Evaluation The Immunological State Of Women's Sera Infected With Toxoplasmosis.

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الخلاصة

جاءت الدراسة الحالية للتحري عن بعض المؤشرات المناعية في مصول المصابات بداء المقوسات الكوندية Toxoplasmosis في مدينة بغداد من خلال الوقوف على الدور المهم لبعض الانترلوكينات التي تتوسط الاستجابة الالتهابية اثناء الاصابة بطفيلي *T.gondii*. تم التحري عن اضـــداد الطفيلي عن طريـــق تقنيـــة (MEIA) ، استخدمت تقنية ELISA في التحري عن المستوى المصلي لكل من IL-1α ، IL-8 ، و IL-10 كما استخدمت تقنية الـ SRID لحساب التركيز الاجمالي لـ IgG، IgM ، C_3 ،و C_4 في مصول المصابات والسبطرة.وفقا للدراسة جاءت نتائج قياس تركيز IgG مرتفعة معنويا في مصول المصابات (1455.68 mg/dl) قياسا مع السيطرة (1158.55 mg/dl) فيما لم يتأثر معدل تركيز IgM في كلا المجموعتين، كما ان نتائج قياس تركيز عوامل المتمم C_3 ، C_4 هي الاخرى جاءت متقاربة وبدون فروقات معنوية. لم تظهر فروقات معنوية في معدل تركيز IL-Iα(0.68 Pg./ml) و IL-8 (20.14 Pg./ml) عند مقارنتها مع الاصحاء (0.187 و 17.0 Pg./ml) على التوالي اما معدل تركيز IL-10 فقد ارتفع بشكل كبير اثناء الاصابة (21.125 Pg./ml) قياسا مع مجموعة السيطرة (9.525 Pg./ml)كونه احد الانترلوكينات الضد التهابية المنظمة التي تمنع الاستجابة المناعية للمضيف من ان تؤدي الى تغييرات مرضية غير مرغوبة .

لم تسجل الدراسة أي تغير معنوي في معدل العدد الكلي لكريات الدم البيض بين مجموعتي النساء قيد الدراسة، كما لم تظهر فروقات في معدل النسبة المئوية للخلايا العدلة في كلا المجموعتين، بينما كان معدل النسبة المئوية للخلايا الحمضة واللمفاوية مرتفع معنويا في عينات المصابات قياسا مع السيطرة.

Abstract

Background: **The ubiquitous protozoan parasite *Toxoplasma gondii* is a major cause of morbidity and mortality in neonates and immunocompromised hosts. Both acute invasion and reactivation of latent infection result in an inflammatory reaction with lymphocytes, macrophages, and neutrophils.**

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(البحث مستل من رسالة الماجستير)

Better understanding of the mechanisms of resistance of the host against this protozoan is
important for development of safe, effective alternative treatment regimens for toxoplasmosis in the future.

***Aim of study*:This study aimed to investigate some immunological parameters of woman's sera infected with Toxoplasmosis in Baghdad city, through investigation the potential role of some interleukins mediating the inflammatory response after *T. gondii* infection.**

***Methods:* Detection of parasite antibodies was achieved by MELA by testing 185 women which referred to central health laboratory during the period from March to July 2004; ELISA technique was used to detect serum concentration of IL-1α, IL-8, and IL-10 , also SRID used to calculate total IgG,IgM, C3, and C4 concentrations in both group understudy.**

***Results:*According to the study, there was significant increasing in mean concentration of IgG whereas IgM concentration do not affected in both patients and control groups, also there were no significant differences in the mean concentration of C3 and C4 component of complement between two groups of study. There were no significant differences in mean concentration of serum IL-1α (0.68 pg/ml) and IL-8 (20.14 pg/ml) in infected women when compared with healthy one (0.187 pg/ml) and (17.0 pg/ml) respectively. However, the mean serum concentration of IL-10 was highly and significantly elevated during *T. gondii* infection (21.125 pg/ml) in comparison to that of healthy control (9.525 pg/ml). The study did not record any significant change in the mean total WBCs count between patients and control groups, also there were no differences in mean percentage of neutrophils in both groups, the mean percentage of eosinophils and lymphocytes was highly significant in infected women's samples as compared with control.**

