

TOXOPLASMA INFECTION AND PREGNANCY OUTCOME IN PREGNANT FEMALES IN SAUDI ARABIA: A SINGLE CENTER STUDY

By

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Abstract

Toxoplasmosis is a disease caused by *Toxoplasma gondii*. It is asymptomatic in the majority of immunocompetent individuals who contract it. When *T. gondii* infection is acquired during pregnancy, the parasite can be transmitted via the placenta to fetus, causing congenital toxoplasmosis. The present study assessed the seroprevalence of *T. gondii* among pregnant females in Al-Baha, Saudi Arabia. Moreover, the aim was also to assess the consequences of infection during pregnancy among those females. Blood samples from 173 patients were used for detection of anti-toxoplasma IgG & IgM. The results showed that 35(20.2%) were positive, of whom 33(19.1%) were IgG positive and 2(1.2%) were IgG & IgM positive. Of the 33 IgG positive cases ten with history of abortion, two history of congenital anomalies, three cases with history of intrauterine growth retardation, and two cases with history of preterm labor. All cases consumed not-well cooked meat, ten had pet-cats.

Keywords: Saudi Arabia *Toxoplasma gondii*, Pregnancy, Outcome,

Introduction

Toxoplasmosis is a protozoan disease caused by *T. gondii*, an intracellular parasite of worldwide distribution (Yamada *et al*, 2011), with about one-third of the world's populations were toxoplasmosis seropositive (Bodaghi *et al*, 2012). Wilking *et al*. (2016) in Germany reported that the Robert Koch Institute surveyed sero-*T. gondii* among adults for the first time and was 49.1%. More than 40 million men, women, and children in the U.S. carry *Toxoplasma gondii*, but very few have symptoms because the immune system usually prevented the parasite from causing illness (CDC, 2018). In France fetal transmission, outcome was live birth in 95% of cases, with latent congenital toxoplasmosis in 90% of cases and symptomatic forms in 10% of cases, of which 1/3 are severe and 2/3 moderate (Picone *et al*, 2020). In humans, the main oral source of infection was be eating or handling inadequately cooked or raw meat containing tissue cysts (bradyzoites), or consuming raw fruit, vegetables, or water contaminated with oocysts (Schlüter

et al, 2014) or the risk of mother-to-child transmission, these zoonotic routes of infection were important where an infected woman for the first time during pregnancy (Cong *et al*, 2015). The close contacts with felines and exposure to soil contaminated with cats' feces were sources of infection (Ben Abdallah *et al*, 2013). Again, congenital toxoplasmosis, reactivated *Toxoplasma*-encephalitis in immunosuppressed patients as the AIDS, HIV or after stem cell or organ transplantation (Coster, 2013), and ocular toxoplasmosis was congenital significant clinical pictures (Saadatnia and Golkar 2012). Besides, congenital toxoplasmosis included hydrocephalus, microcephaly, intracranial calcifications, retinochoroiditis, strabismus, blindness, epilepsy, psychomotor, and mental retardation, petechiae due to thrombocytopenia, and anemia (McAuley *et al*, 1994), but sometimes congenital toxoplasmosis may be fatal (Kieffer and Wallon, 2013).

Epidemiological studies of *T. gondii* in the pregnant women showed significant variation in prevalence from 9% to 67% in Euro-

pean nations (Nash *et al.*, 2005; Maggi *et al.*, 2009; Ramos *et al.*, 2011; Lopes *et al.*, 2012) and up to 92.5% in Ghana (Ayi *et al.*, 2009). Global meta-analysis used strict criteria based on seroconversion and low IgG avidity reported that about 201.600 children annually suffered from congenital toxoplasmosis (Rostami *et al.*, 2019),

As to Saudi Arabia, in Riyadh positive toxoplasmin skin tests among pregnant women were 22.1% (Shoura *et al.*, 1973), and in Mecca positivity was same among female pilgrims (Morsy and El Dasouki, 1977). El Hady (1991) in Abha by IHAT found 31.6% anti-*Toxoplasma* among pregnant women. Sarwat *et al.* (1993) in Mecca, Al Amari (1994) in Abha, Yanaza and Kumari (1994) in Al-Hassa and Makki and Abdel-Tawab, 2010) in Eastern Saudi Arabia detected anti-*Toxoplasma* antibodies among the blood donors. Besides, Abdel-Motagaly *et al.* (2017) in Egypt reported the transmission of toxoplasmosis infection by blood transfusion as well nosocomial by the needle stick-injury. Abdalla *et al.* (1994) in premature infants in Saudi Arabia, with different clinical pictures reported anti-*Toxoplasma* IgM positivity of 23.1%. IgG-positivity varied from 9.13% in Hail Region to 39.43% in the Eastern Region (El Harthi *et al.*, 2006). Between 2002 & 2020, the seroprevalence of toxoplasmosis in humans was assessed by the Saudi demographics. Al Ghazi *et al.* (2002) by torch age-agents in pregnant women detected *Toxoplasma* IgG antibodies in 35.6%, CMV total IgG antibodies were in 92.1%, rubella IgG antibodies in 93.3%, HSV-1 IgG antibodies in 90.9%, HSV-2 IgG in 27.1%, and VZV IgG antibodies in 74.4%, but 0% seroprevalence rate for HIV-1. Ismail (2020) reported that approximately one-third of Saudi Arabia populations had IgG seropositivity, and 6.4% had IgM seropositivity.

