

# Seroprevalence of *Toxoplasma gondii* among AIDS Patients in Saudi Arabia

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## Abstract

**Background:** *Toxoplasma gondii* (*T. gondii*) is an intracellular parasite mainly found in the central nervous system CNS, however, it can persist in multiple tissues in the body. Moreover, *T. gondii* is the commonest protozoans causing infections among individuals with acquired immunodeficiency syndrome (AIDS). Thus, the aim of this study was to investigate the frequency of *T. gondii* infection among AIDS patients in Makkah at Saudi Arabia. **Methods:** Fifty patients with AIDS proved to be positive by ULTRA HIV Ag-Ab Enzyme Immunoassay, and thirty healthy volunteers negative for AIDS by ULTRA HIV Ag-Ab Enzyme Immunoassay were subjected to determination of anti *T. gondii* immunoglobulin M (IgM) antibody seropositivity and anti *T. gondii* immunoglobulin G (IgG) antibody seropositivity using commercially available enzyme-linked immunosorbent assay kits. **Results:** The results showed that the seropositivity rate of anti *T. gondii* IgM antibodies among AIDS patients (18%) was significantly higher than in the healthy volunteers group (3.33%). Regarding the serum level of anti *T. gondii* IgG antibodies among AIDS patients, it was 30% significantly higher compared with those of the seropositive healthy volunteers (6.67%). **Conclusions:** These statistically significant results support the association between *T. gondii* infection and AIDS and suggest the usefulness of providing data for an educational program that will be designed to prevent *T. gondii* infection among AIDS patients.

## Keywords

AIDS, *Toxoplasma gondii*, Anti *T. gondii* IgG, Anti *T. gondii* IgM

## 1. Introduction

Toxoplasmosis is one of the most common parasitic infections affecting approximately one-third of the world's population. This parasite is mainly caused by the obligate in-

tracellular protozoan *T. gondii*. It considered one of the most successful parasites on earth, since it has the ability to maintain a benign coexistence with its host [1]. Moreover, toxoplasmosis is the most common among patients with AIDS. The disease may be a recrudescence of a latent infection or a newly acquired toxoplasmic infection. It may result from rupture of the cyst and conversion of the bradyzoites into the tachyzoites within the tissue of the retina or elsewhere in the body [1]. If not treated early or promptly, Toxoplasmic encephalitis is a fatal disorder. Clinical diagnosis and therapy of toxoplasmosis in patients with AIDS pose a challenge to the managing clinician. *T. gondii* organisms may infect the central nervous system (CNS) and form cysts in the tissue of normal individuals after systemic toxoplasmosis. Release of bradyzoites from the cysts leads to proliferation and foci of the infection. In the CNS, toxoplasmic encephalitis may present as a mass lesion in the brain, toxoplasmoma. In patients with human immunodeficiency virus (HIV) infections, the differential diagnoses of such a focal lesion include toxoplasmosis in 60% of the cases, primary CNS lymphoma in 25%, and multifocal leukoencephalopathy in 15%. Other infections are less frequently encountered [2].

Unfortunately in Saudi Arabia, limited studies have been performed to identify and assess the seroprevalence of *T. gondii* among Saudi's. The seroprevalence rates of toxoplasmosis have been estimated in a number of different studies by serological investigations to be varied from 52.1% in Asir [3] to 37.5% in Al-Hassa area [4], while the prevalence rate of anti-*Toxoplasma* IgG was 25%, and of IgM was 5% in the Eastern region [5]. There is scarce information regarding Toxoplasmosis in the western region of Saudi Arabia, particularly in Makkah. Therefore, the aims of this study are to explore the seroprevalence of *T. gondii* infection among AIDS patients in Makkah city, as a part western region of Saudi Arabia, to provide data for an educational program that will be designed to prevent *T. gondii* infection among AIDS patients.

## 2. Materials and Methods

The present case control study was performed on 80 subjects who attend to King Faisal Hospital and divided into 2 groups according to the result of ULTRA HIV Ag-Ab Enzyme Immunoassay.

