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Effect of *Toxoplasma gondii* infection on the level of Human Macrophage-Derived Chemokine (MDC) in women with Polycystic Ovary Syndrome

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Abstract
Infected samples were obtained from patients at Al-Zahra Maternity and Children's Hospital, Al-Hakim Hospital, and other outpatient clinics in the Al-Najaf Governorate between July 2022 and November 2022. In this investigation, 120 samples were gathered and distributed as follows: As a control group, 30 women were uninfected, 30 were infected with the T.gondii parasite, 30 were infected with PCOS, and 30 were infected with both T.gondii and PCOS. The ages of the patients ranged from 19 to 45. Serum IgG and IgM levels, in addition to AMH (ovarian reserve hormone) levels, were analyzed in women diagnosed with polycystic ovary syndrome.The results of this study showed that when compared to the control group (0.0664 + 1.053 ng/ml), the concentration of MDC in women with T. gondii parasite infections rose considerably ($P<0.05$) (0.0994 + 2.356 ng/ml).The MDC concentration in PCOS rose considerably ($P<0.05$) compared to the control group (0.06644 + 1.053 ng/ml), and the MDC concentration in the blood serum of women infected with T. gondii and PCOS also increased significantly ($P<0.05$) compared to the control group (0.0664 + 1.053 ng/ml).

Keywords: *Toxoplasma gondii*,Chemokine, Polycystic Ovary Syndrome

Introduction

Zoonotic disease caused by parasitic infection with Toxoplasma gondii. This parasite's life cycle is one of the more complicated ones, as it requires two types of hosts: definitive, represented by the feline family Felidae, particularly cats, and intermediate. This includes many endothermic animals, including humans and other mammals, and birds. The infection has no symptoms**[24]**. Toxoplasmosis (cat sickness) is a frequent disease affecting people and animals.The host's immune system may block the parasite's proliferation and the production of tissue cysts in most body tissues, including the central nervous system, and skeletal and cardiac muscles, without any symptoms showing in most cases**[23]**. As for people who suffer from immunocompromised diseases and become infected with this disease, they have a reactivation of a latent infection of the parasite Weiss and Weiss.When infected by women during pregnancy, it is transmitted to the fetus through the placenta, causing great danger to the fetus. It may cause miscarriage or death of the fetus inside the womb**[16]**.The host's immune system plays an essential and prominent role in fighting parasitic infections in general, preventing the parasite that causes cat disease, Toxoplasmosis, from multiplying. This moderate infection causes symptoms such as fever and lymphadenitis, and those infected with HIV or malignant conditions are given immunosuppressive medicines. A parasite infection causes problems and severe symptoms such as hypertrophy and pneumonitis**[1]**.Splenomegaly, pneumonitis, and mortality are all possible outcomes. Infection with the *T. gondii* parasite results in both humoral immunity (the production of antibodies, particularly IgG and IgM) and cell-mediated immunity (the generation of anti-parasite free radicals such as oxygen species by immune cells). ROS may oxidize proteins and lipids, as well as trigger chemical alterations in nucleic acids **[2]**.Some immunological and analytical criteria were used to detect T. gondii infection, which causes cat sickness, to assess the amount of anti-Mullerian Hormone (AMH) in women with PCOS, and to study some common characteristics among them and their influence on the existence of the parasite. Furthermore, PCOS was utilized as the Minividas test to test the enzyme-linked fluorescence test (ELFA), which is one of the automated tests used to study IgM, IgG, and enzyme-linked immunosorbent assay (ELISA)**[3]**.The most prevalent cause of placental anovulation and ovulatory disorder infertility is a polycystic ovarian syndrome (PCOS). Menopause is connected with a metabolic condition, a frequent endocrine illness in women of reproductive

age. The syndrome is the main cause of ovarian dysfunction that leads to associated anovulation, resulting in infertility, as 5%-10% of women of childbearing age are affected by this syndrome**[4]**. This syndrome can be identified by clinical symptoms and biochemical parameters, as well as by the use of ultrasound. Menstrual irregularities, signs of androgen excess, obesity, and hirsutism are all examples of clinical manifestations of PCOS**[5]**. An index value of 8 or above indicates hirsutism. ‹Since the results of this analysis in the blood shows how many eggs are still in the ovaries, it is thought to be a crucial fertility test. As people age, fewer eggs remain in their ovaries, which results in less hormone secretion.The value of the analysis also increases in women who suffer from PCOS due to the increase in the number of small follicles that are Less than 8 mm in the ovaries**[6]**.

