Seroprevalence of *Toxoplasma gondii* Infection among Pregnant Women Attending Antenatal Care in Asmara, Eritrea: Preliminary Report

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Abstract

Background

The majority of human infections with *Toxoplasma* produce no symptoms, but in congenitally infected children can cause devastating effects including eye blindness, brain damage, or miscarriage.

Transmission to the fetus occurs predominantly in women who acquire their primary infection during gestation.

Objective

The study aimed to determine the seroprevalence of *T. gondii* among pregnant women attending Antenatal Care (ANC) in different sites of Asmara, Eritrea, and to identify possible risk factors associated with Toxoplasma infection among pregnant women attending the antenatal center.

Methods

The study has followed a cross-sectional laboratory-based survey. The data were collected from 210 pregnant women in four health facilities. Voluntary sampling technique and a structured questionnaire were used to collect associate factors and socio-demographic information. Cobas e411 clinical chemistry Analyzers was used to test the blood serum for IgG and IgM antibodies. Epi-Info (V 7.0) was used for data entry and SPSS (V 20.0) was used for data analysis.

Results

From the 210 samples, 53.6% (112) samples were seropositive and 46.4% (97) samples were seronegative for toxoplasma specific IgG antibody. Furthermore, 2.9% (6) samples were seropositive and 97.1% (203) samples were seronegative for toxoplasma-specific IgM antibodies.

Conclusion

The seroprevalence was considerably high, 53.6% IgG antibody and 2.9% IgM antibody which requests attention, in order to introduce preventive control measures, screening tests, and health education.

Introduction

Toxoplasma gondii is a microscopic zoonotic and an obligated intracellular protozoan parasite that can infect most warm-blooded animals and causes a disease called toxoplasmosis [1]. It was originally described in a North African rodent called the gundi, from which derives its specific name [2, 3]. Humans

can become infected by ingestion of oocysts released in cat feces; by ingestion of raw or undercooked meat or drinking unpasteurized milk containing *T. gondii* tissue cysts [1, 4]. Transplacental congenital transmission of tachyzoites, blood transfusion or organ transplantation are very rarely [5, 6]. All stages can be infectious to human [2]. In chronically infected women; infection can be reactivated because of their immuno-compromised state [7]. Recently, in immuno-competent individuals more virulent strains that cause severe symptoms have been reported [8].

The diagnosis relies on either indirect serological tests or direct detection of the parasite or by PCR. Serologic tests that indicating recent or past infection are most effective in immune-competent adults. These tests include: ECLIA, ELISA, IFA, LA tests and complement fixation to detect IgG and IgM antibodies [9]. The presence of a high IgM titer in the absence of significant IgG titers indicates early stages of primary infection. A negative IgM titer is helpful for ruling out recent infection. However, a positive IgM test should be verified in a reference laboratory [3, 10]. Current procedures allow diagnosis of an active infection of the fetus in utero by means of PCR of amniotic fluid [11].

Children over 5 years of age with severe acute toxoplasmosis or active chorioretinitis is treated with pyrimethamine. Treatment of pregnant women are controversial but treatment with spiramycin is still advocated [12]. Current evidence is insufficient to confirm that treating seroconvert during pregnancy prevents fetal infection [11].

It is noted that no study on the seroprevalence of *Toxoplasma gondii* in general and/or specific target groups has been done in Eritrea. None is known on what are the associated risk factors for the spread of the disease and its causes. Therefore, the purpose of this study was to estimate the seroprevalence of *T. gondii* and its associated risk factors among pregnant women attending Antenatal Care (ANC) in Asmara City, Eritrea.

Materials And Methods

Study Area:

The study was conducted in Asmara, Eritrea at all the health facilities that provide maternal health services, including delivery, one hospital and other three health facilities were selected for the study to represent the city.

Eritrea is situated in the horn of Africa bordered by the Sudan, Djibouti, Ethiopia and the Red Sea with 1,212 Km seashore. It covers an area of 124,000 sq. km and is divided into three topographic areas (the Western Lowlands, the Central Highlands and the Coastal Plains). Administratively, it is divided into 6 regions or zones, namely Southern Red Sea Zone, Northern Red Sea Zone, Anseba, Gash-Barka, Southern Zone and Maekel (Central Zone). The total population are about 6.1 million [13]. Asmara is in central region (Zoba Maekel), country's capital city, is the targeted area of the study.