Group 1): fifty AIDS patients (35 males and 15 females) were positively diagnosed by ULTRA HIV Ag-Ab Enzyme Immunoassay (Genscreen, Bio.Rad) and selected from in or out patients to the King Faisal Hospital Makkah, Saudi Arabia.

Group 2): thirty healthy (20 males and 10 females). The healthy volunteer group was negatively diagnosed by ULTRA HIV Ag-Ab Enzyme Immunoassay and those were chosen from health care workers and from the relatives/visitors of the patients.

From all subjects included in this study, five milliliters of blood was taken under sterile conditions. Then, blood samples were centrifuged at 1000 rpm, and the sera were stored at  $-20^{\circ}\text{C}$  until the analysis for anti *T. gondii* immunoglobulin M (IgM) antibodies, and anti *T. gondii* immunoglobulin G (IgG) antibodies positivity. Determination of the Anti *T. gondii* IgG & IgM Antibody Positivity Serum samples were analyzed for

Anti *T. Gondii* IgG & IgM antibodies by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (DRG® Toxoplasma IgM (TORCH) (EIA-1799), DRG International, Inc., USA) AND (DRG® Toxoplasma IgG (TORCH) (EIA-1798), DRG International, Inc., USA). The test was performed in the Laboratory of King Faisal Hospital following the manufacturer's instructions. Serum samples were diluted in sample diluent at 1:100. Then, 100 µL of reference calibrator, positive control, negative control, and diluted serum samples, was added to wells of microtiter plate coated with purified *T. gondii* RH strain antigen, incubated for 30 minutes at room temperature, and followed by washing 5 times. Then 100 µL of enzyme conjugate was added to each well, except the blank well, and incubated for 30 minutes at room temperature. After washing, 100 µL of tetramethylbenzidine substrate was added to each well, including the blank well, and incubated for 15 minutes at room temperature, followed by addition of 50 µL of the stop solution. The optical densities were read at 450 nm with a micro-well reader. The mean of duplicated cut-off calibrator value (32 Iµ/ml), positive control, negative control and serum samples were calculated. *T. gondii* index of each determination were calculated by dividing the mean values of each sample by calibrator mean value. A sample was considered positive for IgM when a *T. gondii* index was equal or greater than 1.0 (>32 Iµ/ml), A negative reaction corresponds to *T. gondii* index less than 0.90 (<32 Iµ/ml), a positive reaction to *T. gondii* index of 1.00 or greater (>32 Iµ/ml), and an equivocal result to *T. gondii* Index between 0.91 - 0.99.

### 3. Ethical Considerations

The study purpose was explained to the all participants, then, an informed consent was taken from them. The study was approved by the Research Ethical Committee of the Health affairs and Committee of the King Faisal Hospital in Makkah and the study conducted between the periods of March to September 2015.

### 4. Statistical Analysis

Collected data were numbed, coded, and introduced to a computer using the Statistical Package for Social Science for Windows version 11.0. The  $\chi^2$  test was used to analyze the frequency of anti *T. gondii* IgG and IgM positivity in the studied groups to clarify statistically significant differences. A value of  $P < 0.05$  was considered statistically significant, and value of  $P < 0.001$  was considered statistically highly significant.

### 5. Results

In the present study, 9 (18%) of the 50 cases of AIDS group (G1) and 1 (3.33%) of the 30 healthy volunteers (G2), were positive for anti *T. gondii* IgM antibody. The percentage of the anti *T. gondii* IgM antibody positivity in the AIDS group was significantly higher ( $P < 0.05$ ) than in the healthy volunteers group (**Table 1**). In addition, 15 (30%) of the 50 cases of AIDS group (G1) and 2 (6.67%) of the 30 healthy volunteers (G2) were positive for anti *T. gondii* IgG antibody. The percentage of the anti *T. gondii* IgG

**Table 1.** Results of ELISA anti *T. gondii* IgM in study samples.

Study samples	No. (80)	ELISA results		X <sup>2</sup>	*P-value
		Seropositive No. (%)	Seronegative No. (%)		
AIDS group	50	9 (18%)	41 (82%)	3.687	P < 0.05 Sig
Healthy control group	30	1 (3.33%)	29 (96.67%)		

antibody positivity in the AIDS group was significantly higher ( $P < 0.05$ ) than in the healthy volunteers group (Table 2).