Materials and Methods

Collect blood samples

In this investigation, (120) samples were gathered and divided as follows: 30 healthy women served as the control group, whereas 30 others were afflicted with the Toxoplasma gondii parasite, PCOS, or both. Patients with ages ranging from (19-45) years who visited Al-Zahra Maternity and Children Hospital, Al-Hakim Hospital, and a few outpatient clinics in Al-Najaf Governorate during July and November 2022 provided infected samples.It was explored by measuring serum IgG and IgM levels, ovarian reserve hormone (AMH) in women with polycystic ovary syndrome, and clinical indicators such as obesity, irregular or amenorrhea, and acne. Ultrasound testing confirmed PCOS**[7]**.Women from all the examined groups were given medical syringes to extract 5 ml of venous blood for each sample.After centrifuging at 3000 rpm for 5 minutes, the serum was split into three portions and kept at -20 degrees Celsius until serological testing**[8]**. Age, sex, and marital status information were recorded for each sample. Detection of *T. gondii* using the VIDAS method is a quantitative test used to estimate the amount of *T. gondii* antibodies in blood serum by enzyme link fluorescent assay (ELFA).Human Macrophage-Derived Chemokine ELSIA Kit**[9]**.

The Statistical Analysis

SPSS (Statistical Packages for Social Sciences) version 20 was used to analyze the data. The mean and standard deviation were calculated, and the T-test was used to see whether there were any statistically significant differences between the means**[10]**.

Results

1-Human Macrophage-Derived Chemokine ELSIA Kit in women infected with *T. gondii*

As demonstrated in Figure (1), the concentration of MDC in women infected with the T. gondii parasite rose considerably (P<0.05) (0.0994 + 2.356 ng/ml) as compared to the control group (0.0664 + 1.053 ng/ml).

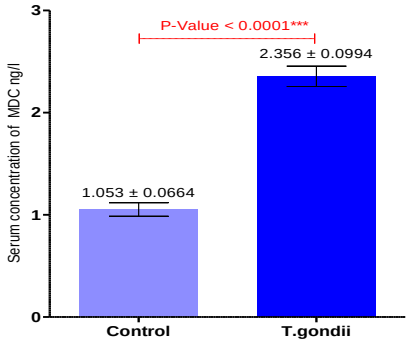

Figure 1: Concentration (ng/ml) of MDC in the serum of women infected with *T. gondi*

2-Estimation of the Human Macrophage-Derived Chemokine ELSIA Kit in Women with PCOS.

When examining women with polycystic ovary syndrome (PCOS) and observing some clinical signs such as obesity, irregular or amenorrhea, and acne, in addition to confirming PCOS by ultrasound examination

and measuring ovarian reserve hormone (AMH), the current study showed that The MDC concentration increased significantly (P<0.05) (0.1340 + 2.573 ng/ml) compared with the control group (0.06644 + 1.053 ng/ml) as shown in Figure (2).

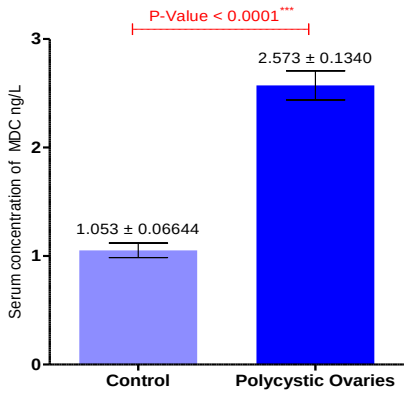

Figure 2: Concentration (ng/ml) of MDC in the serum of women with PCOS

3-Evaluation of the Human Macrophage-Derived Chemokine ELSIA Kit in *T. gondii*-infected women with PCOS.

The current investigation found that the concentration of MDC in the blood serum of women infected with the parasite T. gondii and PCOS was substantially higher (P<0.05) than in the control group (0.0664 + 1.053 ng/ml), as demonstrated in Figure (3).

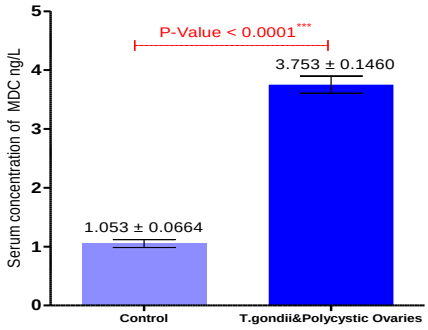

Figure 3: Concentration (ng/ml) of MDC in the blood serum of women infected with *T.gondii* parasite and PCOS syndrome

4-Human Macrophage-Derived Chemokine Concentration in Women Infected with *T. gondii* Parasite and Polycystic Ovary Syndrome and Co-Infection Between Them:

The present investigation displayed that the concentration of MDC in women infected with *T. gondii* and polycystic ovarian syndrome increased significantly (P<0.0001) (0.1460 + 3.753 ng/ml) compared to women infected with polycystic ovary syndrome (0.1340 + 2.573 ng/ml) and women infected with *T.gondii* (0.0994 +2.356 ng/ml) as shown in Figure (4).

