Asian Journal of Research in Infectious Diseases

Volume 12, Issue 1, Page 24-32, 2023; Article no.AJRID.66842
ISSN: 2582-3221

Acute Toxoplasmosis among Pregnant Women Attending Antenatal Clinic in Kaduna State

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Authors' contributions

This work was carried out in collaboration among all authors. Author IUE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors GI and MK managed the analyses of the study. Author EII managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRID/2023/v12i1235

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/66842

Original Research Article

Received: 20/01/2021
Accepted: 29/03/2021
Published: 17/01/2023

ABSTRACT

Introduction: Acute infection of *Toxoplasma gondii* can be transmitted during pregnancy to the foetus vertically which may cause congenital complications like abortion, stillbirth, visual impairment, seizure, hearing impairment and neurological disorders.
Methodology: A total of 357 pregnant women were screened using ELISA method for acute *Toxoplasma gondii* (IgM).
Result: The investigation however shows a prevalence of 2.8% (IgM). Ages 16-20 and 26-30 years have the highest prevalence of 3(0.8%) positive. While ages 21-25 and 31-35 years have prevalence of 2(0.6%) positive. However ages 36-40 years are all negative. (p> 0.05).This did not show any statistical significant with the age groups. Northern Senatorial zone has the highest prevalence of 8(2.6%) followed by the Central Senatorial zone with 2(0.2%) while the Southern zone shows no acute Toxoplasmosis,(p <0.05) hence it shows statistical significant. Women in their second trimesters have the highest prevalence of 7(2.0%) followed by first trimesters with

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prevalence of 2(0.6%) and third trimester with prevalence of 1(0.2%) positive, however it is not statistical significant ($p > 0.05$).
Conclusion: Therefore the chance of acquiring acute infection of *T. gondii* is possible during pregnancy and would have potential tragic outcomes for the mother and new-born despite the fact that it can be prevented. The need for aggressive awareness and necessary facilities available for screening of *T. gondii* during antenatal clinic is necessary.

Keywords: Toxoplasmosis; IgM; serum; trimesters.

1. INTRODUCTION

Toxoplasmosis is a zoonosis, caused by the obligate intracellular protozoan [1,2]. This disease poses major public health challenge in congenital infections causing seizure, mental retardation, hearing impairment and visual loss, it is however transmitted to humans by ingestion of oocysts, or through accidental ingestion of sporulated oocysts from the environment [3,4]. Alternatively, it can result from consumption of water or food contaminated by oocysts excreted in the faces of infected cats [5,6].

The disease is an important food-borne pathogen and may also be transmitted by blood and blood products, organ transplants or by the ingestion of tachyzoites in unpasteurized milk [7,8]. In fact, toxoplasmosis was once a leading infectious cause of food-borne death after Salmonellosis and listeriosis in the USA [9]. Among several domestic animals cat is the definite host and play significant role in the spread of toxoplasmosis because they are the only animals that excrete resistant oocysts into the environment [10]. However pigs, cattle, sheep, goats and rodents may play role in its transmission. Rats and mice are thought to be persistent wildlife host reservoirs of *T. gondii* [10,11]. One of the major challenges of the parasite in human is once they are infected with the parasite, they continually harbor the organism throughout life since human defence mechanisms cannot eliminate the cyst of Toxoplasma [12].

Globally approximately 10% of congenital Toxoplasma infections result in abortion or neonatal death. In 10-23% of congenital infections, signs are present at birth; these may include hydrocephalus, chorioretinitis, hepatosplenomegally, and microcephally [12]. Clinical signs of congenital Toxoplasma infection are not apparent at first in 67-80% of cases [13]. A significant proportion of encephalitic patients can also present with neuropsychiatric disorders including psychosis, dementia, anxiety, and personality disorder [14]. Ocular toxoplasmosis may occur in up to one third of children that survive congenital infection and is the most common cause of intraocular inflammation in the world [15].Hearing loss has also been reported in 10%-30% and developmental delay in 20%-75% of this group of patients [16]. Seroprevalence varies considerably high up to 50% with countries where raw meat is commonly eaten and in tropical regions of Latin America or Sub-Saharan Africa where cats are numerous and the climate is favourable for oocysts survival [17].

The chance of acquiring acute infection with *T. gondii* is high during pregnancy and the infection would have potential tragic outcomes for the mother, the foetus and new-born despite the fact that it can be prevented [18]. Inspite of the wide practice of keeping cats as domestic animals and presence of stray cats around, and suitable climatic conditions favoring survival of the parasite in the study area, to our knowledge, there is no regular serological screening of pregnant women for *T. gondii* infection [19]. Research has been shown that over 90% of women who contract *T. gondii* infection remain asymptomatic and spontaneously recover, only a small proportion will develop clinical signs of the disease [20,21].

The clinical presentation in pregnant women is not more severe than in non-pregnant women and most often occurs as an influenza-like illness with an incubation period of 5-18 days following exposure [22]. Seroprevalence varies greatly in geographical regions within a country and within different ethnic groups according to different environments, social customs, and habits of different populations [23-26].

