Role of IL-1β, Prolactin and DHEA in men Patients Infected with Toxoplasmosis

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Abstract. Toxoplasma gondii has a worldwide distribution and it is one of the most prevalent infectious agents in Iraq, as it is found in various mammals, fish, and terrestrial and water birds. Cats are the only definitive host for the parasite that throws the infective phase into the environment. The primary aim of this study was to determine the serum levels of IL-1β, prolactin, Dehydroepiandrosterone (DHEA) in patients and healthy group. The study was conducted on 260 Males suspected of Toxoplasmosis ages ranging from 20-50 years old. All these cases were examined by measuring Toxo IgG serum levels, who attended AL-Hakeem hospital, and (30) healthy males as the control group, collected randomly from AL-Najaf province, these samples were collected from March 2023 to August 2023. Any patient was using the drug or undergoing disease removal from the current study. The current study revealed that the concentration of IL-1β in patients infected with Toxoplasmosis were significant increase (P<0.05) compared to the control group, but the concentration of (prolactin) in patients infected with toxoplasmosis were significant decrease (P<0.05) is compared to the control group. Also, it revealed that the DHEA levels were elevated but non significantly in samples infected with Toxoplasmosis compared to the control group. The current study has concluded that infection with Toxoplasmosis may be a risk factor. A chronic T. gondii infection is associated with variations in levels of serum prolactin and these variations may influence the immune system by IL-1β increase the susceptibility to Toxoplasmosis infection. Keyword: Najaf, Parasite, T. gondii, IL-1β, DHEA

1 Introduction

Toxoplasma gondii (T. gondii) infection occurs through the digestive system, after the parasite invades the intestine, which is known as parasitemia occurs, and it transforms the bradyzoite stage of reproduction into the tachyzoite stage of reproduction in the layer of epithelial cells of the intestine, and it is transferred to the blood and then to the rest of the body's organs by lymph [1]. Macrophage cells work to eat the parasite after that entry into the blood, and after entering the secretive phase, the proliferation of phagocytes then leads to the formation Parasitophorous vacuole inside the cytoplasm of phagocytic cells and the
vacuole contains protein components that cannot be removed from the proteins of the phagocytic cell membrane, it has an important role in enzyme release from phagosomes and lysosomes when attacking the parasite causing death, thus the parasite remains inhibited inside the cell. Phagocytosis is a factor in survival in the latent state in patients with toxoplasmosis [2]. In response to TLR, activated complement components, other cytokines (including TNF-a), and IL-1 itself, hematopoietic cells like blood monocytes, tissue macrophages, skin dendritic cells, and brain microglia create IL-1 [3]. In patients with certain mutations, known as autoinflammatory disorders, increased IL-1 output is associated with inflammation. However, NLRP3 or caspase-1 activation is not the exclusive cause of IL-1-mediated inflammation. Caspase-1-deficient mice experience the same IL-1-mediated illness as wild-type mice (WT) mice [3, 4]. It is known that the hormone prolactin (PRL), which is released by the pituitary gland, affects the immune system. *Toxoplasma* growth was prevented by prolactin in murine microglial cell cultures [5] and severely constrained in mouse and human cell lines with intracellular toxoplasma proliferation [6]. *T. gondii* prevalence was low among women with hyperprolactinemia [7]. Neither the host nor the parasite is directly cytotoxic by PRL, although it can likely attach to the surface protein of the parasite and disrupt its receptors [8]. Dehydroepiandrosterone (DHEA) is a steroid hormone that is produced in the brain, gonads, and adrenal glands in addition to being produced from pregnenolone by the enzyme 17-20 desmolase. Despite being a hormone, it has proven to be a highly effective antiparasite medication. In vitro, low levels of DHEA prevent *Entamoeba* trophozoites from proliferating, adhering to one another, and moving about, whereas high levels drive the parasite's lysis [9]. DHEA possesses toxoplasmicidal actions on extracellular tachyzoites. Ultrastructural evaluation of the treated parasite demonstrated that the cytoskeleton structures are altered by DHEA. DHEA in vitro therapy reduces both the passive invasion event and the survivability of extracellular tachyzoites [10].

### 2 Materials and Methods

#### 2.1 The subjects

The study was conducted on 260 Males suspected of Toxoplasmosis ages ranging from 20-50 years old. All these cases were examined by measuring Toxo IgG serum levels, who attended AL-Hakeem hospital, and (30) healthy males as the control group, collected randomly from AL-Najaf province, these samples were collected from March 2023 to August 2023. Any patient was using the drug or undergoing disease removal from the current study.

#### 2.2 Blood Specimens collection

Only 50 positive samples out of 260 suspected patients and 30 healthy people attended AL-Hakeem hospital, and (30) healthy males as the control group, collected randomly from AL-Najaf province, these samples were collected from March 2023 to August 2023. 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 three parts and kept in deep freeze at -20°C until utilized for IL-1β, Prolactin and DHEA. The biomarkers in the current study were estimated by Eliza Kits.
2.3 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 [11].

