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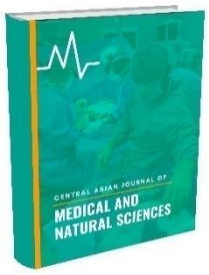

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Role of MIF and HSP70 Proteins in Immune Response for Patients Infected with Toxoplasmosis

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Abstract: The study was conducted on 250 suspected patients and 30 healthy persons who have visited Al-Marjan Hospital in Babil province from September 2021 to till February 2022. This study aimed to evaluation the serum levels of the MIF cytokine and HSP70 protein in females with Toxoplasmosis and control groups. The parasite was recognized after an examination of the Serum sample by using a VIDAS® TOXO IgG II. A total of 250 suspected patients were collected, all of these are females, sixty out of 250 (24 %) 60 females are patients infected with Toxoplasmosis and 30 healthy peoples. The results of current study showed higher significantly P<0.05 in concentration MIF cytokine in patients group (45.52±21.71) ng/ml compared with control group with concentration (16.48±6.86). The concentration of HSP70 level was higher significantly P<0.05 in patients group (3.70±3.06) ng/ml than in control group (1.61±0.92) ng/ml.

Key words: Toxoplasma gondii, MIF, HSP70, Babil, Iraq.

1. Introduction

Toxoplasmosis, a parasitic disease, is caused by the widely distributed parasite known as Toxoplasma gondii, as stated by (Hotez, 2014). According to official reports, more than one billion people worldwide are affected by T. gondii, as noted by (Hofmann et al. 2012). T. gondii belongs to the phylum Apicomplexa and is a highly successful protozoan parasite, as indicated by Tenter et al. (2000). Estimates suggest that up to one-third of the global population is infected with T. gondii, as reported by (Montoya & Liesenfeld, 2004) and (Pomares & Montoya, 2016). Toxoplasmosis is ranked as the third most prevalent foodborne parasitic infection requiring hospitalization, according to (Vaillant et al. 2005).

Additionally, 200,000 cases of congenital toxoplasmosis per year throughout the world, causing a variety of birth problems such as vision loss, anemia, microencephaly and premature birth (Pomares and Montoya,2016). Toxoplasmosis is a zoonotic ailment that holds considerable medical and veterinary implications. It is instigated by Toxoplasma gondii, a protozoan parasite belonging to the exclusive Toxoplasma genus. This disease possesses noteworthy global influence and significance.

The parasite has the capacity to infect a wide range of warm-blooded creatures, including livestock and humans. The primary modes of transmission encompass transplacental transmission, ingestion of raw vegetables or water contaminated with T. gondii oocysts via cat feces, and consumption of tissue cysts present in undercooked or raw meat from infected animals (Almeria and Dubey 2021). The sexual and asexual stages of the *T. gondii* life cycle occur in respective definitive and intermediate hosts. *T. gondii* is only able to establish sexual reproduction in cat species' digestive tract epithelium. The parasite, in contrast, reproduces asexually in all warm-blooded species, such as humans and livestock (Sasai and Yamamoto,2019).

In humans, two types of infections occur: inherited, which is transmitted vertically from the mother to the fetus via placental tissues, and acquired, most commonly through the digestive route by consuming undercooked meat dishes or coming into contact with cat feces, which is the definitive host of parasite(Jones *et al*,2014). *Toxoplasma gondii* infections are common worldwide, but they are usually asymptomatic, on the other hand, can lead to serious illness in humans, particularly in children with congenital infections and others with weakened immune systems, and even immunocompetent people have died from toxoplasmosis (Peyron *et al*., 2016). In the majority of adults, it does not cause serious disease, however in immunocompromised persons, it can induce deadly sickness, and in congenitally damaged children, it can cause blindness and mental retardation. (Hill and Dubey, 2002). The parasite elicits a strong innate immune response in infected hosts which is followed by strong adaptive immunity (Khan and Moretto,2022). Macrophage migration inhibitor factor (MIF) a pleiotropic inflammatory cytokine and one of the earliest cytokines discovered, is regarded as a crucial upstream mediator of innate immunity.

Increased MIF expression is associated with increased immunopathology and inflammation. (Yoo *et al*., 2016). Through promoting the synthesis of pro-inflammatory mediators including IL-8 and TNF-, there is evidence that MIF participates in both innate and adaptive immune responses. (Hertelendy *et al*., 2018). Heat shock proteins (HSPs) are highly preserved molecules that primarily aid in folding other proteins. They play a vital protective role in cells undergoing stress, and most HSPs have their expression induced by stress. While initially thought to function inside cells, recent studies show some HSPs move to the cell surface, especially when the cell experiences stress. They are therefore typically thought of as danger signaling biomarkers (Zininga *et al*., 2018), It's mainly expressed in most organisms under physiological conditions, but their expression can significantly increase in response to four types of stimuli: physical heat shock or radiation, chemical and microbial such as parasites, viruses, bacteria, fungi, and dietary stimuli. These proteins were discovered to protect cells from high temperatures and other forms of stress (Bolhassani and Agi,2019).

