

Sero-epidemiological Study of *Toxoplasma gondii* among Pregnant Women in Akre City

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ABSTRACT

Recently has been observed globally that toxoplasmosis disease was caused by *Toxoplasma gondii* and generally its asymptomatic infection in people except pregnant women. Many previous studies were suggested this pathogen transmits by ingesting of undercooked or raw meat contaminated with the oocytes of this parasite. The study aimed to show the seroreactivity of *Toxoplasma gondii* antibodies among pregnant women and to evaluate association of studied risk factors with infected cases. ELISA technique used for detection of anti-toxoplasma specific IgG and IgM antibodies. A biography form interview for participant was performed to estimate between the risk factors and toxoplasmosis. The present study was carried out from the beginning of October 2018 to the end of March 2019 and included 90 pregnant women visited Akre general hospital. Out of 90 tested samples, 49 (54.46%) and 4 (4.44%) were positive for IgG and IgM respectively. According to residency factor, pregnant women from villages had higher percentages of IgG (57.14%) and IgM (4.08%) than those lived in the city which were recorded 0% and 48.48% of IgM and IgG respectively. A high percentage of IgG (65.64%) and IgM (7.27%) were found among pregnant women with history of miscarriage. Seropositivity for both IgG (61.90 %) and IgM (9.52%) was higher among pregnant women who had cats compared to those without cat. Seroprevalence of IgM and IgG was 9.67% and 54.83% among population who had undercooked meat, while 1.69% and 59.23% were positive for IgM and IgG respectively, in those well cooked meat meal. Highest positive percentage of IgG (77.77%) was

recorded at ages 40-50 years while the lowest rate was (40%) at ages <20 year. Through a seropositive reaction was found that age groups as a possible risk factor by ELISA IgG in which risk increased by rising of age categories. Significant correlation was detected between consumption of undercooked meat and cases positive for IgM. It revealed the association between third trimester and cases with positive IgM. In the conclusion, toxoplasmosis is widespread in the area which requires raising awareness among pregnant women to decrease T. gondii infection and subsequently minimize possibilities of congenital transmission.

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1. INTRODUCTION

In 1908, Nicolle and Manceaux were the first to describe and isolate this parasite and they detected in blood, spleen and liver of a North Africa rodent [1]. The taxonomy has recently changed: Phylum: Apicomplexa, Class : Coccidea, Subclass: Coccidiasina, Order : Eimeriida, Suborder : Eimeriorina, Family: Sarcocystidae, (Sarcocystis), Subfamily : Toxoplasmatinae, Genus : Toxoplasma [2]. Three infectious form have been contributed the life cycle of this protozoan parasite including Tachyzoite , Bradyzoites and Oocysts [3]. *Toxoplasma gondii* cause toxoplasmosis which is one of the main causes of congenital anomalies among infected pregnant women and clinically very important because may cause pregnancy `s fetus death or severity complication such as hydrocephalus or retinoblastomas [4]. It is an obligate intracellular protozoan parasite that has a complex life cycle alternating between asexual reproduction taking place in several tissues of vertebrates (intermediate hosts) and sexual reproduction taking place in epithelial linings of the digestive tract of cats and generally its asymptomatic infection in people except pregnant women [5]. High proportion of seroprevalence has been illustrated among pregnant women and women of childbearing age from different parts of the world [6]. Many previous studies were demonstrated the transmission of *Toxoplasma gondii* by various methods. It was suggested this pathogenic parasite transmitted by ingesting of undercooked or raw meat contaminated with the oocytes, Exposing to contaminated soil and water, passing this infectious from mother to their unborn fetus and others pathway probably involved in transmission of this pathogenic parasite such as blood transfusion and organ transplantation [7]. The definitive hosts of this parasite are member of Felidae (cat) family) and warm blooded animal or human could be an intermediate hosts [8]. It has been considering the congenital transmission of *T. gondii* during pregnancy as a risk factor for the health of newborn infants. While in immune-compromised individuals, the parasite can cause life threatening infections and some nervous system disorder resulted like mental retarders, blindness and fetal death [9]. The rate of transmission of this infection from mother to fetus varies based on gestational period and the severity of infection, According to the trimester of pregnancy, the percentage of infection during the first trimester is 10-15%, which is increase to 45% in the second trimester and the up to 60% in the third stage of trimester [10]. Laboratory Identification is made by two methods including both directly and indirectly. Indirect method by serological methods, while directly methods by amplification of specific nucleic acid sequences (i.e., Polymerase Chain Reaction [PCR]), hybridisation, isolation of organism, and histologic demonstration of the parasite and/or its antigens (i.e., immunoperoxidase stain) [11]. A serological method varies such as Latex agglutination test, indirect fluorescent antibody test , immunochemiluminescence (ICL) or immunochromatography (IC) and ELISA techniques .Other rarely used methods include