Study Design, Study Population and Sample Size:

The study was designed as cross-sectional laboratory based study. That comprises all pregnant women living in the Catchment area of Asmara administration, in the reproductive age group of 16 to 45 years [14,15].

The data was collected between July and August 2016 from 210 pregnant women (in four health facilities) whom attending their ANC follow up. Voluntary sampling technique, was implord following their consents and agreements to participate in the research study.

Data Collection Tools and Techniques:

A structured questionnaire was used to collect the pregnant women demographic information and the other associated risk factors in the study. Pregnant women were interviewed by the enumerators which were health professionals who works in the facilities where the collection had been conducted.

Collection of sample and Laboratory investigation:

A three ml venous blood sample was collected from study participants using standard procedures on Lithium heparin container and was stored in freezing condition (-20⁰c) before analysis. Serum was prepared by centrifugation of blood at 2500 rpm for 3 minutes.

The electrochemiluminescence immunoassay (ECLIA) was used for in vitro quantitative determination of IgG and IgM antibodies to *Toxoplasma gondii* in human serum on Elecsys and Cobas e411 immunoassay analyzers.

Quality control (QC):

For QC; Preci Control was used. Controls for the various concentration ranges were run every day when the test in use and following each calibration. Based on the measurements of cal-1 and cal-2 the cut-off was calculated automatically.

Interpretation: IgG: >1 (non-reactive), ≥ 1.0 - > 30 (Intermediate), ≥ 30 (Reactive).

IgM: > 0.8 (non-reactive), ≥ 0.8 - >1.0 (Intermediate), ≥ 1 (Reactive).

Data entry and Data Analysis:

Questionnaire completeness checking process was followed by data entry into computers using Epi-Info (V 7.0), free software package developed by CDC. To describe the data in terms of frequency and proportion SPSS (V 20.0) computer program was used [16]. To describe the age of participants, which is continuous variable, average and standard deviation were used [17]. In this study, the chi-squared test has been applied to screen for an association between IgG and IgM antibodies seropositive results and the several risk factors included in the study [18].

Ethical Issues; Informed Consent and Confidentiality:

Research approval for the study was obtained from Research and Ethic Committee, Faculty of Medical Laboratory Sciences, University of Gezira. Based on the request of Faculty of Medical Laboratory Sciences, University of Gezira, the Health Research Proposal Review and Ethical Committees, MOH Eritrea had accepted and gave a permission to conduct the research on the specified sites. Ethical Permission was obtained from the Ministry of Health, Zoba Makel.

Participants were briefed about the aim of the study and requested their willingness to participate in the study. The interview was carried out face-to-face to obtain information and were kindly asked for their enthusiastic participation and sign on the prepared consent form.

Results

Socio-demographic characteristics of study population:

Overall 210 samples of pregnant women were considered from Edagahmus mini Hospital, Akria Health Center, Vilajo/ BMCH Community Hospital and Godief Health Center each constituting 80 (38%), 50 (24%), 41 (20%) and 39 (19%) respectively. Majority of the pregnant women were in the age group of 25 to 34 years representing 60%, in the age groups of 15–24 years and 35–44 years were 27% and 13%, respectively. The mean and median age was comparable with 27 and 26 years, respectively with variation of 5 years by SD. Only 10% of the women were reported to be not married being divorced, widowed or separated while the remaining 90% were married. Equal to 98% of the participants were from Tigrigna ethnic group. 70% of the pregnant women were noted to reach secondary level while the rest 16% and 14% were reported to reach elementary and post-secondary educational level respectively. 74% were housewife, and the remaining 14% and 12% women were engaged in daily workers and other type jobs. Majority of the pregnant women were at their 2nd trimester which accounts 42%; while the remaining 37% and 21% were in their 3rd and 1st trimesters respectively (Table 1).

