Z.A. carried out the experiment and verified the analytical methods. E.M. conceived the original idea and contributed to the preparation and diagnosis of the sample. N.M. contributed to the interpretation of the results and supervised the project. Z.A. and E.M. wrote the manuscript in consultation with N.M. All authors discussed the results and contributed to the final manuscript.

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