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Effect of Toxoplasmosis on hematological, biochemical and immunological parameters in pregnant women in Tikrit city, Iraq

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Abstract:
The aim of the present study was to detect the effect of infection with *Toxoplasma gondii* on hematological, biochemical and immunological parameters in pregnant women. Blood samples, 101 in the total number, were subject to serological test (ELISA test) collected between first of September 2013 to the end of March 2014 from pregnant women: 76 seroposative toxoplasmosis and (25) seronegative toxoplasmosis (control group) divided into two groups according to their ages (16-26 years and 27-46 years). Hematological parameters in whole blood samples were examined. Liver enzyme activity such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) also urea and creatinine concentration in serum were determined. Besides, the levels of interleukins (IL-6 and IL-10) were analyzed. In infected groups WBCs counts, ALT, AST, ALP activities, urea and creatinine concentration, IL-6 and IL-10 levels were increased significantly $p \leq 0.05$, while the Hb and PCV levels decreased significantly $p \leq 0.05$ when compared to control groups.

Introduction

Toxoplasmosis is an important disease which is caused by the coccidian parasite *Toxoplasma gondii.* It was reported first in human by Janku in 1923, who described the parasitic cysts in the retina of a child with congenital hydrocephalus. Its infection induces several immunological changes in the body which are characterized by the production of the immunoglobulins IgM, IgG and IgA (1,2).

T. gondii has a worldwide distribution and is one of the most prevalent infectious agents in human, serologic studies which infests nearly one – third of the world human population (3).

T. gondii can be located in every vital organ, and especially in acute stage it can be seen in blood, cerebrospinal fluid, semen, tears, saliva and urine. It is a zoonotic disease that causes abort and fetal destruction due to placental transmission (4).

Human infection leads to an acute disease that is usually oligosymptomatic in immunocompetent individuals. The disease is considered to be self-limited and does not require treatment. Symptoms occur in 10-20% of cases (5).

Many research works have been done on different clinical forms of the disease, an association between toxoplasmosis with hepatomegally and some abnormal liver function tests was found (6). Also the involvement of the kidney in toxoplasmosis leading to impairment of their function(7)

In parasitic invasions, the lymphocytes synthesize many specific cytokines play a major role in the pathogenesis of parasitic diseases. IL-

10 and IL-12 control the type of the immune response. (8–9).

The study objectives were to assess the effect of *T. gondii* on liver and kidney functions, some blood parameters and on chose indices of the immune response.

Materials and methods

Blood samples and ELISA analysis: Blood (5 ml) was collected from 101 pregnant women: 76 seropositive Toxoplasmosis and 25 seronegative toxoplasmosis (Control group) divided into two groups according to their age (16-26 years and 27-46 years) between first of September 2013 to the end of March 2014.The selection was based on visits of the patients to Educational Hospital of Tikrit and on the subsequent referring by the specialist-physician for serological examination. All sera samples of pregnant women were analyzed by using ELISA for *Toxoplasma* specific using immunoglobulin G (IgG) Kit (Human, German) and the final results were recorded by ELISA reader (optical absorbance, OD = 450).

Hematological and biochemical analysis: Anticoagulated blood samples were used to determine total leukocyte counts, concentration of blood hemoglobin (Hb), packed cells volume (PCV) (10)**.** Biochemical tests were carried out to Aminotransferases (ALT and AST) activities according to the recommended method of Young (1990), and for ALP activity was determined by King-Armstrong method (11).

Kidney functions were evaluated by estimation of urea by Batton and Crouch, 1977 (12) and creatinine by Tietz 1999 (13).

Immunological analysis: The levels of interleukins were determined in blood serum using ELISA tecnique with a set of Cusa bio (14).

Statistical analysis:

Data were analyzed using ANOVA and Duncan Multiple Range test, P value was considered significant when it was $p \leq 0.05$ according to SPSS 14 (2006)(15).

Results

Hematological and biochemical analysis: The hematological findings in control and infected pregnant women are presented in table(1). Hb and PCV levels in women infected with *T. gondii* were increased significantly ($p \leq 0.05$), while WBCs count decreased significantly ($p \leq 0.05$) compared to control women in both groups of age.

