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Correlation Study between CCL2 and Cortisol 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 CCL2 and cortisol in patients and healthy groups.
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 IgM and 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 (CCL2 and cortisol) in patients infected with Toxoplasmosis had a significant increase ($P< 0.05$) compared to the control group. Also, the current results revealed that the serum levels of CCL2 (ng/ml) correlated positively and significantly with cortisol (ng/ml) in patients infected with chronic Toxoplasmosis.
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 hormones for can result in inducted behavioural alterations and these variations may influence the immune system by Cortisol thus increasing the susceptibility to Toxoplasmosis infection.

Keywords: Kufa, Men, Toxoplasmosis, CCL2, Cortisol

Introduction
Toxoplasma infection can be brought on by consuming undercooked, contaminated meat, notably deer, hog, and lamb. Inadvertent ingestion of food that has been tainted by raw meat-contaminated hands, knives, utensils, cutting boards, and other food-related things. Toxoplasma cannot be spread through unbroken skin. (Flegr, 2013) [8].
Both the cellular and humoral immune responses overlap in severe infections to control them and the inflammatory response varies depending on the parasite genotype, as the first type, *T. gondii*, induces the humoral immune response, which produces antibodies like IgM and IgG, and the cellular immune response, which is the main response to control intracellular infections like infection with the parasite (Ingram *et al*., 2013) [13].
The parasite must remain dormant to elicit a site immunological response, which can affect the levels of dopamine and neurotransmitters by releasing the pro-inflammatory cytokines interferon C and indoleamine 2,3-dioxygenase (Berenreiterova *et al*., 2011 [5]; Al-Hadraawy *et al*., 2022).
Latent toxoplasmosis is asymptomatic in immunocompetent hosts. However as evidenced by the existence of *Toxoplasma* cysts in the neurological and muscular tissues, the infection is typically chronic. Low amounts of anti-*Toxoplasma* IgG are seen in the serum of infected individuals, and the infection results in lifetime protective immunity (humoral and cellular) to reinfection (Weiss and Dubey, 2009) [30].
After being stimulated by cytokines including TNF- and IL-1, macrophages, dendritic cells, and endothelial cells create CCL2. Monocytes, effector and memory T cells, including Th1 and Th2 cells, and memory T cells in general express the CCL2 receptor (Kallikourdis *et al*., 2007) [16]. It has been demonstrated that CCL2 has a role in luring these cells to the sites of *T. gondii* infection. Through TLR2, the tachyzoite soluble antigen can trigger the host cell's creation of CCL2 in a cascade. RH strain infection was observed to greatly boost the expression and production of CCL2 by human fibroblasts (Aviles *et al*.,

2008) [4].
A glucocorticoid hormone released by the adrenal cortex is cortisol. The glucocorticoids could create proteins that could have stimulatory or inhibitory effects on a particular tissue (Gardner *et al.*, 2011) [11]. The adrenal cortex secretes the glucocorticoid hormone cortisol. The glucocorticoids could create proteins with potential stimulatory or inhibitory effects on a particular tissue (Shahnaz *et al.*, 2011) [25].

Materials and Methods

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.

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 two parts and kept in deep freeze at -20C until utilized for CCL2 and cortisol. The biomarkers in the current study were estimated by Eliza Kits.

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 (Al-Hadraawy *et al.*, 2022).

Results

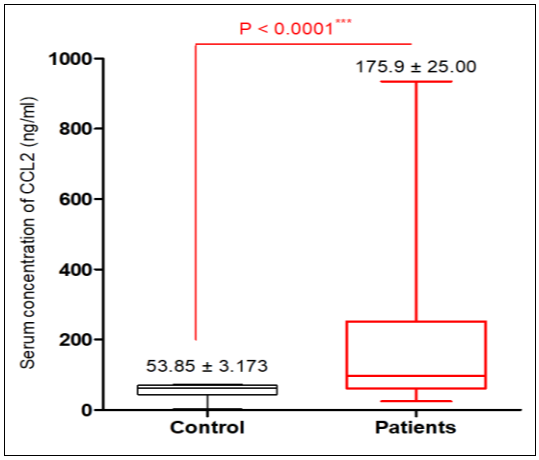

Fig 1: Serum concentration of CCL2 ng/ml in healthy individuals and patients infected with Toxoplasmosis

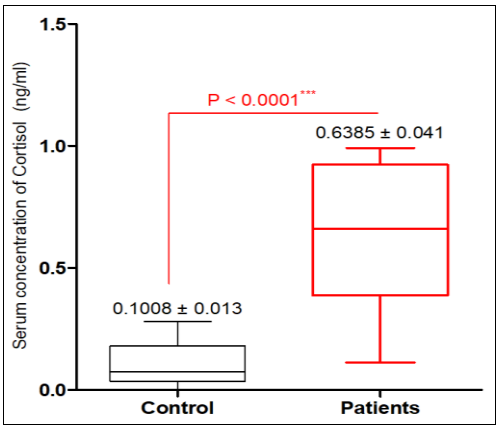

Fig 2: Serum concentration of Cortisol ng/ml in healthy individuals and patients infected with Toxoplasmosis