3 Results

The current study revealed that the concentration of IL-1β ng/ml in patients infected with Toxoplasmosis was a significant increase (P< 0.05) (0.4986± 0.05 ng/ml), in compared to the control group (0.02935 ± 0.003) ng/ml, but the concentration of PRL ng/ml in patients infected with Toxoplasmosis was a significant decrease (P< 0.05) (0.5345± 0.099 ng/ml), in compared to the control group (1.385 ± 0.117 ng/ml), while the current study revealed that the concentration of DHEA ng/ml in patients infected with Toxoplasmosis was a non-significant increase (P< 0.05) (1.913± 0.045%), in compared to the control group (1.877 ± 0.04 %) as seen in figure (1), (2), (3) respectively.

![Fig. 1. Serum concentration of IL-1β ng/ml in healthy individuals and patients infected with Toxoplasmosis.](image1)

![Fig. 2. Serum concentration of prolactin ng/ml in healthy individuals and patients infected with Toxoplasmosis.](image2)
Fig. 3. Serum concentration of DHEA ng/ml in healthy individuals and patients infected with Toxoplasmosis.

4 Discussion

The current study revealed that the concentration of IL-1β ng/ml in patients infected with Toxoplasmosis was a significant increase, compared to the control group. These results may be due to IL-1β production contributing to host control of *T. gondii* infection. IL-1β is an inflammatory cytokine that has been described as a “master regulator” of inflammation, since it can activate downstream inflammatory genes. *T. gondii* has been shown to induce IL-1β in multiple human cell types, including monocytes, foreskin fibroblasts. Previous studies suggest that *T. gondii*-induced IL-1β mediates protection against the parasite, but pathways that lead to IL-1β production in human cells during *T. gondii* infection are not well understood [12], and we have previously shown that *T. gondii* infection of primary human monocytes induces the production of IL-1β transcripts IL-1, may play a significant role in modulating the host's immune defence against *T. gondii* infection [13, 14, 15].

Interleukin-1beta (IL-1) is a key regulator of inflammation that activates a variety of downstream inflammatory genes. *T. gondii* induces IL-1 in multiple human primary cells and cell lineages, and *T. gondii*-induced IL-1 mediates host protection against the parasite. The production of IL-1 is regulated by the inflammasome, a multiprotein complex typically composed of caspase-1, an adaptor protein ASC (apoptosis-associated speck-like protein). The pathways leading to the production of IL-1β in human cells during *T. gondii* infection are not well understood. Described through an in vitro study that *T. gondii* can promote IL-1β transcript, processing and release in human monocytic cell lines. Based on that, IL-1β was proposed as a key regulator of the innate inflammatory response against *T. gondii* in the initial phase of infection. *T. gondii*-induced IL-1β mediates protection against the parasite, but pathways that lead to IL-1β production in human cells during *T. gondii* infection are not well understood[16]. Interleukin-1beta (IL-1) is a key regulator of inflammation that activates a variety of downstream inflammatory genes. *T. gondii* induces IL-1 in multiple human primary cells and cell lineages, and *T. gondii*-induced IL-1 mediates host protection against the parasite. The production of IL-1 is regulated by the inflammasome, a multiprotein complex typically composed of caspase-1, an adaptor protein ASC (apoptosis-associated speck-like protein). Extracellular cleavage of the inactive IL-1β precursor by neutrophil enzymes such as proteinase-3 and elastase generate active IL-1β because the cleavage site is close to that of caspase-1 [16]. The pathway nuclear factor κB (NF-κB), Mitogen-activated protein kinases and IFN regulatory factors play central roles.
in the induction of interleukin IL1β, and interferons (IFN) type I and type II. The activation of the pathway leads to the recruitment of immune cells to the site of infection via the secretion of chemokines, and these recruited cells can kill Toxoplasma via the upregulation of effector molecules. There are important differences in how the innate immune system of different host species detect Toxoplasma and, once it is detected, how it is eliminated via IFNγ-induced anti-parasitic activities [17]. The present study is in agreement with previous studies by Chandrasekharam et al. (2000) [18]. showed secretion of IL-1, IL-6, GM-CSF, and ICAM-1 by HRPE cells in response to T. gondii infection. Elevated production of IL-1 and other molecules by T. gondii-infected resident cells may initiate local immune reactivity during primary infections and recurrent reactivation episodes in Toxoplasma-infection. Also not agrees with Washino et al. (2012) [19]. who showed effects of T. gondii infection on the IL-10, IL-1β, and IL-6 mRNA expression of CD11c+ cells in IL-1 deficient mice were similar to those observed in WT mice. proinflammatory cytokines were of IL-1 deficient mice at an early stage of T. gondii infection because low of immunity. The level of IL-1β mRNA from DC was not statistically different between infected IL-1 deficient and WT mice. The current study revealed that the concentration of PRL ng/ml in patients infected with Toxoplasmosis was a significant decrease, compared to the control group. This decrease in the hormone is due to PRL deficiency in mice and may increase the probability and severity of infections. There was an association between the level of PRL and the frequency of T. gondii infections among women and men. It was shown that PRL has inhibitory effects on Toxoplasma proliferation in mononuclear cells of individuals with high PRL levels [20]. This observation was interpreted to suggest that testosterone primarily acted on the pituitary lactotrophs of both constructs to suppress prolactin secretion. This observation was suggestive of the suppressive effects of testosterone on prolactin synthesis [21]. Prolactin, a hormone, synthesized in the adenohypophyseal lactotrophs, has no known target organ or defined role in male reproduction, expression of prolactin receptors on choroid plexuses and hypothalamus presupposes a latent role for this hormone in the regulation of male fertility. The situation in which large amounts of PRL are in the blood of men or non-pregnant women is called hyperprolactinemia [22]. Dopamine has been shown to inhibit the release of prolactin in a variety of in vitro systems [23]. The current study does not conform with the study conducted by Dzitko et al. (2008) [7] in women with high PRL levels, T. gondii prevalence was lower than the control group (33.9% vs 45.58%). The current study is agreed with similar results reported by Singh (2021) [24] in Bihar, India the prevalence of T. gondii infection in the population of patients with a PRL level below and above the normal with the population of those having normal PRL level, revealed lower seroprevalence in the group of men and women with T. gondii infection. It has been proven that PRL deficiency in mice may increase the probability and severity of infections. Bromocriptine, the inhibitor of PRL secretion, is used in organ transplantation and autoimmune diseases to inhibit the immune system [25]. The current study in disagreement with Mohammadpour et al. (2018) [26,28] showed the prevalence of T. gondii infection in women with high PRL levels was lower than that in the comparison group with normal levels of PRL immunoregulatory role of PRL and indicated that the high levels of PRL could be related to Toxoplasma seronegativity in women. This study does not agree with ASMAA et al. (2021) [29] in Egypt who found a significant decrease in dopamine levels and an increase in prolactin levels in clozapine-infected treated rats. In metoclopramide-infected treated rats' prolactin levels significantly increased. And not agree with studies performed by Benedetto and Auriault (2003) [29, 30] who described that PRL levels are high during inflammatory or innate immune responses to several protozoa.