## 6. Discussion

*T. gondii* is an obligate intracellular protozoan parasite able to cause an infection among different warm-blooded mammals as well as humans. In most cases, human toxoplasmosis is subclinical or appears like a minor viral disease [6]. Infection with *T. gondii* results in the invasion of the brain and the formation of tissue cysts that persist throughout the life of the host without causing symptoms because immunocompetent hosts control this chronic infection with a T lymphocyte driven defense [7]. For this reason, in HIV positive individuals (high-risk populations), public health efforts should focus on early detection and diagnosis of HIV infection and a rapid initiation of HAART to avoid any opportunistic infection, including toxoplasmosis. Seropositivity rate may be used as a quantitative measure of relative risk for disease reactivation in HIV infected individuals. Unfortunately, HIV-infected individuals are more commonly at risk for disease reactivation resulting from cyst rupture than for a newly acquired infection. In the settings with high general population seroprevalence, the relatively high prevalence of *T. gondii* infection in asymptomatic HIV- individuals suggests that toxoplasmosis may represent a frequent opportunistic parasitic disease [8].

All over the worlds, *T. gondii* has a one third affected population [7]. However, there is a very limited data available on the prevalence of *T. gondii* among the Middle East and North Africa (MENA) regions which are made up by approximately 20 countries where almost 400 million people live (5 % of the world's population) [9].

In general population, *T. gondii* seroprevalence measures the accumulated exposure to the parasite during a person's lifetime in a particular social setting. Thus, the seropositivity rate could be treated as a quantitative measure of the relative protection for an individual of this population [10]. In addition, in immunocompromised patients, toxoplasmosis is always a life-threatening disease. In North African HIV-infected individuals, toxoplasmosis represents one of the main causes of death [11].

The results of the present study showed that the frequency of anti-*T. gondii* IgM antibody positivity was significantly greater in AIDS patients (18%) than that in the healthy volunteer group (3.3%). This finding concurs with the other previous studies [12]-[15]. This higher seropositivity supports an association between *T. gondii* infection and AIDS.

Worldwide prevalence rate of latent Toxoplasma infections in HIV-infected patients

**Table 2.** Results of ELISA anti *T. gondii* IgG in study samples.

Study samples	No. (80)	ELISA results		X <sup>2</sup>	*P-value
		Seropositive No. (%)	Seronegative No. (%)		
<b>AIDS group</b>	50	15 (30%)	35 (70%)	6.101	P < 0.05 Sig
<b>Healthy control group</b>	30	2 (6.67%)	28 (93.33%)		

including Ethiopia has been found to vary greatly from 3% to 97% [15] [16]. In this study, 30% of study participants were seropositive for *T. gondii* IgG. This result was lower than study findings of Addis Ababa, Ethiopia (93.3%) [15], Iran (38.01%) [8] and Malaysia (44.8%) [8] [17]. The difference might be due to differences of the population ethics, genetic susceptibility, Toxoplasma strains prevalent in different place or country, as well as the way and period of the parasitic infection either before or after the onset of AIDS. In addition, the sociocultural differences such as keeping dogs and cats at home and consumption of raw and insufficiently cooked meat are might be another risk factor for *T. gondii* infection. All these factors are associated to several disease outcomes, and the study of these related factors will be essential in further defining the relationship between *T. gondii* and AIDS.

## 7. Conclusions

Toxoplasmosis seropositivity frequency was significantly higher in AIDS patients than in the healthy volunteer group. These statistically significant results support the association between *T. gondii* infection and AIDS and suggest the usefulness of providing data for an educational program that will be designed to prevent *T. gondii* infection in AIDS patients.

This study had some limitations. It was the first study carried out in Makkah and it was conducted only at one city, thus the results could not be generalized to all regions of the Kingdom. Also another study is needed to investigate the prevalence of *T. gondii* infection among a larger number of AIDS patients.

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