As to animals, Morsy *et al.* (1994) in Riyadh detected anti-*Toxoplasma* antibodies in rodents. Al Dakhil and Morsy (1996) in the Eastern Region reported anti-*Toxoplasma* antibodies in the Indian grey mongoose (*H.*

edwardi). Also, Amin, AM, Morsy (1997) in Jeddah detected anti-*Toxoplasma* antibodies in butchers and slaughtered sheep and goats. In the human host, the parasites form tissue cysts, most commonly in skeletal muscle, myocardium, brain, and eyes; these cysts may remain throughout the life of the host. Diagnosis is usually achieved by serology, although tissue cysts may be observed in stained biopsy specimens

This study aimed to assess the seroprevalence of *Toxoplasma gondii* among pregnant females in Al-Baha District to determine the infection risk factors and the consequence during pregnancy.

Materials and Methods

Study population: The study was a hospital-based cross-sectional study. It took place in the King Fahd General Hospital (KFH) in Al Baha, Saudi Arabia, between 1st February 2020 and 1st February 2021. Pregnant mothers, in their first and second trimesters, who attended (KFH) the antenatal clinic, were included. Among 173 blood samples collected sera were separated for detection of anti-*Toxoplasma* IgG and IgM by ELISA

Anti-*Toxoplasma* IgG & IgM levels: Specific IgG & IgM antibodies to *T. gondii* were measured using ELISA Kits (Enzywell *Toxoplasma* IgG & IgM Kits, according to the manufacturer's instructions. The dichromatic spectrophotometer (Bio-Tek[®], USA) was used to measure absorbance of tested sera and control ones at 450nm. Results were measured semi-quantitatively by calculating a ratio of optical density (OD) value for samples to OD value of the positive and negative control ones. Sample was considered positive for the specific antibodies if its absorbance was more than the Cut-off (>1.3 for IgG & >1.2 for IgM), and negative if it was less than 0.7 for IgG and 0.8 for IgM. The test was repeated if there was any doubt or if the borderline results were (0.7-1.3 for IgG & 0.8-1.2 for IgM). This ELISA Kit have a sensitivity of 98% and a specificity of 10%, without cross-reactivity when compared to other ELISA kits.

Statistical analysis: Data were analyzed by SPSS 22.0 software package. The data were reported as means and standard deviation. Categorical data are reported as frequencies.

Ethical considerations: This study was approved by the Ethical Committee of King Fahd Specialist Hospital, Saudi Arabia. According to Helsinki (1964) as human experimentation developed originally in 1964 for the medical community

Results

Patients: One hundred seventy-three pregnant females were in the first and second semester, with ages ranged from 30 to 45 years (30.46 ± 8.9). Serologically 35 (20.2%) were positive for anti-*T. gondii* antibodies. Of whom 33 (19.1%) were IgG positive and 2 (1.2%) positive for both IgG & IgM antibodies. Out of the 33 positive females for Anti-*Toxoplasma* IgG, ten cases with history of single and/or repeated abortions, and two women with history of congenital anomalies, another two with a history of intrauterine growth retardation, and two cases had a history of preterm labor. All cases reported the occasional consumption of the undercooked meat. Ten women documented contact with cats. Details were given figures (1 & 2)

Discussion

In the present study, total 20.2% and 1.2% were positive IgG and IgM respectively. A wide range of seroprevalence of toxoplasmosis was reported in different region in Saudi Arabia by several epidemiological studies as in Jazan (Aqeely *et al*, 2014), Makkah (Ismail *et al*, 2016), Al-Madinah (Imam *et al*, 2016), and Qassim region (Rasheed *et al*, 2021). These figures were lower than that reported in others Saudi Arabian regions; Al-Khobar (Al-Mulhim and Al-Qurashi 2001), Al Ahsa (Al-Mohammad *et al*, 2010), Riyadh (Almogren 2011), Khamis Mushait and Abha (Al-mushait *et al*, 2014), Aseer (Eida, 2015), and AD-Dawadimi (Alanazi *et al*, 2017). On the contrary the present results were higher than that reported in Hail (Abdel Galil 2014) and Arar (Alanazi *et al*, 2017). A rise in IgM

titers is sufficient evidence of acute infection. By contrast, a single positive IgM titer can mean that the infection was acquired either during pregnancy or before conception. If the infection is acquired before conception, the fetus is very unlikely to be at risk for congenital toxoplasmosis (Van Kessel and Eschenbach, 2021).

