ORIGINAL ARTICLE

CONTENTS

Effect of some immunological markers on the level of anti-mullerian hormone (AMH) in women infected with *Toxoplasma gondii*

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ABSTRACT

Aim: To study effect of *T. gondii* infection on levels of immunological parameters for possible effects that may appear in infected women in the future, and to study direct correlation of infection in raising AMH value.

Materials and Methods: 80 blood samples were collected and divided into two equal groups. Of unmarried women, their ages ranged from 20-25: 1st group was from infected with Toxoplasma who visited health institutions, 2nd group was from whom were not infected as a control group. Toxoplasma infection was diagnosed using the LAT test and the ELISA test. Detection of human IL-2, IL-10 and IL-12 (pg/ml) ELISA KIT, Assay Max Human was conducted according to manufacturing company.

Results: An increase in the level of human IL-2, IL-10 and IL-12 (pg/ml) in infected women compared to control group. A positive correlation of the human IL-2, IL-10 and IL-12 (pg/ml) with AMH level, which supports possibility of adopting AMH level as an indicator of cases of polycystic ovary cysts and infertility in future.

Conclusions: It was conducted to determine the relationship between the levels specific parameters such as IL-2, IL-12, and AMH and their effect on women with Toxoplasmosis, where the relationship was positive between the immune parameters, its levels increased in the event of infection, which increases possibility of using it as indicators of infection and also to predict the incidence of polycystic ovary syndrome or occurrence of infertility cases, there is a positive correlation between IL-2, IL-12 with level of AMH.

KEY WORDS: Toxoplasmosis, immunological, IL-2, IL-12, anti-mullerian hormone

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INTRODUCTION

An internal parasite belonging to the felidae family, *Toxoplasma gondii* uses humans as its intermediate host. In most cases, patients' feelings are asymptomatic [1]. *T. gondii* is a parasite that mostly affects the central nervous system in mammals, birds and reptiles. It can also infect the skeletal muscles and reproductive system. This parasite is harmful to people who receive organ transplants and causes acquired toxoplasmosis [2]. Additionally, it results in congenital toxoplasmosis in the fetuses of a number of domestic and wild animal species, including pigs, sheep and goats [3]. The virulence and pathogenicity of *T. gondii* strains are categorized into three genotypes. The first genotype is primarily isolated from human hosts, while the second genotype is observed in severe cases of infection [4], when a host ingests *T. gondii* tissue cysts, sometimes referred to as bradyzoites, from an intermediate host that has already been infected, infection results. Inflammation results from *T. gondii* entering the small intestine and changing into the rapidly dividing tachyzoite form [5]. The parasite's life cycle depends on two aspects of the immune response triggered by this technique. *T. gondii* infects immune cells and replicates inside of them. The parasite spreads throughout the body by invading immune cells and then moving on to new hosts in the brain, muscles and other organs [6]. Chronic infection is distinguished by the change of parasites to a bradyzoite transcriptional programme and the elimination of the bulk of tachyzoites [7]. This process entails the formation of a cyst wall rich in saccharides, which is critical for the survival of the parasite during its passage through the gastrointestinal tract of the subsequent host [8]. Thus, the opportunity for transmission is restricted, as the parasite destroys the host prior to this transition occurring in the absence of a robust immune response [9]. Interleukin-2 (IL-2) and interleukin-12 (IL-12) are required for the initiation and expansion of innate and adaptive immune responses in cells such as epithelial or endothelial cells [10]. Inflammasomes regulate the