***Conclusion*:Most cases of toxoplasmosis were diagnosed at the chronic stags of disease, hence there were no clear sign and symptoms that draw attention, however, abortion was the only manifestation that refer to infection with toxoplasmosis, since the concentrations of pro inflammatory cytokines (like IL-1α) and chemokines (such as IL-8) decreased against increase the level of anti-inflammatory and regulatory cytokine IL-10 that prevent the host immune response leading to undesirable pathological changes.**

Introduction

Toxoplasma gondii **is one of the most successful intracellular protozoan parasites and is able to infect a broad range of warm-blooded vertebrate hosts including humans and livestock animals. With its ubiquitous distribution and a seroprevalence of approximately 30%, T. gondii is also one of the most abundant eukaryotic parasites in humans (1, 2).**

After patients ingest infective tissue cysts or oocysts,released tachyzoites first invade and multiply in intestinal epithelial cells (3), and then spread to regional lymph nodes, leading to hematogenous and lymphatic dissemination (4). Tachyzoites can invade any nucleated mammalian cell in an active process requiring release of the contents of specific parasite organelles followed by formation of a parasitophorous vacuole and inhibition of phagosome-lysosome fusion (5). Tachyzoites divide rapidly within the specialized vacuole, resulting in lysis of the host cell, subsequent invasion of adjacent cells, and dissemination. In immunocompetent individuals, infection normally results in a mild to asymptomatic infection, as a potent innateT- cell-independent immune response is generated against the tachyzoite (6, 7, 8, 9). This response results not only in tachyzoite killing but also in the parasite's transforming to the dormant encysted bradyzoite stage within skeletal or heart muscle and the central nervous system, which can persist in these tissues for life without eliciting an inflammatory response.

Although bradyzoites are apparently harmless, sequestered within dormant cysts, a persistent immunity to *T. gondii* is required to avoid reemergence of the tachyzoite stage and accompanying pathologic changes. Indeed, the latter is often observed in chronically infected immunocompromised hosts (10, 11, 12). Bradyzoites are found in proportionately larger numbers in the central nervous system (CNS); indeed, cyst reactivation most often occurs in the brain. This fact is well illustrated by the high incidence of encephalitis induced by *T.gondii* as a major cause of morbidity and mortality in patients with AIDS (10, 11).Toxoplasmosis in immunocompromised patients most often results from reactivation of a persisting *T. gondii* infection due to the severely depressed T-cell-mediated immune response, but can occasionally also result from acute primary infection.Furthermore, the parasite is of major medical relevance after primary maternal infection during pregnancy, eventually leading to congenital toxoplasmosis. It is the ability to transmigrate through the placenta and to replicate within different foetal tissues without being efficiently hindered by the

premature immune system that makes *T. gondii* an important cause of prenatal infections. This can consequently severely injure the foetus, eventually leading to abortion, to considerable pathology at birth or to late sequelae (13).

The intracellular lifestyle of *T. gondii* largely protects the pathogen against the host's humoral immune response. Host cell invasion is an active, parasite-driven event that is accomplished within less than 30 s (14), thereby dramatically reducing the time of exposure to antibodies or complement. Infection with *T. gondii* is predominantly controlled by cell-mediated immunity (15), although antibodies may also be involved (16, 17). Appropriately activated T lymphocytes are crucial for the control of both the acute and chronic phase of toxoplasmosis (18, 19, 20). Specifically, CD8+ T cells play a major role as effector lymphocytes against *T. gondii* (18), whereas CD4+ T cells are crucial for the regulation of the immune response against *T.gondii* (19, 20). T lymphocytes, natural killer (NK) cells, and activated macrophages have been shown to play important roles in resistance to *T. gondii* infection (21, 22, 23). In murine models, depletion of both CD41 and CD81 T lymphocytes causes reactivation of chronic infection (20). NK cells are also critical, as SCID mice, without functional T cells, survive acute *T. gondii* infection for at least 2 weeks (24). NK cells are capable of lysing parasiteinfected cells and releasing cytokines, a key effector mechanism in controlling acute toxoplasmosis (22, 24, 25). Gamma interferon (IFN-γ) activation of macrophages is particularly important, as infection is uniformly fatal in IFN-γknockout mice (26, 27).

