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Role of NLRP3 in Patients Infected with Toxoplasma Gondii Parasite

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***Abstract*---**The study was conducted on 350 aborted women and 30 healthy women who have visited AL-Zahra maternity and paediatrics hospital in AL-Najaf province from November from August to January 2022. This study was designed to determine the effects of *T. gondii* infection on some parameters such as NLRP3, IL-18, IL-1β and TNF-α, The results showed a significant elevation (P<0.001) in serum concentration of The current study revealed that serum concentration of NLRP3 (ng/ml), IL-18 (pg/ml), IL-1β (pg/ml) and TNF-α (pg/ml) in patients infected with *Toxoplasma gondii* were significant increase (P< 0.001) (46. 21642 ± 0.524 ng/ml), (474. 12338± 0.906 pg/ml), (15. 41450±0.218 pg/ml), (25.4145065284 ± 0.218232 pg/ml) in compared to the control group (9.701792 ± 0.555 ng/ml), (97.107565 ± 0. 700 pg/ml), (4.129558 ± 0.190 pg/ml), (6.45839 ± 0. 220 pg/ml). The current study has concluded that the infection with *T. gondii* affects the body's immunity by NLRP3, IL-18, IL-1β and TNF-α.

Keywords*---Toxoplasma gondii*, IgG, NLRP3, Kufa, abortion.

Introduction

Toxoplasma gondii is an intracellular primary parasitic infection that affects one-third of the world's population, immunocompetent people have it settle inside their heads. It is an intracellular parasite of the Felidae family that has intermediate hosts in humans (Arling *et al.,* 2009); in immunocompromised patients, it is usually asymptomatic (Jiang *et al.,* 2013). *Toxoplasma* parasite infects mammals, birds, and reptiles, attacking the central nervous system (CNS) first before spreading to the skeletal muscles and reproductive system (Butty, 2009). This parasite causes acquired toxoplasmosis and is particularly harmful in immunocompromised people, AIDS patients, and organ transplant recipients. Congenital toxoplasmosis occurs in the fetuses of a variety of wild and domestic animals, including sheep, goats, and pigs (Kong *et al.,* 2012). *Toxoplasma*

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parasite strains are classified into three genotypes (types I, II, and III) based on their virulence in mice, and their pathogenicity varies depending on the host. The first type is primarily isolated from humans, whereas the second type is found in severe cases of infection (El-Fadaly *et al.,* 2012). Ingesting *Toxoplasma* oocysts or tissue cysts, also known as bradyzoites, from a previously infected intermediate host infects hosts. *Toxoplasma* enters the small intestine, quickly changing to the tachyzoite form and causing inflammation (Dubey *et al.,* 1998). In two ways, this procedure triggers an immune response that is important to the parasite's life cycle. *Toxoplasma* infects and multiplies invading immune cells first (Courret *et al.,* 2006; Saleem *et al.*, 2019). The parasite disseminates throughout the body via immune cells, reaching tissue areas that support persistent infection, including as the brain, muscle, and other tissues (Bierly *et al.,* 2008). Second, parasites transition to a bradyzoite transcriptional pathway and most tachyzoites are cleared during chronic infection. This entails the creation of a saccharide-rich parasite cyst wall, which is required for the parasite to survive transit through the next host's gastrointestinal tract (Bierly *et al.,* 2008; Gregg *et al.,* 2013). As a result, without a strong immune response, the parasite kills the host before the shift occurs, limiting the possibility of transmission. *Toxoplasma* and the NLRP1 and NLRP3 inflammasomes are recognized by innate immune sensors such as Toll-like receptors. *Toxoplasma* detection by the innate immune system increases the release of IL-12, the synthesis of IFN- γ by NK cells, and a Th1 polarized adaptive immunological response (Sher *et al.,* 1993; Hunter *et al.,* 1994). The cytokines in the interleukin-1 (IL-1) family, particularly IL-1α, IL-1β and IL-18, are required for initiating and enhancing innate and adaptive immune responses in cells such as epithelial and endothelial cells (Sims & Smith, 2010). Inflammasomes control the action of numerous IL-1 cytokines, multi-protein synthesis, and microbial infection resistance. Innate immune cells such as macrophages, dendritic cells, monocytes, and non-hematopoietic immune complexes create these cytokines, which are controlled by the intracellular cysteine protease caspase-1, which cleaves and activates immature forms of these cytokines (Netea *et al.,* 2015). Inflammasomes promote type 1 immune responses by secreting IL-1 and IL-18, which aid immune defense against tiny, easily phagocytozed pathogenic microorganisms (McIntire *et al.,* 2009; Strowig *et al.,* 2012; Arbore *et al.,* 2016). The Pyrin Domain Containing 3 (NLRP3) inflammasome, which belongs to the nod-like receptor (NLR) family, is the most well-studied inflammasome and is required for immunity against bacteria (Muruve *et al.,* 2008; Broz *et al.,* 2010), viruses (Allen *et al.,* 2009; Thomas *et al.,* 2009; Kamada *et al.,* 2014), protozoan parasit (Hise *et al.,* 2009). Other antimicrobial defense inflammasomes, such as NLRP1, AIM2, and NLRC4, are also important (Rathinam *et al.,* 2010; Tomalka *et al.,* 2011; Ewald *et al.,* 2014).