Table 1
Socio-demographic characteristics of study population.

Characteristics		Number	Percent %
Health facility	Edagahamus mini Hospital	80	38%
	Akria Health Center	50	24%
	BMCH Community Hospital	41	20%
	Godaif Health Center	39	19%
Age group	15–24 years	57	27%
	25–34 years	125	60%
	35–44 years	28	13%
Marital status	Married	189	90%
	Not married	21	10%
Education	Primary	33	16%
	Secondary	147	70%
	University	30	14%
Pregnancy stage	1st trimester	77	36.7%
	2nd trimester	88	41.9%
	3rd trimester	45	21.4%
Occupation	Housewife	155	74%
	Daily worker	29	14%
	Others	26	12%
Ethnic groups	Tigrigna	205	98%
	Tigre	2	1%
	Saho	1	0.5%
	Hidarb	1	0.5%

Detection of IgG and IgM antibodies among study population:

From the total 209 pregnant women included in the study 112 (53.6%) were seropositive and 97 cases (46.4%) were seronegative for *Toxoplasma* specific IgG antibody. Similarly, 6 samples (2.9%) were seropositive and 203 samples (97.1%) were seronegative (of which 2 samples were on border) for

toxoplasma specific IgM antibody considered as negative. Out of the total 112 IgG seropositive samples 3 were also seropositive for IgM, and out of the 97 IgG seronegative samples 3 samples were seropositive for IgM (Table 2).

Table 2
Association between IgG and IgM antibodies.

			IgM Status		Total
			Negative (-ve)	Positive (+ ve)	
IgG Status	Negative (-ve)	Cases	94	3	97
		Percent	45.0%	1.45%	46.4%
	Positive (+ ve)	Cases	109	3	112
		Percent	52.1%	1.45%	53.6%
Total	Cases		203	6	209
	Percent		97.1%	2.9%	100.0%

Distribution of IgG and IgM seropositive among the socio-demographic characteristics and Causal Factors for Toxoplasma

Tables 3 and 4 indicated the prevalence of seropositive for *Toxoplasma* socio-demographic categories (*Place, Age, Educational level, Marital status, Occupation, and Stage of pregnancy*) using IgG and IgM antibodies. Chi-square (X^2) was done to assess between seropositive *Toxoplasma* and the factors, but no significant association was observed.

Table 3
Distribution of IgG among the different characteristics.

Socio-demographic characteristics		IgG Status				Total (N)
		Negative (-ve)		Positive (+ ve)		
N	%	N	%			
Health Facility	Edagahamus	45	56.2%	35	43.8%	80
	Akria	20	40.0%	30	60.0%	50
	BMCH	16	40.0%	24	60.0%	41
	Godaif	16	41.0%	23	59.0%	39
Age group	15–24	32	56.1%	25	43.9%	57
	25–34	56	44.8%	69	55.2%	125
	35–44	9	33.3%	18	66.7%	28
Marital status	Married	84	44.4%	105	55.6%	189
	Not married	13	65.0%	7	35.0%	21
Education	Primary	16	50.0%	16	50.0%	33
	Secondary	68	46.3%	79	53.7%	147
	Above	13	43.3%	17	56.7%	30
Occupation	Housewife	68	43.9%	87	56.1%	155
	Daily worker	17	58.6%	12	41.4%	29
	Other	12	48.0%	13	52.0%	26
Pregnancy stage	1st trimester	21	46.7%	24	53.3%	45
	2nd trimester	43	48.9%	45	51.1%	88
	3rd trimester	33	43.4%	43	56.6%	77
Total		97	46.4%	112	53.6%	210

Table 4
Distribution of IgM among the different characteristics.