HSPs are responsible for folding proteins into their proper shapes. Hsps can be found in all organisms with cells and have a high level of similarity across species. Some Hsps may increase in amount during times of stress, but not all Hsps behave this way. HSPs are involved in various processes in the cell, including folding proteins, transporting proteins within the cell, and combining or taking apart protein complexes (Bukau et al., 2016). Additionally, antibodies that recognize some HSPs of parasitic origin have been found in the circulatory system of the host (Cabral *et al*.,2017; Multhoff and Hightower,2011).

2. Materials and Methods

2.1. Study design and patients

The investigation was carried out on a cohort of 250 individuals who were suspected to have the condition, alongside an additional 30 individuals who were deemed healthy. The specimens were obtained exclusively from female patients diagnosed with Toxoplasmosis, who were receiving medical care at Al-Marjan Hospital in Babil province. This data collection process took place between

September 2021 and February 2022. Blood samples were meticulously extracted from the aforementioned individuals using vein puncture techniques, with the utmost care and utilizing sterile tubes, in order to generate a serum that would serve as the foundation for the assessment of the study's various parameters.

2.2. Diagnosis *Toxoplasma gondii* parasite by VIDAS

VIDAS TOXO IgG II is a computerized quantitative test for usage on the intimate apparatuses for estimating quantitation of anti-toxoplasma (IgG) of serum via the Enzyme Link Fluorescent Assay (ELFA) (Hussian, 2022).

2.3. Serum collection

Five milliliters of blood were procured from both healthy individuals and those infected. Blood samples were extracted utilizing aseptic plain tubes and subsequently left undisturbed at ambient temperature for a duration of 30 minutes. The centrifugation process was executed at a rate of 3000 revolutions per minute for a time span of 5 minutes, employing a Memmert apparatus originating from Germany. Following this, the serum was collected and carefully stored in sterile tubes, maintaining a low temperature within a deep freeze environment of -20 degrees Celsius, until it was required for further use.

2.4. Serum biomarkers detection

Two human biomarkers were used in this study: of the MIF cytokine and HSP70. The biomarker kits with catalog numbers E-EL-H1530 and E-EL-H1522 were supplied by Elabscience Company from Bulgaria. These ELISA kits were used to quantify the levels of biomarkers in serum samples. The assays were performed on an ELISA plate reader from Human GmbH Germany according to the manufacturer's protocols from Elabscience.

2.5. Assay Procedure

One hundred microliters of standard or sample was added to each well on the plate, which was then kept at 37 degrees Celsius for an hour and a half. The liquid was removed from each well using aspiration and 100 microliters of a solution containing biotinylated detection antibodies was promptly pipetted into the wells. After incubating the plate again at 37 degrees Celsius for one hour, the contents were aspirated and the plate was washed 3 times. Next, 100 microliters of working solution containing HRP conjugate was put into each well, followed by incubation at 37 degrees Celsius for 30 more minutes. The plate was then aspirated again and washed 5 times.

Ninety microliters of substrate reagent was then pipetted into each well, and the plate was kept at 37 degrees Celsius for 15 minutes. After that, to measure the optical density at 450 nm, 50 microliters of stop solution was put into the wells. Lastly, the results were analyzed. The Assay Max Human MIF ELISA test was performed according to the protocol provided by the manufacturer Elabscience, using the same method as explained before for the HSP70 ELISA.

2.6. Statistical analysis

The T-test was employed in this investigation to make comparisons between samples utilizing Graph-pad prism version 10 computer software. A P-value of less than 0.05 was deemed to be statistically significant according to Al-Hadraawy (2016).