In addition to the resistance conferred by macrophages and T cells, neutrophils also play a role in host resistance to *T.gondii*. Neutrophils can phagocytose and kill opsonized *Toxoplasma* (28). In vivo support comes from the finding that mice deficient in inducible nitric oxide synthase (iNOS) succumbed to acute infection following depletion of neutrophils (29). Thus, it appears that neutrophils contribute to acute resistance against this parasite and might account for the ability of (iNOS) knockout mice to control infection in the apparent absence of macrophage killing function. Neutrophils might scavenge infected cells, secrete toxic products, or produce chemokines required for the recruitment of other effector cell populations (30).Chemokines are a group of chemotactic polypeptides that are key mediators of leukocyte activation and chemotaxis (1,20). They are divided into groups of related families based on the arrangement of cysteine residues in their amino-terminal domain (4, 22). The C-X-C or b-chemokines, of which IL-8

is a prototype, are primarily involved in the recruitment and activation of neutrophils, although they may attract other leukocyte populations (20).In immunocompetent hosts, the parasite induces strong T-cell-mediated type 1 immunity (15), with production of proinflammatory cytokines and IFN-γ. *T. gondii* infection is lethal in the absence of these cytokines (8, 26). However, the strong Th1 response generated during *T. gondii* infection must be tightly regulated by anti-inflammatory factors, without which the immune response triggered by the parasite leads to immunopathology (31). Thus, while IFN-γ is required for resistance to *Toxoplasma*, excessive levels of the cytokine are lethal (32). Interleukin-10 is a potent antiinflammatory and immunosuppressive cytokine, initially described as "cytokine synthesis inhibitory factor" because of its ability to suppress production of IFN-γ, IL-2, and proinflammatory cytokines (33, 34).IL-10's ability to block activation of cytokine synthesis and several accessory cell functions of macrophage renders this cytokine a potent suppressor of the effector functions of macrophages, T cells, and NK cells. In addition, IL-10 likely contributes to regulating proliferation and differentiation of B cells, mast cells, and thymocytes (34).

Because interleukins are important mediators for neutrophils, macrophages, and T cells, which accumulate at the site of *T. gondii* infection, the present study investigated the potential role of some interleukins in mediating the inflammatory response after *T. gondii* infection.

Materials and Methods

Subjects: The subjects in the present study included 70 female patients with toxoplasmosis who were admitted to central health laboratory of Baghdad city during the period from March to July 2004. The control group included 20 individuals whose distribution by age (The age range, 17 to 42 years) and profession was similar to that of the patients with toxoplasmosis but who had no history of abortion and whose serum samples were negative for *T.gondii* antibodies detection test. The diagnosis of toxoplasmosis was based on the following criteria: (1) clinical symptoms that diagnosed by gynecologist (2) detection of *Toxoplasma* IgG antibodies by using microparticles enzyme immuno assay (MEIA).

Sample collection: Blood samples were collected from all the subjects of this study. Venous blood (5 ml) was collected from each toxoplasmosis patient,each control subject. Sera were separated from (3ml) of each blood sample by centrifugation at 1000 rpm for

15 minutes. The serum was divided into three parts and each of which was stored in appendroff s tube to prevent repeated freezing and thawing cycles that may affect serum factors, no preservatives was added, and appendroff s tubes stored in deep freeze at -80°C. Until use. In addition, remnant (2 ml) of peripheral blood was collected in tube containing EDTA or blood test performing.

Detection of Toxoplasma IgG antibodies: This test was performed in central health laboratory depending on MEIA technique for quantitative estimation of IgG antibodies in serum or plasma of toxoplasmosis infected patient, and by using Abbotte AXSYM System.

Study of humoral immune response: Total IgG, IgM, C3, C4 concentrations in serum were determined in all patients and control group by simple radial immunodiffusion (35) by using Biomaghreb supplemented dishes and performed according to its manufacturers' instructions.

Study of cell-mediated immune response: Through calculate the concentration of IL-1α, IL-8, and IL-10 in serum samples of patients and compared result with control group. These tests were performed depending on ELISA technique by using a known conc. of each cytokine under study and its optical density values (O.D.) in drawing a standard curve, then use this curve to find the conc. of that cytokine in serum of all subjects of study(after reading O.D.of each sample). These tests were performed by using Biomaghreb kits for IL-1α, IL-8 detection and Biosourse kit for IL-10 detection according to their manufacturers' instructions.

Total and Differential WBCs Count: Calculation of total WBCs accomplished by using MS9 instrument which is a complex system containing many parts and solutions, a special bar enter through EDTA blood tube and the results of total WBCs, Hb, PCV, blood group….etc. will appeared on the system monitor.