activity of numerous IL-2 cytokines, multi-protein interactions, and resistance to microbial infections [11]. Innate immune cells, including monocytes, dendritic cells, and macrophages generate cytokines of this nature. Nonhematopoietic immune complexes regulate the intracellular cysteine protease-mediated cleavage and subsequent activation of immature forms of these cytokines [12]. It is a protein that is manufactured by the granulosa cells found in the ovarian follicle, and it is considered one of the best tests used to detect ovarian reserve [13]. Its percentage decreases in women as they age, becoming very few and cannot be noticed or detected when menopause is reached, which makes it Also an indicator to predict the approaching of this stage [14]. Sertoli cells generate ovarian reserve hormone, a significant contributor to the process of gender differentiation from the moment of fetal gender differentiation until puberty. Ovarian reserve hormone is a member of the transforming growth factor β family. Granulosa cells additionally generate anti-mullerian hormone (AMH) from the moment of implantation until the cessation of ovarian function [15]. AMH is secreted from granulosa cells during the development of primary eggs in follicles up to 6 mm in diameter, and its expression gradually decreases with follicle development. Serum AMH levels are associated with other clinical features such as cycle duration, average ovarian size, and hormone levels [16]. Testosterone and androstenedione in the blood, therefore AMH is a potential biomarker for polycystic ovarian morphology and PCOS, and can be measured to avoid the need for ultrasound examinations in the early follicular phase [17]. In comparison to healthy women, women infected with the parasite exhibit greater resistance to insulin in the bloodstream as age and weight increase. Research has demonstrated that hyperinsulinemia is progressively more significant in the intricate development of polycystic ovary syndrome. This is supported by the identification of a significant correlation between insulin resistance and abnormalities in ovarian function, and it is evident that the level of insulin resistance increases with the severity of the condition [18]. There is a lower possibility of spontaneous ovulation in PCOS individuals. This suggests that severe hypomenorrhea or amenorrhea is coupled with moderate or severe insulin resistance. Measuring the level of AMH in serum is informative and necessary during IVF treatment cycles [19]. Since it is closely related to the number of oocytes obtained after stimulation and the number of eggs retrieved, it is useful in predicting suboptimal and excessive ovarian responses upon stimulation. Most reports indicate a weak predictive value of AMH on the pregnancy rate in the new cycle of IVF treatment, so it has been suggested that it gives some prediction of pregnancy, especially when fresh or frozen embryo transfers are performed in the field [20]. AMH has recently been studied as a potential new clinical measure for ovarian reserve and gonadotropin responsiveness. Several big prospective studies have been published in recent years, showing highly fascinating new data about the application [16]. In assisted reproductive technologies, the clinical utility of AMH measurement in predicting both the quantitative and qualitative ovarian response is noteworthy. The AMH hormone is a Sertoli cell-specific protein in males. It is secreted by the testicles via the gonads beginning in the eighth week of pregnancy, and at a significant level until puberty. When the development of the Sertoli cells is complete, the synthesis of the AMH hormone diminishes compared to the condition in women. The regulation of testicular activity appears to be AMH's sole physiological function in adult males. AMH is a marker of Sertoli cell function, as it is released in both serum and semen by adult males. AMH can be found in adult males. Finding out about its measurement could be helpful in learning more about spermatogenesis.

AIM

The aim of our research was to study the effect of *T. gondii* infection on levels of immunological parameters for possible effects that may appear in infected women in the future, and to study the direct correlation of infection in raising AMH value.

MATERIALS AND METHODS

Eighty blood samples were collected and divided into two groups (40 in each group). The unmarried women were aged 20–25 years. The first group consisted of women infected with toxoplasma and visiting medical institutions, and the second group consisted of women who were not infected as a control group. Toxoplasma infection was diagnosed using the LAT test and the ELISA test. Sterile plain tubes were used to collect blood samples, which were numbered, and some information was recorded on a special form. Following a five-minute separation of the blood using a centrifuge set at 3000 rpm, the serum was placed in numbered plastic containers and stored at -20°C until various immunological and serological tests were conducted. Detection of human IL-2, IL-10 and IL-12 (pg/ml) ELISA KIT the Assay Max Human was conducted according to the manufacturing company using the same procedure. AMH was measured by a blood sample drawn from the vein using a small needle, and the examination was completed.

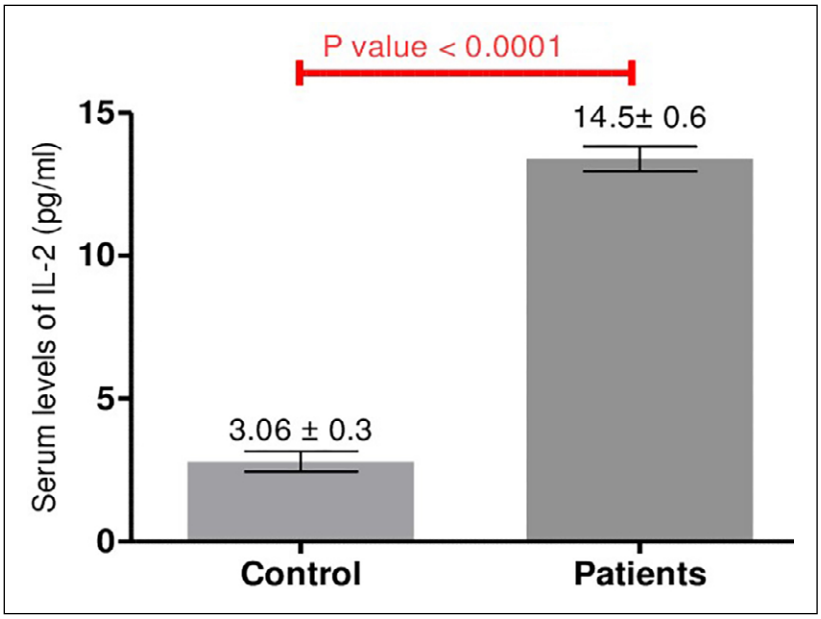

Fig. 1. Concentration of IL-2 (pg/ml) in patients infected with T. gondii compared with the control group (p-value ≤ 0.001).