Table (2) shows the comparison of serum enzymes activities between the patients and control groups.

Serum AST ALT and ALP activities were significantly higher in patients compared to the control at ($p \leq 0.05$).

Concerning the effect of toxoplasmosis on kidney function, the results in table (3) also demonstrate that there is a significant ($p \leq 0.05$) increase in the means of urea and creatinine concentrations in both infected groups compared with control groups.

Immunological analysis: As shown in table (4) the level of IL-6 in *T. gondii* infected women was higher in compared with healthy controls and in both groups. The difference in the values of IL-6 between the infected and healthy subjects is statistically significant ($p \leq 0.05$).The level of IL-10 was found to be most highly differentiated. In seroposative toxoplasmosis was 4.3 and 3.9 pg/ml in tow infected group whereas in healthy controls only 0.09 and 0.15 pg/ml respectively. The difference between these values is highly statistically significant ($p \leq 0.05$).

Table 1: Hematological parameters Level in Toxoplasmosis pregnant women

Categories(year)	**Blood parameters**		
	WBC/ mm^3	**PCV** (%)	**Hb**(g/dl)
Control group	9978 ±709 b	44.5 ± 2.944 a	13.5 ± 0.920 a
Infected women(16-26)	11495 ±93 a	40.9±2.779 b	12.3 ± 0.923b
Control group	7257± 2542 b	43.8 ± 2.217 a	13.3± 0.866 a
Infected women(27-46)	11237 ± 619 a	39.6± 2.658 b	12±0.906 b

*Different letterers refer to significant differences at ($p \leq 0.05$)

Table 2: liver enzymes activity in Toxoplasmosis pregnant women

Categories(year)	**Liver enzymes**		
	ALT(IU/L)	**AST**(IU/L)	**ALP**(IU/L)
Control group	13 ± 1.291 b	13 ± 5.447 b	65 ± 3.08 c
Infected women(16-26)	43± 6.043a	30 ± 6.330 a	72.5 ± 23.72.5 b
Control group	10 ±3.017 b	10.5 ± 6.377 b	58.2 ± 1.71 c
Infected women(27-46)	42 ± 6.496 a	26 ± 9.379 a	97 ± 14.86 a

*Different letterers refer to significant differences at ($p \leq 0.05$)

Table 3: Urea and creatinine concentration in Toxoplasmosis pregnant women

Categories (year)	**Urea** (mg/dl)	**Creatinine**(mg/dl)
Control group	23.2 ± 4.573 b	2.5 ± 1.0134 b
Infected women(16-26)	47.9 ±7.950 a	1.4±0.4340 a
Control group	26.1 ± 8.183 b	2.7 ± 0.2608 b
Infected women(27-46)	46.9 ± 7.769 a	1.3 ±0.4151 a

*Different letterers refer to significant differences at ($p \leq 0.0$)

Table 4: Interleukins Level in Toxoplasmosis pregnant women

Categories(year)	**IL-6**(pg/ml)	**IL-10**(pg/ml)
Control group	2.8 ± 0.311b	0.09 ± 0.096 b
Infected women(16-26)	4.6 ± 0.747 a	4.3 ± 4.375 a
Control group	0.6 ± 0.096 c	0.15 ±0.152 b
Infected women(27-46)	4.8 ±1.426 a	9.3 ± 3.976 a

*Different letterers refer to significant differences at ($p \leq 0.05$)

Discussion

The causes of increased in the rates of Hb and PCV are the differential of physiological and immunity state in pregnant infected women with toxoplasmosis, but other studies found that the blood parameters some time remains stable and not changeable during period of infection (16). The results show decrease in WBCs in infected women which might because these cells had been affected by toxoplasmosis. Which it considered as one of the important factors which control the natural and acquired immunity response in the body of pregnant infected women (17).

Toxoplasmosis causes severe and progressive damage to the liver (18,19), remarkable proliferations of organisms such damage in the liver metabolism (20).We found that liver enzymes activities ALP, AST, and ALT were increased in *T. gondii*-infected women, Serum AST and ALT activities are excellent markers of hepatocellular injury and serum ALT activity is more specific than serum AST for assessing liver injury and this results consistent with previous studies conducted on experimental animals (21,22,23). The significantly elevated serum activities of liver enzymes also agreed with (24). Increased plasma AST and ALT activities reflects impairment of the liver, where this infection can cause round cell infiltration in the portal areas, cholestasis, swollen endothelial cells and focal necrosis of liver cells (25).When the liver is impaired the liver cells release

the enzymes into the blood raising the enzyme activities (26).