Blood smear were performed onto a glass slide. The smear was stained with a leishman stain for 2 minutes, drowned in dye buffer for 18-20 minutes then washed under tap water, then leaved to dry, the slides examined under high power magnification of microscope and each cell types were counted.

Statistical analysis: All data obtained from recent study analyzed statistically by using SPSS program. Differences were recorded as significant whenever the probability (P) was less than 0.05 ($p<0.05$).

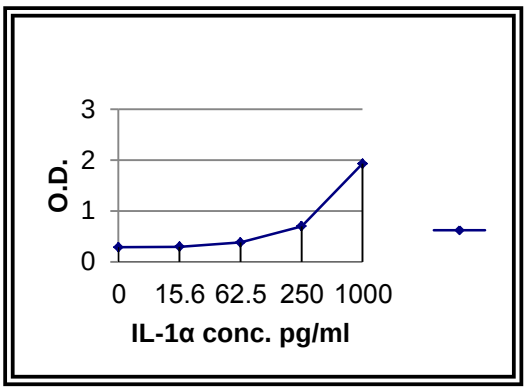

Figure-1.standard curve of IL-1α

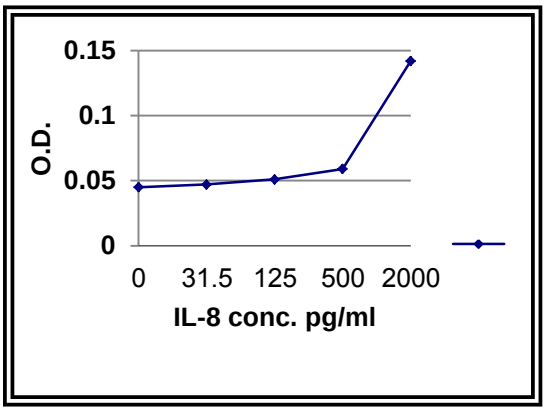

Figure-2.standard curve of IL-8

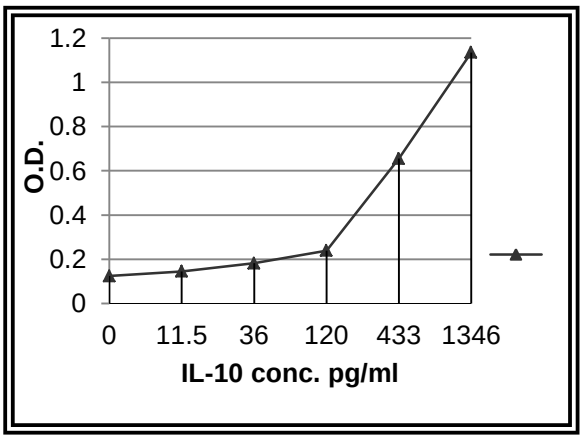

Figure -3.standard curve of IL-10

Results

The resent study showed an obvious elevation in the mean concentration of IgG (FIG.4) in patients group (1455.68 ± 411.28) as compared with (1158 ± 340.26) of control group, this elevation was of significant differences (p<0.05);however there were no differences in mean concentration of IgM in both patients and control groups.(FIG.5)

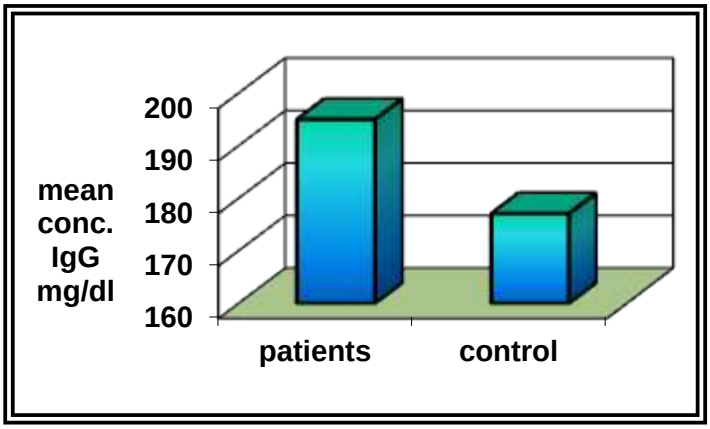

Figure-4.mean concentration of total IgG in patients and control sera (mg/dl)