The subjects

The study was conducted on 350 suspected patients with *T. gondii* parasites and 30 healthy people as control groups. The institutional ethics committee approved collecting samples of the faculty of science at the University of Kufa, and all participants signed informed consent forms. Toxo specific IgM and IgG methods examine all suspected samples. These samples were collected from suspected patients who attended the AL-Zahra maternity and paediatrics hospital in AL-Najaf province from August to January 2022.

Blood Specimens collection

Only 60 positive samples out of 350 suspected patients and 30 healthy people attended the AL-Zahra maternity and paediatrics hospital clinics in AL-Najaf province from August to January 2022. The blood samples were taken from patients via vein puncture into test tubes and kept at room temperature for 30 minutes. After that, the samples were centrifuged at 3000 rpm for 5 minutes (Backman/counter, Germany) to separate the serum and collected in other sterile tubes; each sample of serum was divided into five parts and kept in deep freeze at -20C until utilized for NLRP3, IL-18, IL-1β and TNF-α.

The Kits

The biomarkers in the current study were estimated by Eliza Kits such as Human NLRP3 ELISA/ Elabscience / (Catalog No: E-EL-H2557), Human Interleukin-18 (IL-18) ELISA/ Elabscience / (Catalog No: E-EL-H0253), Human Interleukin-1β (IL-1β) ELISA/ Elabscience / (Catalog No: E-EL-H0149), Human Tumer necrosis factor- α (TNF-α) ELISA/ Elabscience / (Catalog No: E-EL-H0109).

Statistical analysis

Graph pad prism for Windows (5.04, Graph pad software Inc. USA) was used to analyze the data, and the results are reported as the mean standard error (SE). A student t-test was used to examine the differences between the patient and control groups.

Results

The current study revealed that serum concentration of NLRP3 (ng/ml), IL-18 (pg/ml), IL-1β (pg/ml) and TNF-α (pg/ml) in patients infected with *Toxoplasma gondii* were significant increase ($P< 0.001$) (46. 21642 ± 0.524 ng/ml), (474. 12338± 0.906 pg/ml), (15. 41450± 0.218 pg/ml), (25. 4145065284± 0.218232 pg/ml) in compared to the control group (9.701792 ± 0.555 ng/ml), (97.107565 ± 0. 700 pg/ml), (4.129558 ± 0. 190 pg/ml), (6.45839 ± 0. 220 pg/ml) as seen in figure (1, 2, 3, 4).