The current study revealed that the concentration of DHEA ng/ml in patients infected with Toxoplasmosis was a non-significant increase, compared to the control group. This
non-significant increase due to DHEAS levels during human toxoplasmosis has not been seen, indicating that its protective effects are the result of potent immunoregulatory mechanisms during infection [31]. Dehydroepiandrosterone-sulfate (DHEA-sulfate) is the most abundant hormone in peripheral circulation. It is synthesized from dehydroepiandrosterone (DHEA) by steroid sulfotransferase (SULT) and hydrolyzed back to DHEA by stearyl-sulfatase (STS). DHEA-sulfate and DHEA rise with adrenarche and approach the nadir around the age of 70. DHEAS regulates cytokine production by both myeloid and lymphoid cells. Thus, most reports suggest this steroid is a potent inducer of IL-2 secretion by Th1 cells and human T cell cytotoxic function [32]. Many beneficial effects of DHEA, include improved immune function and memory and prevention of atherosclerosis, cancer, diabetes, and obesity. Many of the benefits seen in animal studies have yet to be shown in humans and cause functional activation of T cells (increases in CD8+ and CD56+ cells [natural killer cells] and enhanced cytotoxic activity [33].

The role of DHEA on *T. gondii* has not been explored. Here, we demonstrated for the first time the toxoplasmicidal effect of DHEA on extracellular tachyzoites. Ultrastructural analysis of treated parasites showed that DHEA alters the cytoskeleton structures, leading to the loss of the organelle structure and organization as well as the loss of the cellular shape role as an antiparasitic drug in *T. gondii* infections and the possible role of the immune system in the DHEA response in both acute and chronic infection [9]. Previous findings suggest that DHEAS levels are not effective in signalling activity and are not significantly impacted in ocular toxoplasmosis, despite the intrinsic association of DHEAS and the immune system prompting additional inquiry into this hormone's role in human toxoplasmosis [34].

The present study does not agree with the previous study achieved by Hernández et al. (2021) [9] reported that DHEA levels started to decrease in the early luteal, and returned to basal levels by the late luteal subphase. DHEA, however, did not vary across the menstrual cycle. The present study deep-mapped trajectories of DHEA and DHEA-sulfate across the entire menstrual cycle, demonstrating a significant decrease in DHEA-sulfate in the mid-luteal sub-phase. The current studies do not conform with previous studies that DHEA increases the macrophage's function via improving NO content and up-regulating TNF-α expression levels, but also evokes a Th1 immuno-response and represses a Th2 immuno-response through promoting a shift in Th1/Th2 balance toward Th1-dominant immunity *in vivo* and *in vitro*, regulates the immune function by decreasing ROS production and increasing anti-oxidative enzyme activity in mice [35]. The present studies do not agree with a previous study done by Izawa et al. (2012) [36] who indicate that the steroid hormone environment of patients, particularly: the DHEA ratio, is linked to the malnutrition associated with HIV infection during Toxoplasmosis. The decreased DHEA in patients with the advanced stages of the disease could be associated with increased protein catabolism. This model also accounts for reduced basal levels of DHEA(S) found in studies of chronic stress [37].

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