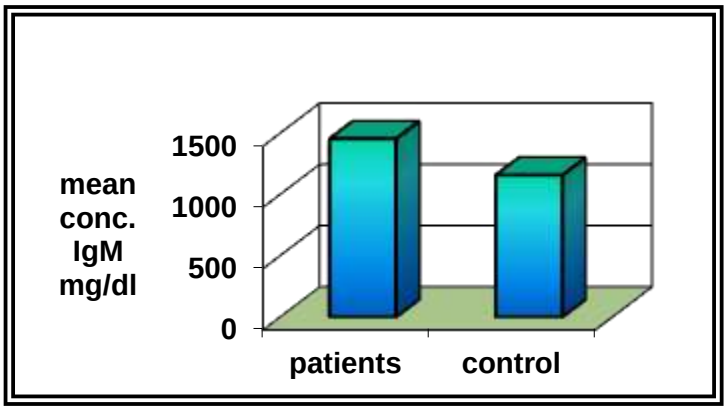

Figure-5.mean concentration of total IgM in patients and control sera (mg/dl)

This study could not record significant differences in mean concentration of C3component in patient's sera (107.99± 27.118) with control one (100.25 ± 3.529); (FIG.6).

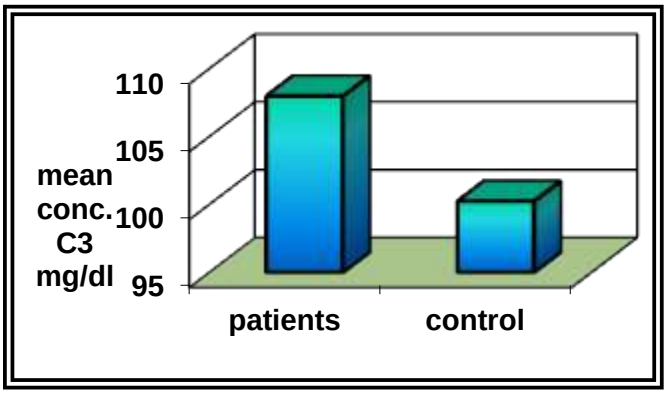

Figure-6.mean concentration of total C3 in patients and control sera (mg/dl)

In the same way, there were no differences in mean concentration of C4 in both groups under study. (FIG.7.)

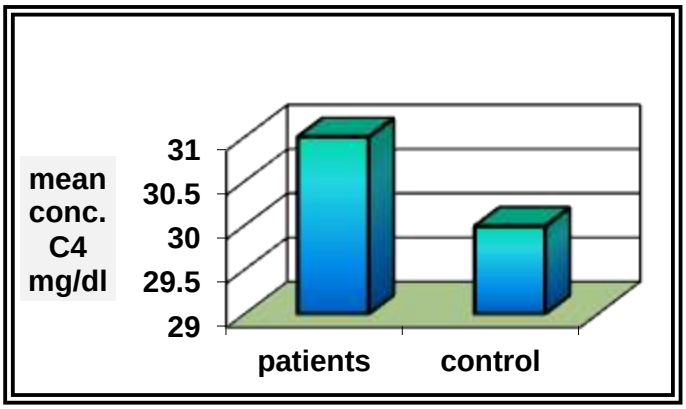

Figure-7.mean concentration of total C4 in patients and control sera (mg/dl)

Results of measuring the concentration of IL-1α by using ELISA technique of patients showed no significant differences in the mean concentration of this interleukin (0.68 ± 6.13) as compared with healthy control (0.18 ± 0.805).(FIG.8.)

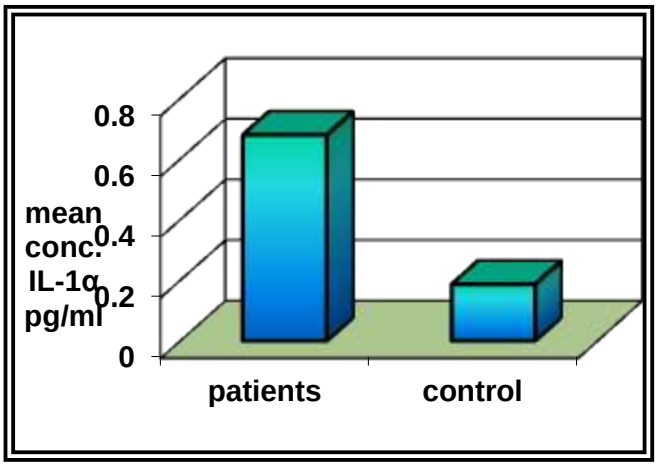

Figure-8.mean concentration of IL-1α in patients and control sera (pg/ml)

Also the result referred that there were no significant variation in conc. of IL-8 in patients and controls sera, it were (20.14 ± 164.9) and (17.0 ± 29.2) respectively. (FIG.9.)