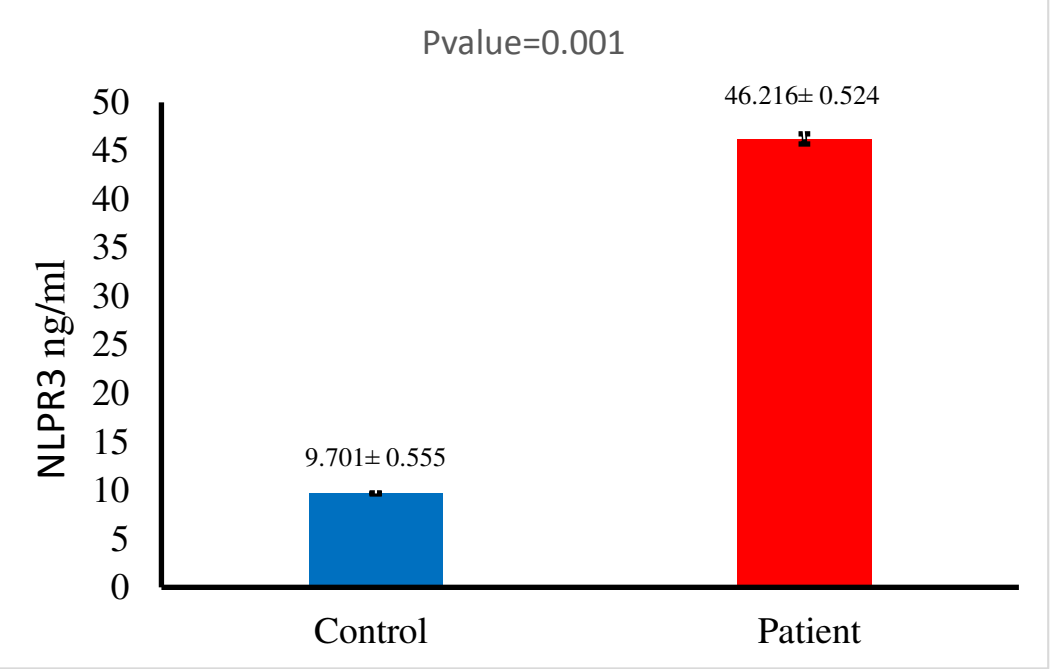

Figure 1. Concentration of human NLRP3 (pg/ml) Comparison between Patients Suffering from *Toxoplasma gondii* Infection and Control Group

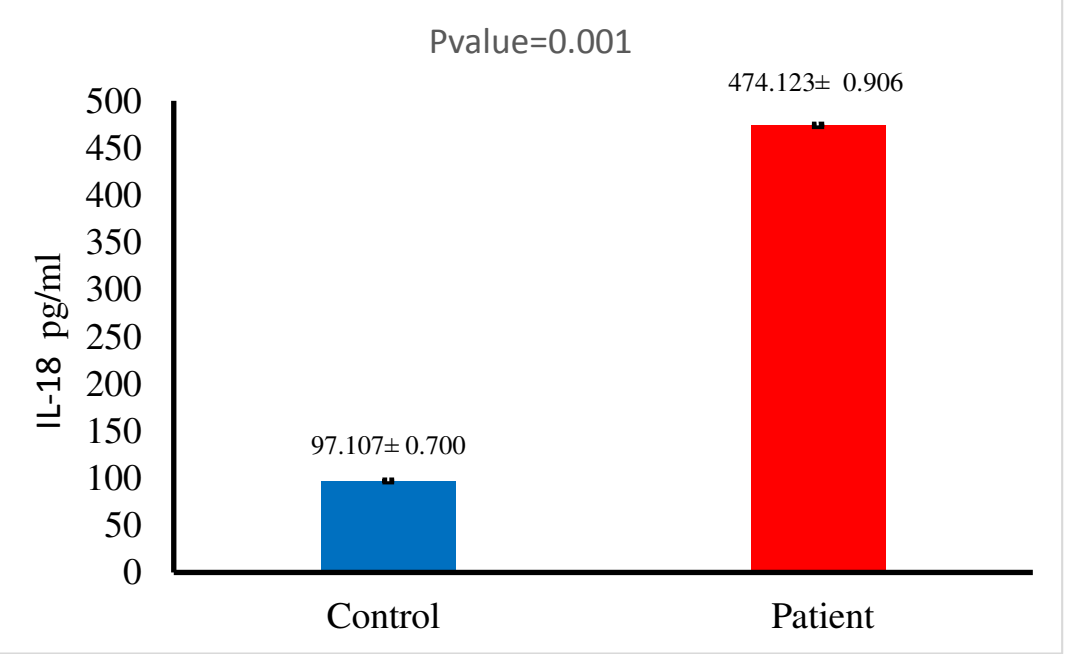

Figure 2. Concentration of interleukin-18 comparison between patients suffering from *Toxoplasma gondii* infection and control group