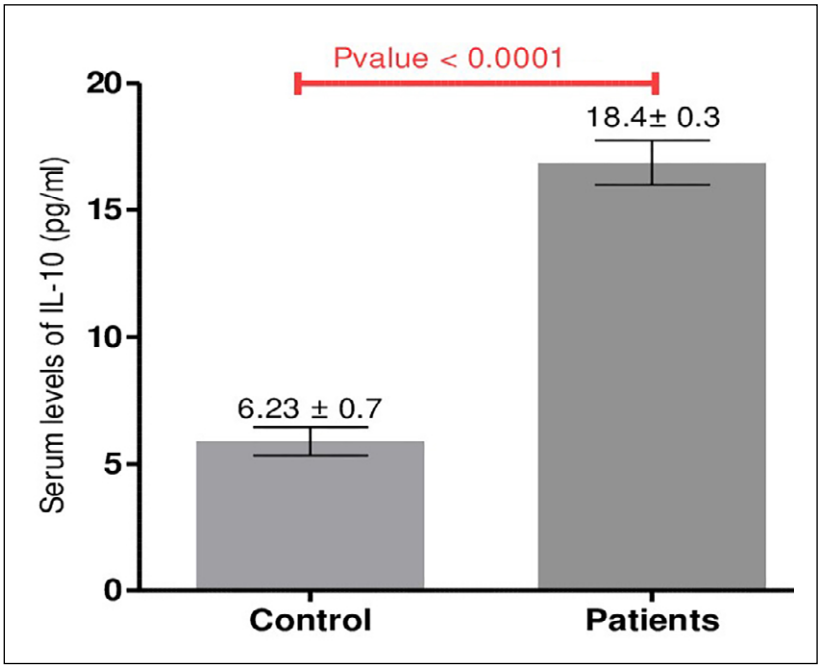

Fig. 2. Concentration of IL-10 (pg/ml) in patients infected with T. gondii compared with the control group (p-value ≤ 0.001).

STATISTICAL ANALYSIS

The mean ± standard error (SE) is used to represent the data. Patient and control group data were compared using a Student`s t-test. The relationships between variables were determined using both (r) and Pearson's correlation. A p-value ≤ 0.001 was considered significant.

RESULTS

Human IL-2 level estimation (pg/ml) KIT ELISA (Fig.1). The current study found that the concentration of IL-2 (pg/ml) in patients with *T. gondii* infection was substantially higher (P≤ 0.001) at 14.5±0.6 pg/ml in comparison to the control group's – 3.06±0.3 pg/ml.

Human IL-10 level estimation (pg/ml) KIT ELISA (Fig.2). The results of the current investigation showed that the concentration of IL-10 (pg/ml) in patients with T. gondii infection was considerably (P≤ 0.001) elevated to 18.4±0.3 pg/ml compared with 6.23±0.7 in the control group.

Human IL-12 level estimation (pg/ml) KIT ELISA (Fig.3) in the current investigation shows that the concentration of IL-12 (pg/ml) in *T. gondii*-infected patients was considerably (P≤0.001) higher at 17.32±0.3 pg/ml than in the control group at 5.04±0.5 pg/ml.

As shown in fig. 4, the concentration of AMH (pg/ml) in patients with *T. gondii* infection was substantially higher (P≤ 0.001) at 5.2±0.1 pg/ml in comparison to the control group's level of 2.5±0.04 pg/ml.