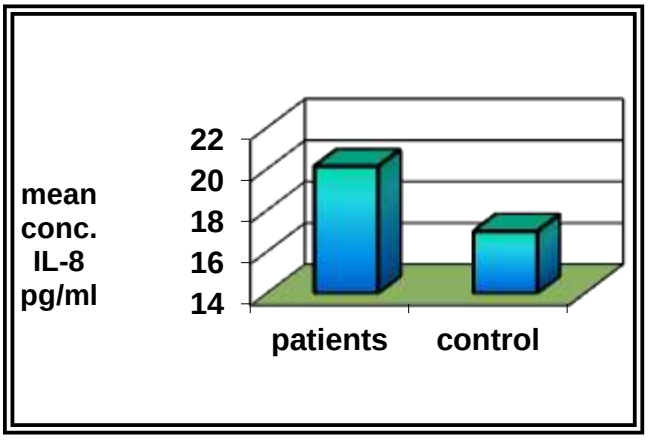

Figure-9.mean concentration of IL-8 in patients and control sera (pg/ml)

There were significant difference in mean conc. of IL-10 of infected women's sera (21.125 ± 40.540) when compared with healthy control (9.525 ± 9.129). (FIG.10.)

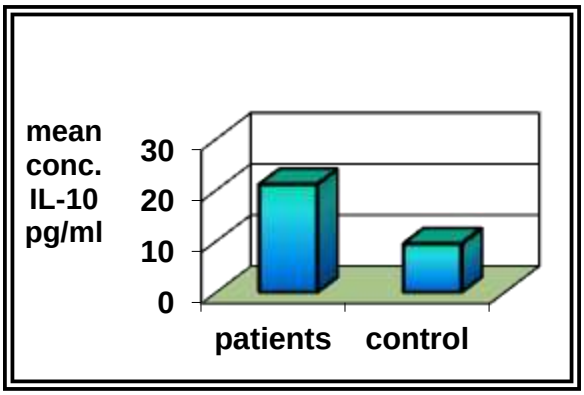

Figure-10.mean concentration of IL-10 in patients and control sera (pg/ml)

No differences were recorded in both total WBCs count and mean percentages of neutrophils for each cubic milliliter of peripheral blood of infected and healthy individuals (Table 1, 2).

Table -1: mean total WBCs count in peripheral blood of patients and control

Groups	No. of samples	mean	Standard deviation	Z-test
Patients	70	6923.83	3004.06	N.S.
control	20	6390.0	1070.93	1.23

Table -2: mean percentage of neutrophils in peripheral blood of patients and control

Groups	No. of samples	mean	Standard deviation	Z-test
Patients	70	52.3	4.18	N.S.
control	20	51.78	3.3	1.09

On another hand, there were a highly significant differences ($p < 0.05$) in mean percentage of eosinophils(7.86 ± 1.4) and lymphocytes(41.1 ± 4.78) in peripheral blood of patients when compared with control(4.16 ± 1.28) and (34.6 ± 4.12) respectively.(Table 3, 4).

Table -3: mean percentage of eosinophils in peripheral blood of patients and control

Groups	No. of samples	Mean	Standard deviation	Z-test
Patients	70	7.86	1.4	H.S.
control	20	4.16	1.28	12.8

Table -4:mean percentage of lymphocytes in peripheral blood of patients and control

Groups	No. of samples	Mean	Standard deviation	Z-test
Patients	70	41.1	4.78	H.S.
control	20	34.6	4.12	12.4

Discussion

Toxoplasmosis triggers both humoral and cellular immune responses and the later being more important for the development of protective and persistent immunity (36, 37).

