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Assessment of Alkaline Phosphatase in Aborted Women Infected with Toxoplasmosis in Erbil city

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Abstract
Background: Previous studies in Iraqi community suggested an association between toxoplasmosis and abortion.
Aim: To determine the levels of Alkaline Phosphatase enzyme, IgG and IgM antibodies in sera of aborted women infected with *Toxoplasma gondii*.
Materials and methods: A total of 81 aborted women were included in the study. Samples were divided into three groups. The first group included 25 patients having previous (chronic) infection with *Toxoplasma gondii*, the second group included 11 patients having new (acute) *Toxoplasma gondii* infection and the third group included 15 patients having both (chronic and acute *Toxoplasma gondii* infection). Thirty healthy matched women were selected as controls group.
Results: IgG levels was increased significantly in patients with (chronic) toxoplasma infection as compared to control (125.8±7.0 *v.s* 5.7±o.5), whereas IgM levels was increased significantly in patients with acute infection in comparison with control (136±6.6 *v.s* 6.6±1.1).While antibodies (IgG & IgM) levels increased significantly (198±15.3 and 172.5±13.4 respectively) in patients with both acute and chronic as compared with control. With respect to Alkaline phosphatase (ALP) the highest specific activity was observed in patients with both acute and chronic (140 ± 9.0) followed by patients with new infection (acute) (134.3 ± 9.0) as compared with none aborted healthy control women group (45 ± 0.8).
Conclusion: The study results suggest that infection with *Toxoplasma gondii* during pregnancy may play a role in the induction of abortion. In addition, Alkaline Phosphatase could play a role in induction of inflammation during *Toxoplasma gondii* infection in pregnant women.
Keywords: *Toxoplasma gondii*, IgG, IgM, Alkaline phosphatase, abortion.

Introduction

Toxoplasma gondii is a widespread protozoan obligate parasite that infects all species of endothermic animals and humans with a high prevalence [1]. Human infections are primarily obtained by ingesting under cooked or raw meat containing viable tissue cysts, ingesting food or water contaminated with *T. gondii* Oocysts [2]. The incidence of infection depends on the immunological state of the population [3]. It also depends on a favorable environmental conditions, such as hot weather that enhance the survival of the oocysts, which are eliminated in cat feces [4]. The biological success of *T. gondii* is associated with the formation of a specific relationship between the parasite and host cells leading to the establishment of a latent, chronic infection [5].

T. gondii is capable of infecting almost all the internal organs and tissues of the mammalian host. In the host cells, *T. gondii* causes DNA damage and rapid cell death with rupture and release of the organisms and soluble antigens that cause many pathological changes ranging from mild congestion to severe degeneration within the affected organs [6]. As well as several relatively studies have detected association between *T. gondii* infection and various hepatic pathologies, such as hepatitis, granuloma, necrosis, hepatomegaly, jaundice, and cirrhosis [7]. Also recently, reports have indicated a possible association of *T. gondii* infection with cancer risk [8].

Chronically infected individuals who possess defects in cell-mediated immunity are at risk for reactivation of the latent infection and its dissemination, causing serious complications with the occurrence of high morbidity and mortality rates among these patients [9]. *T. gondii* spreads to a number of organs of the infected host and is able to cross biological barriers and enter into the brain, eye and placenta [10].

Primary infection with *T. gondii* during pregnancy may lead to infection of the fetus. The clinical spectrum of *T. gondii* infection varies from asymptomatic to severe disease with lymphadenopathy, chorioretinitis and meningoencephalitis [11]. Parasite still remains viable in the tissue cysts throughout the whole life of the host during this stage, cellular immunity mediated by T cells and macrophages, and the activity of type 1 cytokines (IL-12 and IFNγ), plays a crucial role in controlling the tissue cysts and the development of chronic *T. gondii* infection [12]. Recent studies in Iraq indicated an association between *T. gondii* infection and bad obstetric outcomes [13-18]. Thus this study conducted to study if there was an association between *Toxoplasma gondii* infection during pregnancy and abortion in other Iraqi governorates..