Socio-demographic characteristics		IgM Status				Total (N)
		Negative (-ve)		Positive (+ ve)		
N	%	N	%			
Health Facility	Edagahamus	77	96.2%	3	3.8%	80
	Akria	48	96.0%	2	4.0%	50
	BMCH	41	100.0%	0	0.0%	41
	Godaif	38	97.4%	1	2.6%	39
Age group	15–24	57	100.0%	0	0.0%	57
	25–34	119	95.2%	6	4.8%	125
	35–44	28	100.0%	0	0.0%	28
Marital status	Married	185	97.9%	4	2.1%	189
	Not married	19	90.5%	2	9.5%	21
Education	Primary	31	93.9%	2	6.1%	33
	Secondary	143	97.3%	4	2.7%	147
	Above	30	100.0%	0	0.0%	30
Occupation	Housewife	151	97.4%	4	2.6%	155
	Daily worker	29	100.0%	0	0.0%	29
	Other	24	92.3%	2	7.7%	26
Pregnancy stage	1st trimester	43	95.6%	2	4.4%	45
	2nd trimester	86	97.7%	2	2.3%	88
	3rd trimester	75	97.4%	2	2.6%	77
Total		204	97.1%	6	2.9%	210

Distribution of IgG and IgM seropositive among Causal Factors for Toxoplasma:

Based on a different research study, possible risk factors were identified and included in this research study. And these are: source of water, use of raw meat, soil contact and availability of the domestic animal in general and presence of cat in particular. In addition to that three (3) possible health factors history of abortion, HIV status and presence of mentally abnormal child were also included (Tables 5 and

6). Chi-square showed some significant association between seropositive Toxoplasma and soil contact, but no significant association was obtained for most other factors.

Table 5
Distribution of IgG among the different cause factors of Toxoplasma.

Casual factors		IgG Status				Total	
		Negative (-ve)		Positive (+ ve)			
		N	%	N	%	N	%
Source of water	Pipe water	70	46.7%	80	53.3%	150	71.4%
	Well	5	62.5%	3	37.5%	9	4.3%
	Other	22	43.1%	29	56.9%	51	24.3%
Use of raw meat	No	92	46.9%	104	53.1%	197	93.8%
	Yes	5	38.5%	8	61.5%	13	6.2%
Availability of cat	No	71	45.5%	85	54.5%	157	74.8%
	Yes	26	49.1%	27	50.9%	53	25.2%
Availability of other domestic	No	73	48.7%	77	51.3%	150	71.4%
	Yes	24	40.7%	35	59.3%	60	28.6%
Soil contact	No	94	48.2%	101	51.8%	196	93.3%
	Yes	3	21.4%	11	78.6%	14	6.7%
HIV status	No	97	100.%	111	99.1%	209	99.5%
	Yes	0	0.0%	1	0.9%	1	0.5%
Abortion status	No	87	49.2%	90	50.8%	178	84.8%
	Yes	10	31.2%	22	68.8%	32	15.2%
Child mental problem	No	91	46.7%	104	53.3%	195	92.9%
	Yes	6	42.9%	8	57.1%	15	7.1%
Total		97	46.4%	112	53.6%	210	100.0%

Table 6
Distribution of IgM among the different cause of Toxoplasma.

Casual factors		IgM Status				Total	
		Negative (-ve)		Positive (+ ve)			
		N	%	N	%	N	%
Source of water	Pipe water	148	98.7%	2	1.3%	150	71.4%
	Well	9	100.0%	0	0.0%	9	4.3%
	Other	47	92.2%	4	7.8%	51	24.3%
Use of raw meat	No	192	97.5%	5	2.5%	197	93.8%
	Yes	12	92.3%	1	7.7%	13	6.2%
Availability of cat	No	152	96.8%	5	3.2%	157	74.8%
	Yes	52	98.1%	1	1.9%	53	25.2%
Availability of other domestic	No	147	98.0%	3	2.0%	150	71.4%
	Yes	57	95.0%	3	5.0%	60	28.6%
Soil contact	No	190	96.9%	6	3.1%	196	93.3%
	Yes	14	100.0%	0	0.0%	14	6.7%
HIV status	No	203	99.5%	6	100.0%	209	99.5%
	Yes	1	0.5%	0	0.0%	1	0.5%
Abortion status	No	173	97.2%	5	2.8%	178	84.8%
	Yes	31	96.9%	1	3.1%	32	15.2%
Child mental problem	No	190	97.4%	5	2.6%	195	92.9%
	Yes	14	93.3%	1	6.7%	15	7.1%
Total		204	97.1%	6	2.9%	210	100.0%