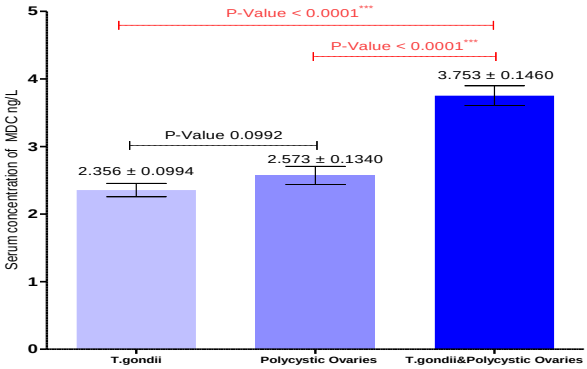

Figure 4: Concentration (ng/ml) of MDC in the blood serum of women infected with *T. gondii* and women with polycystic ovary syndrome and their co-infection.

Discussion

The current study showed that chemokine levels were significantly higher in T. gondii parasite-infected women. Lysis of the host cells and inflammatory infiltrates of lymphocytes, macrophages, and neutrophils are credited with this rise**[11]**. The host responds similarly when a latent infection is awakened in immunocompromised people. Moreover, individuals with compromised cellular immunity, particularly AIDS patients. Among the signals of cellular infiltration following T. gondii infection is the release of proinflammatory chemicals from infected cells and the induction of infection of primary fibroblasts and transformed epithelial cell lines with T. gondii secreting proinflammatory chemicals IL-8, GRO, and MCP-1. IL-1 production and the presence of uninfected cells were critical in mediating the IL-8 response of HeLa cells to infection [19]. When live tachyzoites invade and kill off host cells, the chemotactic response occurs, and host cells respond by releasing IL-8, which is associated with higher mRNA expression**[21]**. In addition, fibroblasts exposed to supernatants or lysates from T.gondii-infected fibroblasts can trigger significant IL-8 production.Chemokine release may contribute to acute toxoplasmosis' inflammatory infiltration or the reactivation of IL-8 and GROa, two neutrophil chemoattractants that accumulate in T. gondii infection sites**[24]**. Neutrophils play a part in stopping the reproduction of parasites, which helps to increase resistance to acute infection**[20]**.

Chemokines increased in T. gondii-infected women. The immune response within 4-10 hours of parasite infection and IL-8 synthesis cause rapid cell lysis. Hosts with IL-8 secretion can be detectable within 2-4 hours, with higher quantities released between 4-8 hours **[22]**.The central nervous system is the principal location of complete T. gondii infection, and chemokine receptor signaling is crucial for the immune response against parasite infection both in the peripheral and central nervous system**s [18]**.Chemokine subtype CXCR3, crucial in the peripheral immune system, is associated with infection-site activation of both CD4+ and CD8+ T cells, IFN- γ release from T-cells, and subsequent activation of inflammatory monocytes**[12]**.Additionally, it encourages the growth of epithelial cells and contributes to the activation of microglia at infection sites. In the brains of infected individuals with Alzheimer's, CXCL10 is markedly raised in astrocytes, while CXCR3 is primarily expressed in neurons and neuronal processes **[17]**.The recent investigation discovered a considerable rise in chemokine levels in women with polycystic ovarian syndrome. Chemoattractant proteins, which activate leukocytes throughout the inflammatory phase, are responsible for this rise**[22]**.Patients with obesity have more monocyte cells in their adipose tissue. In adipose tissue, the differentiation of monocytes into macrophages is accompanied by**[15]**. These chemokines secreted by adipose tissue are linked to increased levels of the chemokines MIF, MCP-1**[24]**, and MIP-1, as well as greater inflammatory activity of adipose tissue in individuals with hyperandrogenism and higher chemokine production. MCP-1 production in the skin's visceral adipose layer is implicated in follicle development**[13]**, ovulation, steroidogenesis, and corpus luteum function, as well as chemokine and sex hormone secretion**[16]**. The current investigation discovered an increase in chemokine levels in the serum of T. gondii parasite carriers with polycystic ovarian disease (PCOS).

Several increased indicators, such as inflammatory cytokines and chemokines, suggest low-grade chronic inflammation in PCOS**[16]**.The immune system is influenced by oestrogen, progesterone, and AMH levels. Individuals with the polycystic ovarian syndrome have low progesterone and AMH levels due to a lack of ovulation or ovulation. Thus, the immune system can be overstimulated by increased oestrogen, which promotes various cytokines and chemokines in these patients**[14]**.

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