In Arab Countries, Kamal *et al*. (2015) in Egypt reported high prevalence seropositive cases in age group of 21-30 years. Post-delivery adverse outcome was in 80.3% of high-risk pregnancy group compared to 20% of normal pregnancy group. Statistically significant was between seropositivity and living in rural area, low socioeconomic level, and the consumption of the undercooked meat ($P < 0.05$). Al-Adhroey *et al*. (2019) in Yemen reported 20.0% of women of which 12.9% were positive for only IgG and 7.1% were positive for both IgG and IgM antibodies. All the 546 pregnant women were seropositive for anti-CMV IgG. Of the 546 women, 40 (7.3%) were positive or equivocal for IgM antibodies. All sera from the 40 women (IgG+/IgM+) showed a high or intermediate CMV IgG avidity index.

Others countries with low IgM seroprevalence were New Zealand; 0.2%, South Korea, 0.1%, and USA, 0.01%, and IgG wide range of prevalence was (53.8%) whereas the three lowest were Mexico; 7.2%, South Korea; 2.1%, and Canada; 0.2% (Bigna *et al*, 2020). Environmental factors that favor *T. gondii* transmission and infectivity, as well as differences in study populations, numbers of cats, diagnostic procedures, and living styles, may all play a role in these differences in the reported prevalence rates from different localities (Saleh *et al*, 2014).

The only definitive hosts for *Toxoplasma gondii* are members of family Felidae (domestic cats and their relatives). Unsporulated oocysts are shed in cat's feces, Although oocysts are usually only shed for 1-3 weeks, large numbers may be shed. These can live for a long period in the environment, especially in water or soil. Domestic cat contact

is frequently listed as a risk factor (Rifaat et al, 1981). This agreed with the present study as the pet cats were found to be a risk factor for *T. gondii*. This also agreed with studies reported in France (Baril et al, 1999) and China (Liu et al, 2009). However, others found no link between Toxoplasma infection and the presence of pet cats (Gebremedhin et al, 2013; Mwambe et al, 2013). Mohammed et al. (2019) in Saudi Arabia reported *T. gondii* antibodies among stray cats (39%) and pet ones (13%). Frequent exposure to feline feces or failure to take preventive measures (e.g., not washing hands or wearing gloves) can significantly increase the chance of infection. Inadequate inspection and infrequent check up with the veterinary clinics could increase the risk of zoonotic diseases spread like Toxoplasma from pet to owners. Not only can stray cats contaminate the environment in an indiscriminate manner, but also many owners allow their indoor cats to defecate outside their homes. As a result, there is a considerable risk of *T. gondii* oocysts contaminating the environment and being transmitted to humans, and the prevalence was projected to be higher (Cong et al, 2015).

In the present study, all positive *T. gondii* cases reported the consumption of meat, suggesting that consumption of meat may be a risk factor for toxoplasmosis if it was not properly cooked. Significant association between toxoplasmosis and consumption of meat were reported (Liu et al, 2009; Wu et al, 2011; Gebremedhin et al, 2013; Andiappan et al, 2014). This recommended that *T. gondii* infection in cattle, sheep, and chicken in Saudi Arabia may be contributing risk which is supported by high prevalence rates of toxoplasmosis in these animals (Al-Anazi 2011; Al Nasr et al, 2018).

The present study showed that toxoplasmosis during pregnancy may be a cause of several forms of bad pregnancy outcomes. Out of 35 toxoplasmosis cases, ten cases had a previous history of abortion, 2 cases had history of congenital anomalies, three cases

had a previous history of intrauterine growth retardation and finally two cases had a previous history of preterm labor. All cases reported the consumption of meat. Ten cases documented contact with cats. Previous reports supported the role of toxoplasmosis acquired during pregnancy as a risk factor for bad pregnancy outcome (Sahwi et al, 1995; Freeman et al, 2005; Olariu et al, 2011; 2019; Yamamoto et al, 2013).

Conclusion

The feasible strategy awareness to prevent congenital toxoplasmosis measures is needed to reduce the risk of infection. The main preventive measures relate to reducing the pathogen burden in the food chain; improving food hygiene; public education, especially of women of child-bearing age; and various precautions in immunosuppressed persons (including in transplantation medicine). Screening for *T. gondii* antibodies before marriage and during pregnancy, is a must to avoid the sources of infection. Avoiding stray and pet cats is a must to minimize zoonotic toxoplasmosis.

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Explanation of figures

Fig. 1: Map of Saudi Arabia, red circle surrounds Al-Baha Province, Saudi Arabia,
 Fig. 2: Prevalence rates of anti- toxoplasma IgG and IgM among participants.
 Fig. 3: Illustrative *Toxoplasma gondii* life cycle after CDC