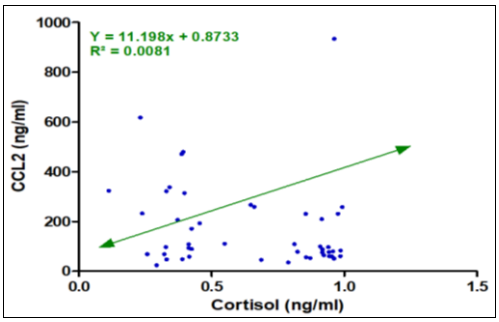

Fig 3: Correlation between CCL2 (ng/ml) Levels and cortisol (ng/ml) in patients infected with Toxoplasmosis

The current study revealed that the concentration of CCL2 and cortisol ng/ml in patients infected with Toxoplasmosis were a significant increase ($P < 0.05$) (175.9±25.00 ng/ml), (0.6385±0.041 ng/ml), respectively in compared to the control group (53.85±3.173 ng/ml), (0.1008 ± 0.013 ng/ml) respectively as seen in Fig 1, 2. Also, the current results revealed that the serum levels of CCL2 (ng/ml) correlated positively and significantly with cortisol (ng/ml) in patients infected with chronic Toxoplasmosis (R^2 = 0. 0081), as seen in Fig 3.

Discussion

The current study revealed that the concentration of CCL2 ng/ml in patients infected with Toxoplasmosis was a significant increase, compared to the control. This increases that the C-C chemokine CCL2 enhances macrophage responses to pro-inflammatory stimuli. CCL2 increases macrophage expression. is a critical component in guiding tissue inflammatory processes, and guiding chemotaxis of leukocytes to sites of infection (William *et al.*, 2017) [31]. Chemokine signalling can also similarly induce activation of leukocytes to cytokine stimulus, and CCL2 expression is increased to facilitate locomotion and leukocyte activation, for example, chemokines drive up-regulation of adhesion molecules essential for tethering of leukocytes to endothelial cells before extravasation from peripheral blood into interstitial tissues (Jin and Hereld, 2008) [15].
CCL2 was one of the first chemokines described and has since been extensively studied for its chemoattractant function. Multiple effects of CCL2 are owed to the types of

cells it recruits. However, increasing evidence has shown that CCL2 may be far more than merely a guidance cue for leukocytes. CCL2 has been shown to enhance the cell-killing properties of monocytes and macrophages, enhance the survival of macrophages and neutrophils, and have a profound influence on macrophage polarization and corresponding effector molecule secretion (Sierra *et al*., 2014) [26].

In the present study in agreement with the study achieved by Santos *et al.* (2023) [24] in Brazil, we identified the highest plasma concentrations of CCL2, CXCL16, and IL-33 in Brazilian pregnant women infected by *T. gondii*.

The current study differs from Rey *et al.* (2013) [23] that relieved decreased serum levels of CCL2 may be associated with active ocular toxoplasmosis and could therefore serve as a marker of disease activity.

The current studies also agree with Najafi *et al.* (2021) [22] who showed the elevation of CCL2, and IL33 gene expression in the early stage of toxoplasmosis is associated with the occurrence of neuropathological alterations. Detection of these genes as an indication of brain damage in the early stages of *Toxoplasma* infection.

The current study does not agree with Dunay *et al*. (2008) [7] who showed that the numbers of parasites were much lower than in the intestine and were not as highly elevated in CCl2 mice (less than 3-fold) when compared to those in control CD 56 mice.

The current study revealed that the concentration of cortisol ng/ml in patients infected with toxoplasmosis was a significant increase compared to the control group. The increase is due to a relationship between anti-IgG *toxoplasma* antibodies and cortisol elevation in toxoplasmosis patients. These results indicated that infected individuals had greater cortisol levels than non-infected individuals. A high blood cortisol level impairs the immune system's ability to operate normally, making patients more susceptible to illnesses like toxoplasmosis. In addition to impairing thyroid gland function, high cortisol production can raise blood pressure and cause hyperglycemia (James, 2010) [14]. Previous studies proved that cortisol increase in these people lasted for a long time after the infection. Cortisol titer is not high in usual circumstances, stressful behaviour results from a cortisol increase which can be considered an acceptable factor (Shahnaz *et al*., 2011) [25].

Cortisol, a steroid hormone, is synthesized from cholesterol. It is synthesized in the zone fasciculate layer of the adrenal cortex. Cortisol has many functions in the human body, such as mediating the stress response, regulating metabolism, the inflammatory response, and immune function (Angelousi *et al*., 2020) [3].

The results also agree with Terpsidis (2009) [28] reported that cortisol levels were greater in infected men and women than in non-infected men and women. Elevated cortisol levels are a symptom of significant stress in a person, and if they continue, they may cause them to respond in ways that are stressful or anxious. On the other hand, this increase in hormone dosage can affect the nervous system in several different ways.