There were quantitative differences in IgG level in toxoplasmosis patients as compared with control, whereas no significant differences in level of IgM of both groups was recorded, such result indicate that the collected samples might be belong to women in the chronic stage of infection and ensured by results demonstrated by Attia *et.al.* (38) of increase serum IgM immunoglobulin after primary infection making this immunoglobulin of diagnostic value to detect acute infection, so that titer of these immunoglobulins (IgG , IgM) used as diagnostic markers to differentiate acute from chronic infection. These finding was differ from Shani (39) which indicate that there were significant increase in both IgG and IgM titers during toxoplasmosis, and this may be due to differences in immunological of women understudy at sample collection time and this agreed with what Kang *et.al.* (16) suggested that both humoral and cell – mediated immune response changed according to stage of infection and its anatomical location inside body.

Specific antibodies (Abs) in present of complement system act on lyses of extra cellular tachyzoites (40), these Abs plus parasite antigen will produce immunological complexes that activate the classical pathway of complement system which elicit parasite killing (41), hence C3 and C4 are important components that mediate this pathway in activation (42).

No significant differences were recorded in IL-1α and IL-8 levels in both toxoplasmosis infected or healthy women groups and this may be due to that samples understudy belong to women in chronic stage of infection and this agreed with previously obtained result of high titer of IgG of infected women s sera , also this finding is supported by Denney *et.al.* (43) whom study effect of IL-1α and IL-8 production during induced toxoplasmosis in Hela epithelial layer and recorded that highest production of IL-1α and IL-8 was after 24 hr. post infection and the intact tachyzoites represent an essential factor of increase IL-8 conc. from its normal level, also they indicate that IL-1α play crucial role as a mediator in IL-8 response to *T.gondii* infection.

This study referred that IL-10 level was significantly increased in *T.gondii* infected women's sera when compared with healthy control, and such a result appear accepted because cell-mediated immunity (CMI) against the toxoplasmosis must be regulated to resist infection on one side, and to avoid immunopathological changes from another one (15), this regulation is achieved through IL-10, TNF-α (Tumor Necrosis Factor-α) that regulate expression and function of IL-12 and other monokines (44).

In 1997, Neyer *et.al.*(31) indicate that IL-10 regulate the synthesis of IL-12 and INF-γ {which is important in controlling of tachyzoites division during both acute and chronic stages of infection(45, 27)}so they will help in avoiding excessive immune response that may lead to extensive inflammatory response and host tissues damages(44).

In the same way, high levels of IL-10 during chronic infection play a role in the down regulation of inflammatory response through decrease cyst burden that serve the host (46) and this agreed with Gazzinelli *et.al.* (7) which referred that there were an increase in IL-10 mRNA expression in CNS during murine toxoplasmosis.

Total WBCs count results appeared within the normal values (4000-11000 cell/ml.blood)in most population in world(47)and such result ensured that patients under study are in the chronic stage of infection, hence there were no clear sign and symptoms that draw attention, however, abortion was the only manifestation that refer to infection with toxoplasmosis, and this seem an appropriate because chronic infection characterized by tachyzoites replication failure and bradyzoites cysts formation, so that no clinical presentation may be elicited (48) because parasite may disappear inside host cells so that the immune system members could not recognize it or its antigens(49).

No differences were recorded in neutrophils levels in patients' blood as compared to control group, and that may be due to fact that neutrophils take part in resistance during acute stage of *T.gondii* infection (43)and could be supported by study of Scharton-Kersten *et. al.*(29)whom demonstrated that iNOS deficient mice submit to acute infection after neutrophils depletion. This result agreed with what concluded previously of lacking any increasing in IL-8 level, because this chemokine (if present) act as a chemoatractant leading to accumulation of neutrophils on acute toxoplasmosis site (11),and this also fit with what Denney *et.al.*(43) obtained that neutrophils are the first agitated cell during inflammatory response which appeared several minutes after chemokines liberation at tachyzoites infection site.

There were significant elevations of eosinophils levels in patients' blood and such a result may be due to fact that eosinophils play a potent role in parasitic infection because they possess an antibodies surface receptors like IgE which is important in hypersensitivity reaction type1, and IgA antibody which perform an essential role in mucus barrier protection, also these cells (eosinophils) have receptors to several cytokines like IL-3, IL-5 which are important in eosinophils differentiation themselves (50). The significant increasing in lymphocyte mean percentage obtained in this study agreed with Mcleod *et. al.* (51) whom indicate to the crucial role of T- lymphocyte in determining *T. gondii* infection outcome, since during chronic infection, parasite cysts can be controlled by host immune response and chiefly via CD8+ effector lymphocyte (52) and those cells are essential to establish a long term memory against *T.gondii* infection (53).

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