The study showed increase in both urea and creatinine levels infected pregnant women as compared with control groups and tis result agreed with h ,j . The increase in the urea concentration may due to *Toxoplasma* deleterious effects on the kidney which decrease the excretion of urea from the body and subsequently increased its serum level [29]. Histopathological changes induced by this parasite in different organs observed in kidneys (30,31). Furthermore, the increase in urea and creatinine concentrations in infected group may be explained as *Toxoplasma* parasite causes glomerular lesions and urinary abnormalities which lead to renal failure. Biochemically, renal failure is typically detected by an elevated serum creatinine level in the urine [32].

Interleukin 6 (IL-6) is an interleukin that acts as both a pro–inflammatory cytokine and an anti-inflammatory myokine. In humans, it is encoded by the IL6 gene and it is secreted by T cells and macrophages to stimulate immune response during infection also plays a role in fighting infection (33). Toxoplasmosis women had higher level of IL-6 as compared to seronegative women as shown in our result. This results agreed with (34) which found that the level of IL-6 was twofold higher in the course of *toxoplasmosis* than in healthy controls, and with (35) who shown that IL-6 required for resistance against *T. gondii*. Infection with *T. gondii*, results in elevated levels of IL-6 in serum and infected tissues seems to confirm the presence of an early and sensitive, although nonspecific, marker of inflammatory states (33).

Interleukin-10 (IL-10), also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine(35). Our study reveals that IL-10 is associated with protection or increased susceptibility to infection with *T. gondii* and this agree with (34k).IL-10 plays an essential role in the inflammatory response during acute *T. gondii* infection, since it is a cytokine with multiple, pleiotropic, effects in immunoregulation and inflammation. It down regulates the expression of Th1 cytokines, MHC class II antigens, and co-stimulatory molecules on macrophages. It also enhances B cell survival, proliferation, and antibody production.[36]. IL-10 is able to deactivate macrophages, induce IFN-γ by *T. gondii*, and facilitate intracellular parasite survival.IL-10 induces immunosuppression during *T. gondii* invasion, which is beneficial both for the host and the parasite [35].

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تاثير الخمج بداء المقوسات الكوندية على بعض المعايير الدموية، الكيموحيوية والمناعية لدى النساء الحوامل في مدينة تكريت ، العراق

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الملخص

هدفت الدراسة الحالية الى كشف تأثير الخمج بطفيلي *Toxoplasma gondii* على بعض المعايير الدموية والكيموحيوية والمناعية في النساء الحوامل. تم جمع 101 عينة دم من اجل اجراء الأختبارات المصلية لها (تقنية الاليزا) وللفترة من بداية شهر ايلول 2013 ولغاية نهاية شهر اذار 2014، وبشكل 76 عينة دم خمجة بالطفيلي و 25 عينة دم غير خمجة (مجموعة سيطرة)، وزعت الى مجموعتين وحسب الفئات العمرية (16– 26 سنة) و(27– 47 سنة). أجريت الأختبارات الدموية على جميع العينات، كما تم قياس فعالية بعض انزيمات الكبد مثل انزيمات النين امينوترانسفريز (ALT)، اسبارتيت امينو ترانسفريز (AST) والالكلاين فوسفاتيز (ALP)، الى جانب قياس تركيز الكرياتنين واليوريا. هذا فضلا عن تحليل مستوى الأنترليوكينات 6 و10. وجدت النتائج في العينات الخمجة بالطفيلي ان عدد خلايا الدم البيض، فعالية انزيمات الكبد وتركيز كلا من الكرياتنين واليوريا ومستوى الأنترليوكينات قد ارتفعت معنويا $p\leq0.05$، بينما انخفض مستوى هيموغلوبين الدم وحجم كريات الدم المرصوص بشكل معنوي $p\leq0.05$ مقارنة مع مجاميع السيطرة .