The current research results are in agreement with Abdelazeem *et al.* (2015) [1] in Sudan who indicated that cortisol titer in the infected individuals is higher in both infected men and women than in uninfected ones. Cortisol increase is a symptom of significant stress in a person, it will lead to stress-induced behaviours and anxiety. This hormone titer increase can damage different parts of the nervous system.

The results of the present study agreed with Mahbodfar *et al*. (2015) [19] that showed the concentrations of testosterone and cortisol increased in individuals seropositive for toxoplasmosis cortisol increase in anti-IgG *Toxoplasma* antibody-positive patients. In this manner, this protozoan parasite probably affects human behaviour, personality and phenotypic traits. Moreover, the infected rats had higher levels of cortisol than the healthy rats.

The present study does not agree with Fukuda and Morimoto (2001) [10] who showed the relationship between cortisol and mental stress. Cortisol appears to be an adequate index for mental stress**.** Subjects who experienced flight simulator tasks did not show increases in cortisol levels as compared with their pre-task levels. However, subjects with high levels of cortisol showed poor performance.

The present study agreement with Al-Saqur *et al.* (2014) [2] in Baghdad who showed that cortisol hormone levels were also measured using an ELISA test for all samples, and the results found a significant increase in the cortisol hormone in patients with toxoplasmosis compared to non-toxoplasmosis individuals.

The present study agrees with Flegr *et al.* (2014) [9], who reported that the highest mean cortisol level in anti-*Toxoplasma* IgG seropositive patients was detected in the psychiatric control group, especially in patients suffering from schizophrenia and bipolar disorder with manic episodes. This was attributed to the fact that cortisol might be a part of the adaptive manipulation of *T. gondii* to suppress immunity and increase the chance of its survival in the host.

The current results revealed that the serum levels of CCL2 (ng/ml) correlated positively and significantly with cortisol (ng/ml) in patients infected with chronic Toxoplasmosis.

There is evidence to suggest a positive and significant correlation between the serum levels of cortisol and CCL2 in patients infected with Toxoplasmosis, The bidirectional relationship between cytokines and cortisol contributes to the maintenance of immune system homeostasis and is critical to regulating inflammation and maintaining health. The association between cortisol level and cell responsiveness to different stimuli, by evaluating chemokine production, as a mediator of immune responses (Liberman *et al*., 2018) [18]. It is known that cortisol may display an immunomodulatory activity, causing changes in proliferation and chemokine secretion (DeSantis *et al*., 2012) [6].

A study by Miller *et al*. (2013) [20] found that high cortisol levels might be related to the CCL2 balance, resulting in immune deregulation rather than immunosuppression. High cortisol levels might suppress the -mediated cellular. immune response, increasing the risk of infectious diseases. Cortisol is immunosuppressive in function, and elicits its immunosuppressive effects by down-regulating key inflammatory transcription factors, essentially weakening the pro-inflammatory response. Cortisol secretion can be positively regulated by stress signals, macrophage-secreted IL-1 and T cell secretion of glucocorticoid response modifying factor (Stephens *et al*., 2012) [27]. Sero-positive patients tended to have high cortisol during toxoplasmosis. Stressful behaviour that results from cortisol increase acts as an immune suppressor factor, motivating recurrent acute

toxoplasmosis through a double upturn number of *T. gondii* tachyzoites and brain cysts (Shahnaz *et al*., 2011) [25].

According to a review by Webster *et al*. (2002) [29], research has consistently shown that cortisol was suggested to act via inducing *T. gondii* higher susceptibility and elevating sequence to *Toxoplasma* infection that is likely considered as an important stress stimulus irritate blood cortisol upturn. These results indicated that cortisol level in infected persons is higher than in uninfected ones. High level of cortisol in the blood disrupts the normal functioning of the immune system and people with elevated cortisol levels may become more prone to infection such as toxoplasmosis.

High levels of inflammatory cytokines stimulate the CCL2 axis to release cortisol which inhibits inflammatory cytokine production. Deregulation of the CCL2 axis results in the lack of suppression of the immune system, known as glucocorticoid resistance (Irwin *et al*., 2011) [12].

Yeager (2011) [32] found that cortisol has anti-inflammatory effects following a systemic inflammatory stimulus. However, a cortisol concentration that acts acutely to suppress systemic inflammation also has a delayed effect of augmenting the inflammatory response to the subsequent, delayed stimulus. The infected rats had higher levels of cortisol than the healthy rats. Although corticosteroids do not directly affect behaviour, they have an important role in fear and anxiety control centres. Cortisol levels increase through tachyzoites, cysts, and cystozoites in infected individuals (Moroda *et al*., 2017) [21].

Karrer-Voegeli *et al*. (2009) [17], found that increased cortisol levels in patients may cause harmful psychological side effects. Individuals with increased levels of cortisol (stress hormone) may suffer from emotional disorders like depression, paranoia and anxiety. *Toxoplasma* infection can induce an elevation in cortisol levels in infected patients and in this manner, this protozoan parasite probably affects human behaviour, personality and phenotypic traits.

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