Subjects, Materials and Methods

Subjects

A total of 81 women with abortion attending to different hospitals around Erbil city were included in the study. They were clinically examined for *T.gondii* infection and evaluated by the consultant medical staff at the hospitals. Serum samples were divided into three groups according to the presence or absence of specific anti- Toxoplasma antibodies: The first group included 25 samples having previous (chronic) infection (containing anti IgG antibodies), the second group 11 samples having new (acute) infection (containing anti IgM antibodies) and the third 15 samples having both (chronic and acute) IgG and IgM antibodies. Thirty healthy matched non aborted women were selected as healthy controls group.

Blood samples

Venous blood was collected from women for serum separation during the period between March 2016 and March 2017. All patients and controls serum were tested for anti – Toxoplasma antibodies. The test was performed by the use of two kits (Omega Diagnostics Company, Scotland), one for the detection of IgG antibodies against *T. gondii* antigens in the

patients' serum, and the other for the detection of IgM antibodies against *T. gondii* antigens in the patients' serum.

Biochemical Analysis

Serum samples were tested for alkaline phosphatase (ALP) enzyme using ELISA kits (BioMerieux, Lyon, France) according to the manufacturer's instructions.

Statistical Analysis

Statistical analysis included calculation of the mean ± standard error (SE), the confidence interval that puts a lower and upper limit to the mean and considered upper 99% confidence limit. The t-test was adopted to check for any significance of the difference between infected women and controls.

Results and Discussion

In present study and as shown in Table (1), alkaline phosphatase activity level was significantly increased (P≤0.05) in women with acute and chronic infection (140±9.0 U/L) as compared with control (45 ± 0.8 U/L). Additionally, its activity was 134.3±9 U/L in patients with acute infection and 88.5±4.4 u/l in women with chronic infection, Table.1.

Table.1. Alkaline phosphatase activity serum level in patients groups in comparison to controls.

Groups	IgG	IgM	ALP U/L
	Mean ± S.E.		
Controls (Healthy)	**5.7 ± 0.5**	**6.6 ± 1.1**	**45 ± 0.8**
Patient with old infection (chronic)	**125.8 ± 7.0**	**-**	**88.5 ± 4.4**
Patient with new infection (acute)	**-**	**136 ± 6.6**	**134.3 ± 9.0**
Patients with both Chronic & Acute)	**198 ± 15.3**	**172.5 ± 13.4**	**140 ± 9.0**

In pregnancy the immunologic changes may induce a state of increased susceptibility to certain intracellular pathogens, including viruses, intracellular bacteria, and parasites. *T. gondii* was the most common parasite that infected women during pregnancy and these findings are consistent with animal data showing that pregnant mice have lower resistance to *T. gondii* than none pregnant control mice [19]. This could be attributed to the increased fluid volume during pregnancy, which may lead to dilution of antibodies with subsequent increased susceptibility to infection. In addition, hormonal changes may increase women susceptibility to infection.

Toxoplasmosis is important due to the possibility of transplacental transmission, harming the fetus. The rate of risk for pregnancy depends on infection prevalence among women at childbearing age [20]. The liver is one of the most important organs involved and affected during the parasitaemia stage of *T. gondii* infection and the mechanisms of liver damage and the histological changes that induced by *T. gondii* infection could be due to a direct proliferative effect of the parasite on the tissues, leading to cell death and tissue damage, or could be related to the indirect effect of infection due to the excessive immunological response to the parasite [20].

Many studies have established a relationship between Alkaline phosphatase enzyme levels with several diseases. The most related was in bone, liver, kidney and other inflammatory diseases. However, they may also indicate malnutrition, kidney cancer tumors,

intestinal issues, a pancreas problem, or a serious infection. In addition many studies on animals infected by *T. gondii* suggest that serum alkaline phosphatase levels were significantly increased. Afshari and his colleagues [21] observed that *T. gondii* has augmenter effects on alkaline phosphatase activity in rats inducing Toxoplasma infection.