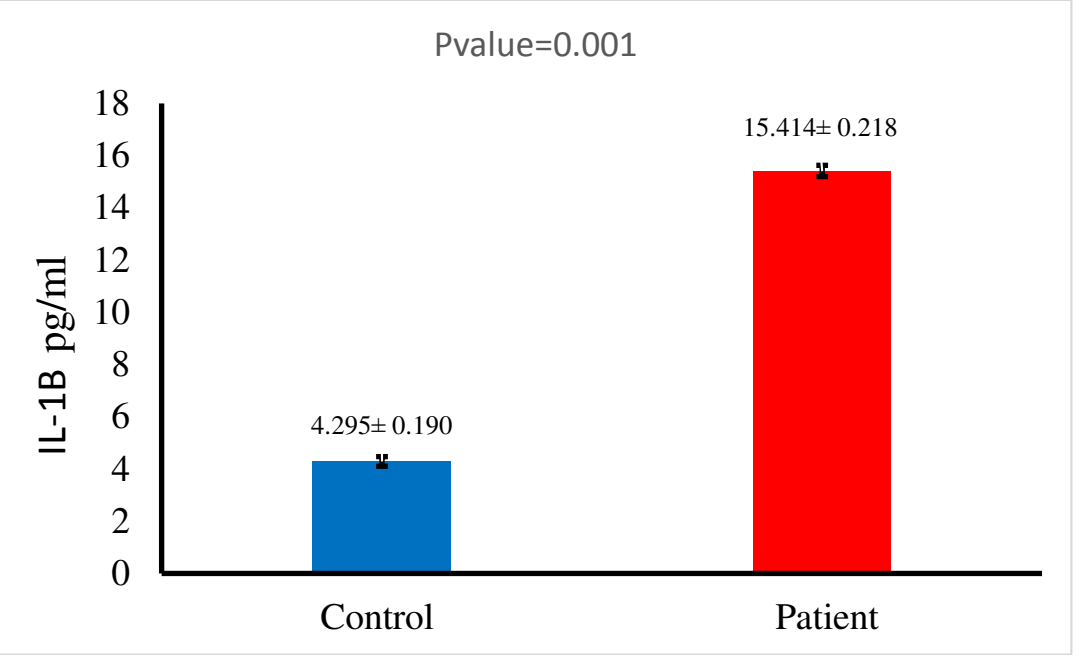

Figure 3. Concentration of interleukin-1β Comparison between Patients Suffering from *Toxoplasma gondii* Infection and control group