demonstration of antigenemia and antigen in serum and body fluids, toxoplasmin skin test, and antigen-specific lymphocyte transformation [12]. The study aimed to show the sero-reactive antibodies of *T. gondii* infection among participant enrolled and to assess the association between the risk factors and toxoplasmosis infected cases in the studied region.

2. LITERATURE REVIEW

Toxoplasmosis is a widely prevalent zoonosis caused by *Toxoplasma gondii*. The definitive hosts are domestic and wild cats while intermediate hosts are all mammals including human [13]. Toxoplasmosis regarded as a main cause of miscarriage and serious health problem in different countries [14], and usually asymptomatic except in immunocompromised people may show some clinical manifestations such as encephalitis, myocarditis, pneumonitis [15]. Several methods have been used for diagnosis of *Toxoplasma gondii* including serological tests, polymerase chain reaction, Histological examination or by isolation of the organism [16]. Recently this epidemic has shown the dangerous picture; unfortunately the range of infection was increased and recorded various substantially among counties [17]. Anti-*Toxoplasma gondii* IgG was detected in 220 (44.4%) and IgM in 55 (11.1%) from 496 serum samples taken from pregnant women by ELISA technique [18]. [13] also used ELISA technique and found IgG in 36.45% and IgM in 4.52% tested samples, while [19] found varies percentage of anti-toxoplasma antibodies 29.4% (58 out of 197) of IgG whereas IgM positive was 5.6%. [20] Found that 18.3% samples were positive for IgG and 1.0% for IgM using ELISA method.

3. METHODS AND MATERIALS

The cross-sectional study design was to achieve the aim and objectives of current study. From each patient about 5ml of venous blood samples were collected by laboratory technologists using a sterile disposable syringe and then separated into serum for serological diagnosis under aseptic condition using IgG (Biorex Diagnostic Limited) and IgM antibodies (Bioactive diagnostic) ELISA test kits. All sample tubes were appropriately labelled with patient's name. The present study enrolled patients who were referred to Gulan general hospital laboratories and Emergency Hospital laboratories in Akre. All data were recorded on survey form for each patient: included age, residential area, stage of pregnancy, history of miscarriage, *toxoplasma* knowledge, presence of pet animals such as cats, undercooked meat. Data was statistically analyzed using (Statistical package for social sciences) SPSS software version 21 (SPSS, IBM Company, Chicago, USA). $P \leq 0.05$ was considered statistically significant. Logistic regression analysis was used to predict the association between *T. gondii* seropositivity and risk factors.

4. RESULTS

The results in the table 1 reveal about 90 participants were investigate and the seroprevalence of infected women and non-infected women to *Toxoplasma gondii* was 49 (54.46%) and 4 (4.44%) were positive for anti-*Toxoplasma* specific IgG and IgM respectively, of the total 90 samples.

Table 1: *Toxoplasma gondii* IgM and IgG Seropostivity

Results	Prevalence of <i>Toxoplasma gondii</i> IgM / %	Prevalence of <i>Toxoplasma gondii</i> IgG / %
Positive	4 / 4.44	49 / 54.46
Negative	86 / 95.56	41 / 45.55
Total	90 / 100	90 / 100

According to the age groups high percentage of IgG was 77.77% at age group more than 40 years old whereas the lowest percentage was 40% for those who were less than 20 years, IgM was 5.26 in both age groups 20-29 & 30-39. Based on the significant correlation, the infection increases progressively with increasing of age categories, as shown in the table 2.

Table 2: *Toxoplasma gondii* IgM and IgG Seropostivity In different Ages

Age	No. of P.	IgM Positive		IgG Positive	
		No.	%	No.	%
<20	5	0	0	2	40
20-29	38	2	5.26	19	50
30-39	38	2	5.26	21	55.26
>40	9	0	0	7	77.77
Total N.	90	4	4.44	49	54.46

It can be seen that the higher rate of seroprevalence were recorded by ELISA immunoglobulin (IgG) than immunoglobulin (IgM) in rural zones. Similarly, 16 (48.48%) of IgG and zero of IgM were recorded of those who live in urban zones, as revealed in table 3. significantly no difference was found between residency and ELISA tests.