Epidemiological studies suggest that prevalence of *T. gondii* infection in pregnant women varies greatly among different countries with prevalence estimates from US studies having a range of 3%–42%, Britain 22%, Netherland 80%, Korea 3.7%,Sudan 34.1%, Senegal 40.2%, NewZealand 33%, Iran 38.1%, Ethiopia 93.3% Dutch 26% and 41.6-66.9% in other Asian countries such as India and Jordan [27-35,17]. In Southern Turkey anti-Toxoplasma IgG and IgM

antibody was found to be 52.1% and 0.54% respectively. Therefore, infections due to *T. gondii* are considered a worldwide zoonosis of great public health importance [36,37].

Worldwide prevalence rate of latent *Toxoplasma* infections in HIV-infected patients varies greatly from 3% to 97% [38,19]. In sub-Saharan Africa, toxoplasmosis often remains undetected and untreated due to insufficient diagnostic procedures [39]. Several studies have shown a consistently high *T. gondii*-seroprevalence for this region, ranging from 35% to 84% in different African countries south of Sahara [28].

In Nigeria the seroprevalence rates of toxoplasmosis by serological investigations have been estimated from 7% to 51.3% in normal pregnant women to 17.5% to 52.3% in women with abnormal pregnancies and abortions, while in Lagos 16.7% prevalence was reported for IgM antibodies in First trimester and 46.7% for IgG at third trimester. A study conducted in Zaria also reported prevalence of 29.1% for chronic and 0.8% for acute infections respectively. [40-42,38].

However, despite the recognized public health importance of *T. gondii* in different parts of the world, studies on the prevalence of toxoplasmosis among people and congenital disease danger posed on neonate and children there is no measures taken for prevention on pregnant women and even children who are venerable to the disease in Nigeria

2. MATERIALS AND METHOD

2.1 Study Area

The study was a cross sectional study carried out in some selected Hospital in Kaduna State, spread across the three Senatorial political Zones. Kaduna State is a old Capital of Northern Nigeria, is located in the north-western geopolitical zone of Nigeria and lies between Longitude 605 and 838 east of Greenwich meridian and latitude, 903 and 1132 north of equator, with average annual temperature of 25.2°C/77. 4°F. It has an estimated population of six million people with a total land mass estimated at 46,020 sq Km in 23 local Government Areas. It shares borders with Zamfara, Katsina, Kano, Bauchi, Plateau, Nasarawa Niger States and Abuja [43].

2.2 Inclusion Criteria

Pregnant women of all ages at all trimesters.
Those attending antenatal clinic in Government.
Hospitals selected in Kaduna State.
Those that gave consent for the investigation.

2.3 Exclusion Criteria

Non pregnant women.
Those not attending antenatal in Government.
Hospitals selected within Kaduna State.
Those that decline consent for the investigation.

2.4 Study Population

Multistage sampling was used in the selection of the study hospitals one each in the three Senetorial district of Kaduna state using random sampling method. In view of the above Gambo Sawaba general hospital in the northern senatorial district, Yusuf Dan Tsoho general hospital in the central senatorial district and Kafanchan general hospital in the southern senatorial district were selected.

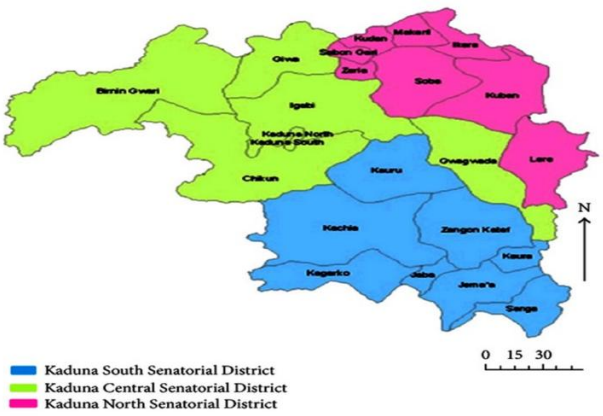

Fig. 1. The study population was pregnant women attending antenatal clinics in some selected State General Hospitals in the three senetorial district of Kaduna State

2.5 Sample Size

The sample size was calculated using the descriptive studies formula [38].

$$n = \frac{z^2pq}{d^2}$$

Where the P = Value of proportion of interest (If no information is known about p then p= 0.5).

A prevalence of 29.1% was used for the calculation [38].

d= Tolerance eg: within 0.05

Hence:

$$\{1.96^2 \times 29.1/100(1-29.1)\}/0.05^2 = 317 \text{ samples}$$

Therefore a total of 349 samples was collected across the three geopolital zones of Kaduna State due to 10% additional anticipated non response rate and to minimize sampling error.

2.6 Materials

The materials used are Vacutainers, serum microtubes, cotton wool, methylated spirit, specific Toxo- IgM EIA Kits, micropippetes of different sizes, distilled water, absorbent paper, micro-titer plate, strip well washer and micro-plate reader with 450 nm wavelength and structured questionnaire.

2.7 Statistical Analysis

Results and data from questionnaires were analysed using the SPSS (version 16 and the Pearson Chi square test at 95% confidence interval and a significance level of 0.05 was used to determine the relationships between the variables and seroprevalence rate.