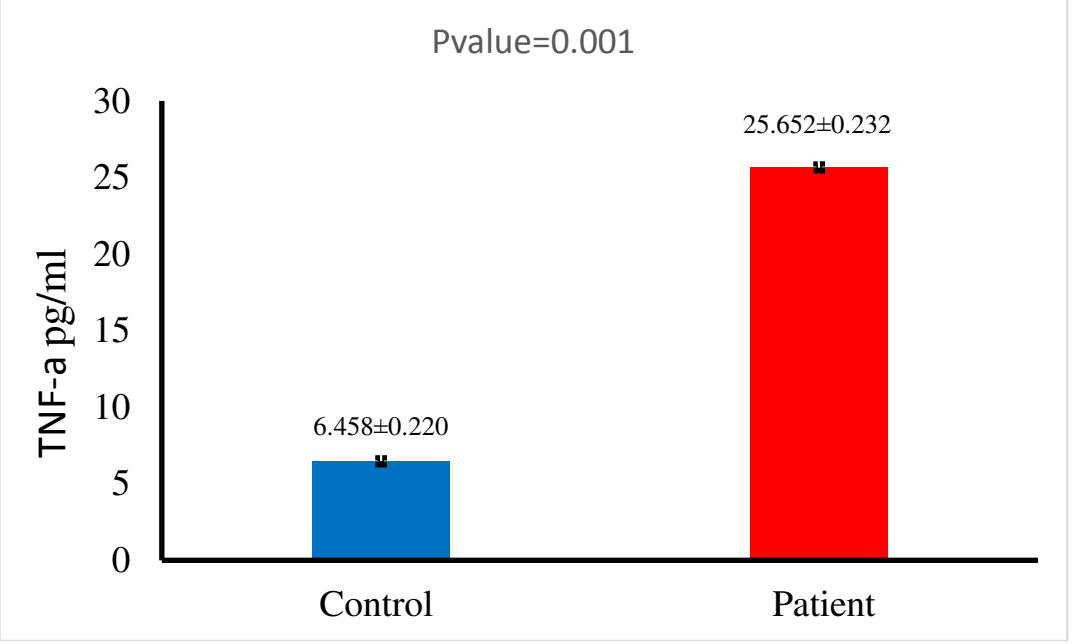

Figure 4. Concentration of TNF-α comparison between patients suffering from *Toxoplasma gondii* Infection and control group

Discussion

Compared to the control group, the serum level of (NLRP3) patients infected with the *T. gondii* parasite increased significantly. This increase could be related to the oligomerization and recruitment of the adaptor molecule ASC, caspase-1 activation, release of the active forms of IL-1β and IL-18, and promotion of the cell-mediated death pathway pyroptosis when pathogen stimuli activate inflammasomes. Inflammasome recognition of pathogen ligands is thus considered an important host innate immune system for detecting invading microorganisms. According to previous research, *T. gondii* is identified by the NLRP1 and NLRP3 inflammasomes, resulting in the production of IL-1β and IL-18 *in vitro* and *in vivo* (Ewald *et al.,* 2014; Gorfu *et al.,* 2014). TLR identification of the parasite by DCs is also essential for IL-12 synthesis, according to research (Yarovinsky *et al.,* 2005; Pifer *et al.,* 2011; Andrade *et al.,* 2013). The generation of IL-12 by *T. gondii* is required for a robust CD4+ TH1-derived IFN- response, which leads to the activation of IFN-mediated genes required for parasite clearance (Collazo *et al.,* 2001; Taylor *et al.,* 2000; Khaminets *et al.,* 2010; Yamamoto *et al.,* 2012). Furthermore, earlier research has demonstrated that the inflammasome-dependent cytokine IL-18 might function with IL-12 during *T. gondii* infection, enhancing IFN- responses and aiding parasite limitation (Cai *et al.,* 2000; Yap *et al.,* 2001; Gorfu *et al.,* 2014). As a result, we expected that NLRP3, ASC, or Casp1/11 loss would drastically reduce CD4+ T cell-derived IFN- responses, resulting in fast host mortality.

Two previous studies found conflicting results regarding the role of the inflammasome in *T. gondii* host immunity. Hitziger *et al.* (2005) finding are congruent with the current finding, demonstrating that inflammasome impairment does not significantly affect parasite limitation. on the other hand, Gorfu *et al.* (2014) finding, show that the lack of either the NLRP1 or NLRP3 inflammasome leads to increased parasite burden and host death.
Furthermore, both IL-1β and IL-18 have been shown to increase IFN- production during *T. gondii* infection (Hunter *et al.,* 1995; Cai *et al.,* 2000). As a result, we postulated that inflammasome activation is essential for *T. gondii* resistance in humans.

Humans lack a functional TLR11, and TLR12 is absent from the human genome (Roach *et al.,* 2005); however, parasite infection in immunocompetent individuals is generally asymptomatic, suggesting that TLR11- and TLR12-independent innate recognition of *T. gondii* is sufficient for human immunity against the parasite. When TLR11-dependent immunity is lacking, both CCL2-dependent recruitment of human monocytes and NLRP1, NLRP3, ASC, and caspase-1-dependent production of IL-1 and IL-18 by these cells show that *T. gondii* triggered inflammasome activation is essential for parasite resistance (Witola *et al.,* 2010; Gov *et al.,* 2013; Gov *et al.,* 2017). The study results demonstrated a significant increase in the serum level of (IL-18) individuals infected with *T. gondii* parasites compared with the control group. It may be attributed to *T.gondii* being an intracellular parasitic protozoan that spreads via ingestion and vertical infection. Oral or intraperitoneal infection of low dosages (<20 cysts) of *T. gondii* triggered IFN-γ production from Th1 and NK cells. However, oral infection with a larger inoculum dose (50–100 cysts) resulted in a detrimental Th1 cell response