3. Results

A total of 250 suspected women patients were collected, sixty out of 250 which patients as seen in figure (1)

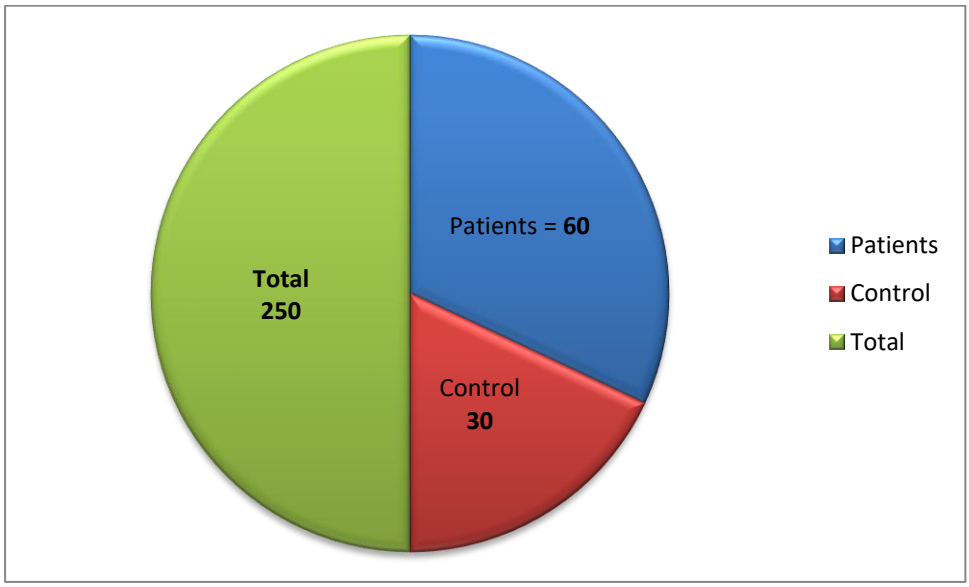

Figure (1): Number of women patients with *Toxoplasma* gondii N= 60.

3.1. MIF Concentration

The present investigation unveiled that there was a noteworthy augmentation (P< 0.05) in the levels of the cytokine MIF in patients compared to the control groups, with concentrations of (45.52±21.71) ng /ml and (16.48±6.86) ng /ml, respectively. This disparity is visually represented in figure (2).

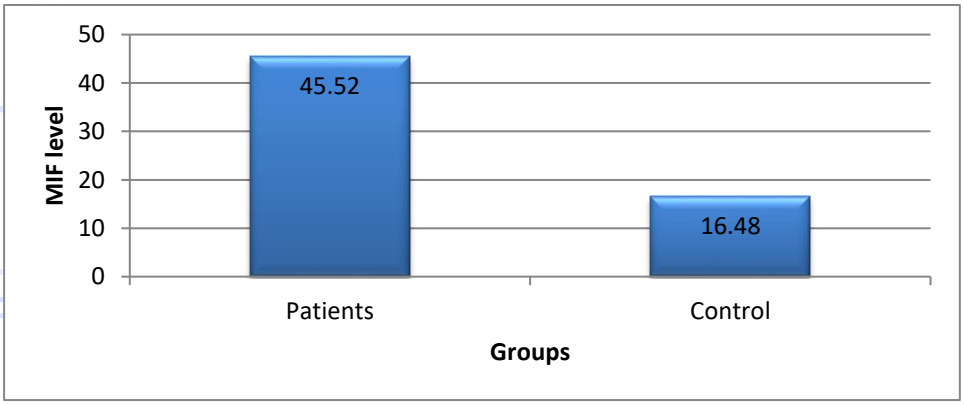

Figure (2): Comparison of mean concentration serum MIF cytokine between patients with Toxoplasmosis and control Group

3.2. HSP70 Concentration

The current study found a significant increase (P≤0.05) (3.70±3.06) ng /ml, (1.61±0.92) pg/ml between patients and control groups respectively as seen in figure (3) :

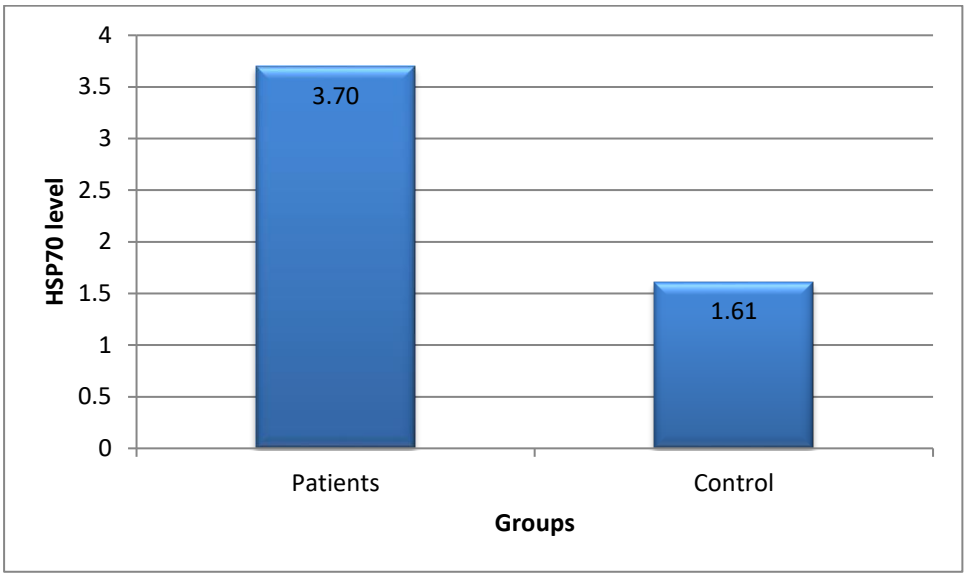

Figure (3): Comparison of mean concentration serum HSP70.