As it known antibodies very important for diagnosing toxoplasmosis in humans. Therefore systematic serological screening for T. gondii IgG and IgM antibodies should be performed in all pregnant women as early as possible in gestation period (ideally in the first trimester) and in seronegative women each month, the differences in antibodies levels is very obvious specially patients with chronic and acute infection. The present study indicated a significant increase in both types of antibody (IgG: 198 ± 15.3 and IgM: 172.5 ± 13.4) as compared with control (IgG: 5.7 ± 0.5 and IgM: 6.6 ± 1.1), Table.1.

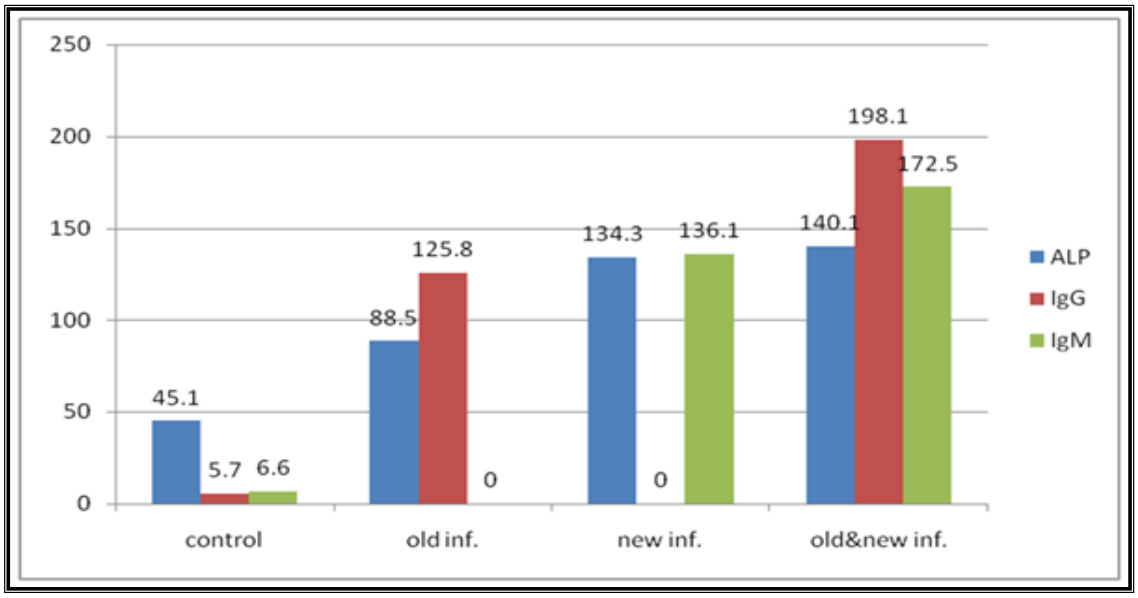

Figure.1. Illustrate serum antibodies (IgG & IgM) and ALP levels in patients groups and control.

Anti *T. gondii* IgM are detectable about one week after the infection and remain for several months or years. So the detection of IgM antibodies alone is insufficient for the establishment of acute infection. Many authors have shown that a positive IgM results is not enough by itself to support a diagnosis of acute infection, since the test can remain positive for a long time after the primary infection [22].

In conclusion, Depending on the present study and previously reported studies for other Iraqi governorates, we recommended screening of the pregnant women during antenatal care. Periodic liver enzyme tests such as Alkaline phosphatase is warranted as an early predictor of liver disease in pregnant women with toxoplasmosis.

ETHICAL APPROVAL: College of Medical and Health Technology Ethical Committee
CONSENT TO PARTICIPATE: Informed consent was taken from each subject before their enrolment in the study.
HUMAN AND ANIMAL RIGHTS: The study conducted in adherence with Helsinki Ethical standards.
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