characterized by severe small intestine necrosis induced by the overproduction of proinflammatory mediators (Liesenfeld *et al.,* 1996). (Liesenfeld *et al.,*1996). On the other hand, IL-18 contributed to the development of immunopathological features in the small intestine after the oral high-dose infection of *T. gondii* via the activation of IFN-γ production (Vossenkamper *et al.,* 2004). (Vossenkamper *et al.,* 2004). By this method, IL-18 operates on IL-15-dependent NKp46+ $NK1.1^{+}$ cells to trigger CCL3 synthesis, which is implicated in accumulating CCR1 positive inflammatory monocytes (Schulthess *et al.,* 2012). IL-22 is also an essential cytokine for ileitis caused by *T. gondii* infection, maybe because of it. Intestinal epithelial cells respond to IL-22 by producing IL-18, which causes innate lymphoid cells to produce IL-22 (ILCs). This positive feedback leads to increased IFN-γ production and a significant increase in neutrophil infiltration in the ileum (Munoz *et al.,* 2015). IL-18 is a proinflammatory cytokine that increases the production of IFN- by NK and T cells and is elevated in response to infection, according to previous research (Monteleone *et al.,* 1999; Pirhonen *et al.,* 1999; Pizarro *et al.,* 1999). Infection with *T. gondii* increases blood levels of IL-18 mRNA but not of IL-18 mRNA, according to Ghayur *et al.* (1997); Gu *et al.* (1997) these findings point to a posttranslational mechanism that controls IL-18 secretion. The elevated serum level of interleukin-18 (IL-18) this could be due to IL-18 potential to increase chemokine synthesis or upregulate adhesion molecule expression (Fehniger *et al.,* 1999; Kohka *et al.,* 1998). IL-18 can have both protective and pro-inflammatory effects (Dinarello, 2009). Infiltrating macrophages and lymphocytes release IL-18, which boosts Th1cell-mediated immunity and inflammation in chronic and severe colitis (Chikano *et al.,* 2000). When intestinal epithelial cells are the predominant source of IL-18, however, it acts as a protective factor during the early, acute phase of mucosal immune responses (Takagi *et al.,* 2003; Zaki *et al.,* 2010). Myeloid cells and epithelial cells are the principal cellular sources of IL-18 (Egan *et al.,* 2011). Although activation of particular innate immune signalling receptors increases IL-18 transcription in myeloid cells, in most other cells, IL-18 is thought to be constitutively produced and stored as intracellular proIL-18 protein (Kolinska *et al.,* 2008; Nakanishi *et al.,* 2001). Pro-inflammatory signals activate inflammasomes and caspases, causing IL-18 to be proteolytically processed to its active form, which is then released by cells via a non-classical secretion pathway (Dinarello, 2009).

According to the findings, the serum level of (IL-1β) patients infected with *T.gondii* parasites increased significantly compared to the control group. May be due to IL-1β production contributing to host control of *T. gondii* infection (Chang *et al.,* 1990; Hunter *et al.,* 1995; Brunton *et al.,* 2000 ;Witola *et al.,* 2011), and we have previously shown that *T. gondii* infection of primary human monocytes induces IL-1β transcript production and NLRP3 inflammasome activation (Gov *et al.,* 2013 ;Gov *et al.,* 2017). *T. gondii* infection, on the other hand, does not activate any known human TLRs, and the signalling pathways involved in TLR-independent IL-1β generation during infection, especially in human cells, are yet unknown. This study shows that *T. gondii* infected primary human monocytes generated IL-1β via a Syk-PKC-CARD9/MALT-1-NF-B signalling pathway and triggered the NLRP3 inflammasome for IL-1β release from viable cells in a GSDMD-independent manner. Furthermore, we have defined discrepancies in Syk's involvement in *T. gondii* Compared to LPS-stimulated primary human monocytes, Syk was required for IL-1β production during *T. gondii* infection. In contrast, Syk primarily