Table 3: *Toxoplasma gondii* IgM and IgG Seropostivity According to Setting Location

Residency	No. of P.	IgM Positive		IgG Positive	
		No.	%	No.	%
Rural	57	4	7.01	33	57.89
Urban	33	0	0	16	48.48
Total	90	4	4.44	49	54.46

Table 4 shows the second trimester recorded higher rate of anti-*toxoplasma* antibodies IgG and IgM in comparison to others trimester. Notable seroreactivity was identified in the Third gestation stage by ELISA IgM. (p value = 0.025)

Table 4: *Toxoplasma gondii* IgM and IgG Seropostivity according To Pregnancy Stages

Trimester	No. of P.	IgM Positive		IgG Positive	
		No.	%	No.	%
Trimester – i	30	0	0	13	43.33
Trimester – ii	38	3	7.89	23	60.52
Trimester – iii	22	1	4.54	13	59.09
Total	90	4	4.44	49	54.46

As shown in the table 5, the seropositivity of IgM was detected 4 (7.27%) and IgG was 36 (65.45%) of patients who had past history of abortion, whereas the result was 13 (37.14%) and zero of enrolled pregnant women of IgG and IgM, respectively of those who had no

miscarriage in their life. No notable influence was identified on the toxoplasma infection in relation to miscarriage factor by ELISA method.

Table 5: *Toxoplasma gondii* IgM and IgG Seropostivity in aborted and non-aborted women

Miscarriage	No. of P.	IgM Positive		IgG Positive	
		No.	%	No.	%
Yes	55	4	7.27	36	65.45
No	35	0	0	13	37.14
Total	90	4	4.44	49	54.46

Comparatively highest rate of seroprevalence of *toxoplasma* antibodies 4 (9.52%) of IgM and 26 (61.90%) of IgG were observed in pregnant samples who had domestic cat at their home compared with those who had not. However, not statistical association was found between Handling of pet animals like cat and ELISA IgG and ELISA IgM, as show in table 6.

Table 6. *Toxoplasma gondii* IgM and IgG Seropostivity in Women that have or have not Cat in their house

Pet animal (cat)	No. of P.	IgM Positive		IgG Positive	
		No.	%	No.	%
Yes	42	4	9.52	26	61.90
No	48	0	0	23	47.91
Total	90	4	4.44	49	54.46

In accordance with toxoplasma knowledge most of the enrolled participants were not aware of being previously tested for Toxoplasma. it revealed that 1 (9%) of IgM and 4 (36.35% of IgG were positive for those who have information about *Toxoplasma gondii* .while, this pathogenic parasite was detected 3 (3.79%) and 45 (56.26%) by ELISA IgM and IgG respectively of those who had not knowledge about this parasitism . as shown in table 7. No statistically significant was detected between Knowledge efficacy and ELISA test.

Table7: *Toxoplasma gondii* IgM and IgG Seropostivity depend on toxoplasma knowledge

Toxoplasma knowledge	No. of P.	IgM Positive		IgG Positive	
		No.	%	No.	%
Yes	11	1	9	4	36.35
No	79	3	3.79	45	56.26
Total	90	4	4.44	49	54.46

Among seropositive pregnant women who have consumed under cooked meat 3 (9.67%), 17 (54.83%) were recorded as a positive case for IgM and IgG, respectively. Relatively to those who had not consumed under cooked meat 1 (1.69%) were detected IgM and 32 (54.23%) of IgG , as shown in table 8 . Significant association was found between seropositivity of ELISA IgM, IgG and risk factor of consumption of under cooked meat .

Table 8: *Toxoplasma gondii* IgM and IgG Seropositivity in related to consumption of undercooked meat

Consumption of undercooked meat	No. of P.	IgM Positive		IgG Positive	
		No.	%	No.	%
Yes	31	3	9.67	17	54.83
No	59	1	1.69	32	54.23
Total	90	4	4.44	49	54.46

5. DISCUSSION

It is crucially important to understand the status of *Toxoplasma* infection in the general population. *Toxoplasma gondii* causes toxoplasmosis around the world and the majority of infections are asymptomatic and without complications except in immunocompromised and pregnant women. Various methods are used for diagnosis of *Toxoplasma gondii*. The main focus of the current study is to screening anti-*toxoplasma* antibodies among surveyed population and to evaluate the association of studied risk factors with infected cases.