4. Discussion

In current study which showed the mean concentration of MIF was highest significant (P< 0.05) between patients and control groups, (45.52±21.71) pg /ml versus (16.48±6.86) pg /ml respectively. The virulence factor MIF, which parasites produce, is crucial for interactions with hosts and parasites and leads to pathogenesis (Moonah *et al*., 2019). Additionally, it serves a function similar to that of a chemokine by promoting the directed recruitment and migration of leukocytes to inflammatory and infectious sites, where they are then quickly released in response to microbial stimuli (Grieb *et al*., 2010). Macrophage migration inhibitory factor (MIF) induces the release of inflammatory cytokines such as interleukin-6 (IL-6) from immune cells and increases tumor necrosis factor (TNF) production in response to lipopolysaccharide (LPS). MIF can also counteract the anti-inflammatory effects of glucocorticoids (Moonah et al., 2014).

Neutrophil movement is guided by chemokines released from epithelial cells. One such chemokine is interleukin-8 (IL-8), which causes inflammation in mucous membranes by robustly drawing in neutrophils in various infectious and inflammatory diseases. Macrophage migration inhibitory factor (MIF) makes human intestinal epithelial cells secrete IL-8, which then summons neutrophils (Sierra-Puente et al., 2009).

MIF can bind to the cell surface receptor CD74, which leads to the secretion of proinflammatory cytokines including IL-8 that cause multiple inflammatory effects (Calandra & Roger, 2003; Ghosh et al., 2018). Some disease-causing protozoan parasites like Toxoplasma, Entamoeba, Plasmodium, and Leishmania produce versions of the MIF cytokine (Ghosh et al., 2020). These parasites try to avoid the immune response to stay inside the host, intensifying the damage from the ongoing inflammatory reaction to parasitic attack (Moonah et al., 2013).

Research demonstrates MIF's role in promoting inflammatory cytokines like IL-8 and TNF-alpha, showing its involvement in innate and adaptive immunity (Hertelendy et al., 2018). Multiple studies link increased levels of certain cytokines like IL-1, IL-8, and TNF-alpha to heightened MIF expression, suggesting this contributes to human diseases (Kang & Bucala, 2019).

Research showing E. histolytica infection raises MIF levels is consistent with findings by (Ngobeni et al. 2017). A study by Rodrigues Oliveira et al. (2018) also found higher MIF levels in parasite-infected

individuals. Kozaci et al. (2005) observed increased serum MIF levels in acute cutaneous leishmaniasis patients compared to controls.

The average concentration of total HSP70 was significantly higher in patients infected with Toxoplasmosis compared to their respective control groups in the present study. The results showed a notable increase ($P<0.05$) with a mean concentration of 3.70±3.06 pg/ml in the patient group, whereas the control group had a mean concentration of 1.61±0.92 pg/ml.Toxoplasmosis, an infectious disease caused by the parasite Toxoplasma gondii, affects various organs within the body. The manifestations of this disease vary depending on the location of the infection and the immune status of the host, as stated by (Al-Abodi 2019). The current findings align with the study conducted by Al-Abodi (2019) in Al-Qadisiyah province, Iraq, which reported a significant elevation of HSP70 protein levels in the blood samples of patients infected with Toxoplasmosis (28.21 ng/ml) compared to the control group (6.96 ng/ml). Additionally, the results from Al-Ubaydi (2011) demonstrated a high concentration of HSP70 in women who experienced abortions, with a significant increase in HSP70 protein levels in the blood samples of patients infected with Toxoplasmosis (6.26 ng/ml) compared to the control group (2.76 ng/ml). Additional studies have indicated that the release of TgHSP70 into the bloodstream is contingent upon the death of the parasite triggered by the host's immune response. On the other hand, the upregulation of TgHSP70 expression in the brain appears to be influenced by the rate of parasite multiplication, as explained by (Barenco et al. 2014). HSP70 plays a significant role in the immune response against Toxoplasma gondii, working together with macrophage cells, cytokines, and specialized T cells, as described by (Hideyuki, 2002).

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