promoted IL-1β release in LPS-stimulated monocytes. Recent research suggests that this CD14+CD16- inflammatory monocyte population is primarily responsible for pathogenic inflammation in arthritis and sepsis (Ziegler-Heitbrock, 2015; Geng *et al.,* 2016); The present findings support this hypothesis. Inflammatory monocytes may regulate the expression or function of receptors or signalling molecules involved in *T. gondii* induced IL-1β production differently than other monocyte subsets, making them more susceptible to inflammatory stimuli. Apoptosis, an inflammatory form of cell death characterised by cell swelling and lysis, is the most well-studied release mechanism (Cookson *et al.,* 2001). IL-1β can also be secreted from live cells without pyroptosis) Evavold *et al.,* 2018). This is true for human monocytes treated with LPS, but not for mice (Gaidt *et al.,* 2016). The discovery and characterization of GSDMD (Kayagaki *et al.,* 2015; Shi *et al.,* 2015), which belongs to the inflammasome and can act as a pyroptosis effector protein by forming tiny pores in the cell membrane (He *et al.,* 2015; Liu *et al.,* 2016; Ding *et al.,* 2016), provided a molecular basis for this inflammatory form of cell death. GSDMD cleavage and cell death did not appear to drive IL-1β release from primary human monocytes in the context of *T. gondii* infection, as there was no difference in the percent of viable *T. gondii* infected or mock-infected monocytes at four hours post infection, the time when functional IL-1β was detected in the supernatant. Furthermore, glycine administration had little effect on *T. gondii* induced IL-1β production, despite glycine reducing ion flux and pyroptosis.

Compared to the control group, the serum level of (TNF-α) patients infected with the *T.gondii* parasite increased significantly. In recent research, TNF- α has been implicated in the etiology of psoriasis disease (Gottlieb *et al.,* 2005; Baliwag *et al.,* 2015; Salvi *et al.,* 2016). TNF-α levels in both skin lesions and blood are higher in patients with active skin disorders (Bradley *et al.,* 2008). TNF-α is produced provisionally in the plasma membrane of cells that are subjected to antibody-dependent cell cytotoxicity (ADCC) (Silva *et al.,* 2010; Horiuchi *et al.,* 2010). ETN contains the Fc portion of IgG1, which causes ADCC to occur (Horiuchi *et al.,* 2010). On the other hand, *Toxoplasma* infection decreases the production of TNF-α to begin infection (Studenicová *et al.,* 2006). The findings of this study were similar to those of El-Hashimi *et al.* (2014) in Iraq, who looked at the levels of TNF- α and IL-6 in the sera of three groups of women (aborted pregnant women who were seropositive for toxoplasmosis, aborted pregnant women who were seronegative for *T. gondii*, and healthy uninfected women), and found a significantly higher level of *T. gondii* .The current findings were consistent with Al-Kady (2011) who researched Egyptian women infected with *T. gondii*. They observed an increase in TNF- levels in these patients compared to the healthy group. Furthermore, the current findings were congruent with those of Alfonzo *et al.* (2005) on toxoplasmosis infected French women, who found an increase in TNF- α compared to healthy women who were not infected with this parasite. Matowicka-Karna *et al.* (2009) found a substantial rise in both IL-10 and TNF- α in the sera of pregnant women infected with toxoplasmosis to women in the control group who were *T. gondii* seronegative. In comparison to the control group, Lang *et al.* (2007) found a drop in TNF- α concentration in the sera of women who were seropositive for *T. gondii* (non-infected women). El-Sherbini *et al.* (2019) investigated the gene expression profile of some pro-and anti-inflammatory cytokines in *Toxoplasma* seropositive women with various pregnancy conditions.

They found that the sera of women who had multiple abortions had higher levels of both IFN-γ and TNF- α than those who had no abortions, implying that the aborted women were in a more inflammatory state. The authors suggested that these cytokines may play a role in toxoplasmosis prognostic or therapeutic issues in pregnant women.

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