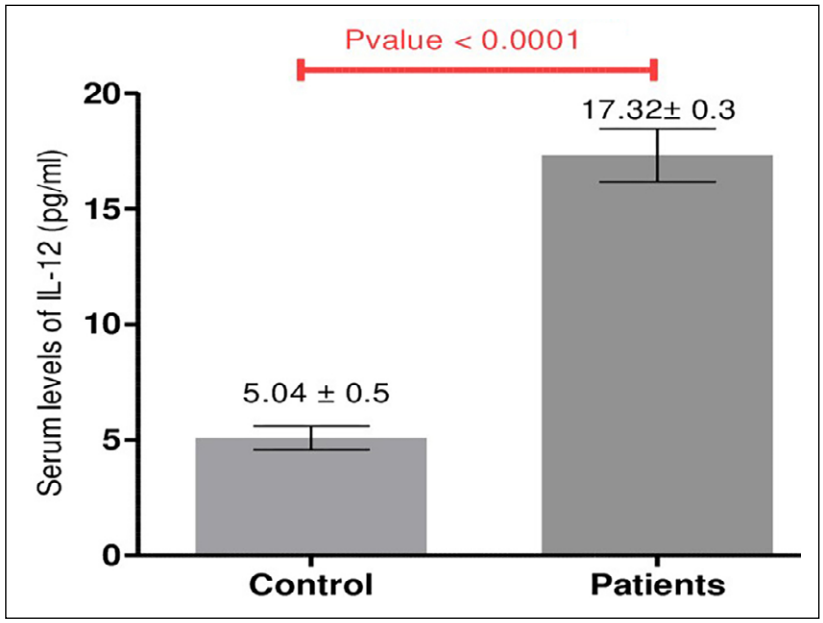

Fig. 3. Concentration of IL-12 (pg/ml) in patients infected with T. gondii compared with the control group (p-value ≤ 0.001).

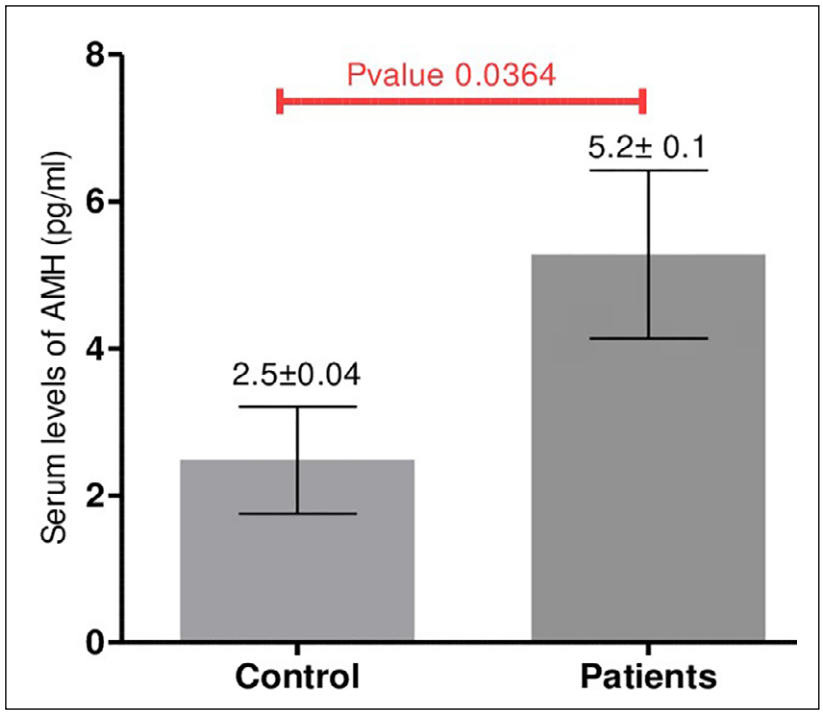

Fig. 4. Concentration of AMH (pg/ml) in patients infected with T. gondii compared with the control group (p-value ≤ 0.001).

DISCUSSION

The study's findings revealed a significant increase in the serum levels of AMH, IL-2, IL-10 and IL-12 in individuals infected with *T. gondii* parasites as compared to the control group. These findings could be attributed to IL-2, IL-10, and IL-12 production aiding in host control of *T. gondii* infection, as we previously demonstrated that *T. gondii* infection increases the synthesis of IL-2 transcripts and activation of primary human monocytes [21]. The current study's findings revealed a considerable increase in the blood serum of those infected with the *T. gondii* parasite as compared to the control group. The reason for this increase was that positive CD4 and CD8 T lymphocytes produce interferon-gamma (IFN-g) naturally, as well as natural killer cells (NK), which are activated when the infection begins by stimulating dendritic cells (DCs) and neutrophil leukocytes for the early production of IL-12, which stimulates natural killer cells to produce interferon (IFN-γ) and is produced in high concentrations by T-lymphocytes during chronic toxoplasmosis infection to prevent reactivation of the parasite's tissue cyst and stimulate macrophagous cells provide antigen presentation, increase the effectiveness of lysosomes in macrophage cells [22], stimulate the effectiveness of natural killer cells,