Discussion

Several research studies has noted, toxoplasmosis is food-borne infectious which is leading cause of death ranked in third after salmonellosis and listeriosis [19]. Though it is highly neglected, congenital toxoplasmosis particularly is a major problem in most communities with a high prevalence of *T. gondii* infection. Epidemiological studies reporting prevalence of *T. gondii* infection in pregnant women across the world suggest considerable variation between countries ranging from 9–67% in Europe and reaching as high as 92.5% in African countries [6]. Thus, conducting a study of the seroepidemiology of this

infection among females of childbearing age could provide appropriate approaches which could help to design the preventive measures [3].

The present study showed a seroprevalence of anti-*T. gondii* IgG antibody of 53.6% among 210 pregnant women in Asmara. Even though these pregnant women were not at risk for toxoplasmosis, because they have already developed IgG antibodies, the remaining 46.4% are at risk of the infection. Also the seroprevalence of toxoplasmosis IgM antibody was noted to be 2.9%, which is lower than many countries. In Saudi Arabia, the prevalence of *T. gondii* during pregnancy for IgG and IgM antibodies were 32.5% and 6.4% respectively [14].

As it was indicated, the study was done in pregnant women in Asmara. Asmara is the capital city of Eritrea where majority of its habitants have better access to different social service such access to clean drinking water, hygiene and sanitation, education and health [15]. Had it been in other rural parts of the countries where access to social services is relatively low, the seroprevalence would have been skyrocketed.

Relatively, the result of seroprevalence IgG antibody in this study done among the pregnant women was 53.6%, which is, comparatively higher than others studies done in other countries. For instance, prevalence was 30.9% in Tanzania [20], Eastern China 15.2%, [6], in Sudan 27% [9], and France 43.8% [21] and comparatively lower than other countries like Cameron 77.1% in 1992, Ethiopia 80.0% in 1998, Argentina 72% in 2001, Indonesia 58% in 2000 [22] and in the city of Kinshasa, D.R of Congo 80.3% [23].

Though, the result revealed that there was no association between seropositivity of toxoplasma antibody and different age groups, the seropositivity rate in 35–44 years age group was obviously higher than other two groups. Despite small variation in age group, the result is in line with studies from other countries [20, 24]. However, in the comparative study done between pregnant women in Malaysian and Myanmar has identified that *Toxoplasma* seropositive was found to have an association with aged 30 years and above [18]. The same Malaysian study conducted among the pregnant women has also identified that mothers' education, gestational age, gravida, disease awareness, obstetrics history, having contact with cats or soil, consumption of undercooked meat, unpasteurized milk or untreated water have a relation with toxoplasmosis. Though this is the first study ever done on Toxoplasmosis in the country, the result showed that the seroprevalence of *Toxoplasma* infection was considerably high (53.6%) using IgG antibody. When IgM antibody was considered, the disease prevalence was noted to be 2.9%. It has been noted that the areas of the study was in Asmara where the exposure to risk factors is relatively minimal. Thus, even considering this, the result was higher which requests an attention. Though some of the risk factors had included in the study, test result has showed that except soil contact, none of them had any significant association with the disease pattern.

Conclusion

The absence of a statistically significant relationship between the prevalence of *Toxoplasma* infection and many of the factors included in this study, does not necessarily mean that these factors have no

influence on the transmission of toxoplasmosis. This could be due the test method where it demands higher number of disease cases. Probably a larger sample size would have revealed significant relationships. However, in this case some of the cases were lower when results were further segment among the various socio-demographic characters and causal factors.

Declarations

Conflict of interest: We declare that we have no conflict of interest.

Ethical Issues: In this study, ethical considerations have been fully observed.

Acknowledgment: We would like to acknowledge the staff of Antenatal Care (ANC) in Asmara City, Eritrea and all the patients who participated in the study.

Authors' Contribution: AST and AAT designed and did data collection. AST, AAT and KAM did writing of the original draft. KAM and ADA did editing and reviewing of the original article.

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