Out of a total 90 patients who were referred to laboratory investigation included in the present study and semi-automated ELISA technique was used for detection anti-*toxoplasma* specific IgG antibodies and anti-*toxoplasma* specific IgM antibodies. *T. gondii*-specific IgG is associated with previous exposure to the parasite and is used as a marker for latent infection while IgM is used as a marker for exposure or acute infection. Based on the results obtained, the percentages of IgG and IgM were 54.46% and 4.44% respectively. The outcome results were agreeing to those recorded via [21] who diagnosed that 42.5% of IgG and 7.5% of IgM from cases were positive by ELISA method and [22] found that 29.2% and 3.1% of pregnant were positive for IgG and IgM respectively. While the results of this study were disagreed with those found by [23] who detected high percentages of IgM more than IgG by ELFA (Enzyme Linked Fluorescence Assay)-IgG/IgM. As well dissimilar to study done by [24] who identified higher rate of IgM than IgG in female gender. These discrepancies in the results of diagnosed *T. gondii* may referred to many reasons including number samples, socioeconomic status, ethical considerations, genetic characteristics and methodology. Seropositive cases in the results of current study was observed the infection of toxoplasma increased by increasing of age and which is similar to the results were recorded by [25], while the results of this study were disagree to the concluded other studies such as [26]. These differences may be due to many factors such as in the last interval age women more active with family and house holding matters so that more susceptible to endangering agents and pass the infection. where as low proportion of infection at younger age group due to less contact causative agent of toxoplasmosis infection, immunocompromised with advanced of age, the kind of test, low number of sample, climate, and cultural differences regarding hygienic and feeding habits. Sero-reactive and non sero-reactive cases in relation with risk factors like residency, handling of pet animal like cat, history of abortion, toxoplasma knowledge, there were not statistically significant according to the present results and these results were identical to those found by [27]. Our findings were dissimilar to those found by [28, 29] and numerous causes contribute to this differences such as the, high awareness, care and hygiene may decrease the exposure to infection, Epidemiology varies widely by geographical areas of the study, mode of transmission A significant relationship was found between consumption of undercooked meat and IgM which were similar to those found by [30]. The current outcomes were in correspond to other investigators who found no influence of eating undercooked meat on the present outcomes of ELISA IgM like [31]. These dissimilarities based on the prior of highlighted analyzes. A considerable correlation was detected between third trimester of pregnancy and IgM which was comparable to [32]. The results were differing to those studies who found no

statistical association such as [33]. The differences in the results can be attributed to obstetric factors, congenital transmission for women with primary and reactivated infections acquired during pregnancy, higher vulnerability to *T. gondii* infection during gestational period may be due to combination of pregnancy-associated immunosuppression as well as hormonal and physiological factors, number analyzed samples and methodology, effectiveness of treatment during gestation, individual variation and also pertains to the cumulative effect of exposure to the infective stages of the parasite.

6. CONCLUSION

The study found the statistically significant between age categories and ELISA. *T. gondii* infection was more among patients who reside in the rural area compared to those who reside in the urban area. Toxoplasmosis is widely spread in the area which requires a raising awareness among pregnant women to decline and reduce the rate of *T. gondii* infection and subsequently to reduce the possibilities of transmission rate. Despite the lack of statistical correlation between infection and most of associated risk factors, preventive measures among pregnant women should be followed to prevent consequences of Toxoplasmosis.

7. RECOMMENDATION

Early diagnosis of congenital toxoplasmosis in both symptomatic and asymptomatic conditions, in neonatal period, initiation of adequate treatment and long-term, multi-specialist monitoring, including multi-organ rehabilitation of children may prevent or reduce the complications of congenital toxoplasmosis. The high awareness and health education should be targeted for pregnant women to avoid from the contamination during any contact with soil, water with cat waste. Extraordinarily recommend further study for the purposes of aiding the present research is needed to fully clarify the links between latent toxoplasmosis and risk factors and a high number of serum samples should be tested in future studies to obtain more precise results. To date there is no vaccine suitable for human use to prevent this infection from breakout; therefore, the primary health prevention is the necessity method for health protection from the infection which is performed by health and educational establishment.

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