inhibit the effectiveness of Th2 helper T lymphocytes, and also produce IL-12 positive CD4 T lymphocytes. It works to stimulate the production of IFN-γ from CD8-positive T-lymphocytes; both IL-12 and IFN-g are of fundamental importance in generating host resistance against the toxoplasmosis parasite, and thus, cellular immunity has a role in controlling the infection [24]. The current investigation found a considerable rise in IL-12 in women with polycystic ovarian syndrome. The reason for the increase is that androgen levels rise, which causes macrophages to change into proinflammatory macrophages, which causes the emission of more proinflammatory cytokines and worsens the clinical signs of polycystic ovarian syndrome. One is said to be resistant to obesity. Clinical PCOS is characterized by insulinemia and a change in macrophage polarisation from an anti-inflammatory to a pro-inflammatory M1 state [23]. The current study's findings are in line with another study that found that IL-12 concentrations increased significantly in pregnant women experiencing acute and chronic infections, as well as in women who had abortions. The primary host response to *T. gondii* may involve the generation of derived IL-12, as the level of IL-10 can limit the production of IFN-γ via IL-12 synthesis in host cells. Macrophages, which then increase the number of natural killer cells, and IFN-γ helps start the acute infection that causes the parasite to make IL-10 in the host. This stops the production of IL-12 and lowers the amounts of IFN-γ and IL-2 [24]. The results of the study demonstrated a statistically significant increase in the concentrations of IL-12 and IL-10 in the serum of women infected with toxoplasmosis. These levels of IL-12 and IL-10 stimulate the secretion of the disease from monocytes, which in turn encourages the production of IFN-γ, a key immune response mediator. Against the parasite, IFN-γ controls the inside of the parasite cells in a cell-autonomous manner. In addition, IFN-γ prevents parasitic reproduction in the human body by increasing tryptophan degradation in fibroblasts. Conversely, IL-10 secretion is necessary to maintain normal pregnancy and reduce risks of spontaneous miscarriage [2]. Ovarian reserve analysis is performed to measure the levels of AMH present in the body, which is a protein that is manufactured by granulosa cells located in the ovarian follicle. There is a direct relationship between its percentage and the woman's level of fertility, the higher the percentage, the higher the number of eggs in the body [8]. It is worth noting that its highest levels are usually recorded when a woman reaches approximately 25 years of age. Then, its levels begin to decrease after reaching the age of thirty. It is noteworthy that an AMH analysis can be performed on any day of the menstrual cycle, meaning that its levels remain constant and are not affected during it [18]. Examining the ovarian reserve means trying to predict a woman's ability to reproduce by inferring the number and quality of her remaining eggs. At the time of a female's birth, the ovary contains approximately 2 million eggs, and when she reaches puberty, her egg reserve becomes 400,000 eggs. At the age of thirty, their number decreases [20]. To approximately 25 thousand eggs, so as women age, their chance of conceiving decreases due to the decrease in the number and quality of eggs. Eggs may also be affected by some genetic and environmental factors, some medical conditions, early menopause, and smoking, which are among the most important analyses that predict the number and quality [20]. Ovarian reserve hormone AMH is secreted by small follicles that are less than 4 mm in size in the ovaries. For follicles larger than 8 mm, hormone release first declines and eventually ceases. Because the AMH test's value is constant, it may be performed on any day of the cycle. It is regarded as a crucial fertility test because it shows how many eggs are still in the ovaries based on the analysis of blood samples [25]. Leukemia patients with toxoplasmosis, which is considered a major risk factor for their lives, revealed that the patient's serum levels of IgM and IgG infected with the *T. gondii* parasite were high. At the same time, the concentration of IL-12 in the cells was constant [22]. Hematopoietic cells, along with transplanted or moved cells, increased the production of IFN-γ and NK cells. These cells work together to stop leukemia from growing in the body. It shows that IL-12 can be permanently produced in hematopoietic cells, including transplanted and transferred cells. It also increased the production of IFN-γ and activated natural killer cells, all of which may work together to stop the growth of leukemia in living organisms.

CONCLUSIONS

The current study was conducted to determine the relationship between the levels specific parameters such as IL-2, IL-12, and AMH and their effect on women with toxoplasmosis, where the relationship was positive between the immune parameters. Its levels increased in the event of infection, which increases the possibility of using it as indicators of infection and also to predict the incidence of polycystic ovary syndrome or the occurrence of infertility cases. There is a positive correlation between IL-2, IL-12 with level of AMH.

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CONFLICT OF INTEREST
The Authors declare no conflict of interest

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