2.8 Sample Collection

Five millilitres of blood was collected by a qualified Medical laboratory Scientist/Technician via the ante cubical vein by applying torniquet on either of the arm for visibility of the vein then swapping the area with cotton wool soaked in alcohol after which using sterile vacutainer/syringe and a needle is pierce into the vein for blood collection and then transfer the blood into a sterile plain tube and EDTA tubes 2.5 ml each and labelled appropriately. The blood in the plain container was centrifuged at 3000 rpm for 5 minutes and the sera was harvested into clean cryovials and stored at -20^{o}C until it is required for use.

2.9 Serology

Calbiotech (A Life Science Company) USA Commercial Enzyme Linked immunosorbent assay (ELISA) kits specific for *Toxoplasma gondii* IgM were used according to manufacturer's instruction.

2.10 Principle

Calbiotech *Toxoplasma gondii* IgM kit is ELISA based. Sample were incubated with mouse monoclonal antibody against human IgM bound to the solid surface for a microtitre well. Patient IgM is captured by the surface bound antibody. Unbound serum component are washed away,patient anti*toxoplasma gondii* IgM antibodies are detected and bound by an immunocomplex enzyme conjugate, consisting of *Toxoplasma gondii* antigen which is conjugated to horse radish peroxidase. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated in the presence of bound enzyme the substrate is converted to end product. The absorbance of this end product is read spectrophotometerically at 450 nm and is directly proportional to the concentration of IgM antibodies to *Toxoplasma gondii* antigen present in the sample.

2.11 Questionaire Administration

The patient's demographic information were collected using a designed structured questionnaire. The study was however explained to the patients and informed consent obtained before administering the questionnaire. In order to ensure confidentiality, names of patients were not recorded. The questionnaire was interpreted in local language for those who could not understand English.

3. RESULTS AND DISCUSSION

Acute infection of *Toxoplasma gondii* can be transmitted during pregnancy to the foetus vertically which may cause congenital complications like abortion, stillbirth, visual impairment, seizure, hearing impairment and other neurological disorders [44]. This study observed 2.80% prevalence of Toxoplasmosis IgM antibodies similar to the work that reported 3.9% cases in India, 3.26% in Brazil, 2.6% in Gabon, 2.4% in NewZealand, and 0.8% in Zaria,

[45-49,30,38). The findings in this study is however not similar to studies of 13.08% reported in Kano, 5.2% in Qatar, 11.5% in Portharcout, 11.9% in Trinidad Tobago, 5.4% in Gabon, 7.6% in Lagos, and 7.2% in Maiduguri [50-53,42]. The difference in the various prevalence rates could be due to geographical location, climate condition, and cultural behaviour even within same country because the parasite oocyst sporulation is prevalent in warm and humid condition [54].

The observed prevalence of IgM antibodies in the age group 16-20 and 26-30 years is in agreement with Kefale et al. [19], who reported 20%, prevalence in 15-19years and Ballah et al. [55] who reported < 20 years 52.86%. This may be attributed to several factors which could have been responsible for variation among the different age groups. Some of which may include the level of maturity, personal hygiene and socio-economic status of the family which is common in Northern Nigeria. The high prevalence of Toxoplasmosis in the Northern Senatorial zone relative to the other zones may be attributed to the practice by most household in the area of domesticating animals in their compound and keeping of cats as pet, which is in agreement with the findings by Ishaku et al. [38], who reported that 70.1% of ante-natal women in Zaria are susceptible to primary infection compare with other zones. Other reasons that could have accounted for the sero-prevalence in the different Senatorial zone could be due to the geographical and climatic condition of those areas. This is also in agreement with findings by Ogo, [54] who reported that Hausa/Fulani ethnic group who are predominant have the highest sero-prevalence among the major ethnic groups living in the Northern Senatorial district. The practice of keeping pets observed among those living in the Northern Senatorial zone is also a factor that can account for the prevalence of toxoplasmosis in the Northern senatorial district which is a major reserviour of the parasite [38,55].

The study reveals that women in their second trimesters have the highest prevalence of 7(2.0%) followed by first trimesters with prevalence of 2(0.6%) and then the third trimester with prevalence of 1(0.2%).This is in agreement with findings by Ballah et al. [55], Malarvizhi et al. [49] and Ishaku et al. [38] who reported a prevalence of 33.5%, 4.0% and 63.9% respectively in pregnant women during the second trimester. This could attributed to the fact that usually pregnant women don't attend antenatal in their first trimester due to cultural believe, therefore most details concerning pregnancy can only be gotten during their second trimester when they must have started their antenatal clinic. First trimester is also associated with several challenges due to the physiological changes of the woman which include nausea and general body weakness that may influence their level of hygiene, this could account for high prevalence of toxoplasmosis in the first trimester than the third trimester.

Table 1. Prevalence of *Toxoplasma gondii* (IgM) in pregnant women based on age group in Kaduna state

Age Group(year)	No. Examined	IgM pos (%)	IgM neg (%)	*P-Value*
16-20	47	3(0.8)	44(12.3)	0.630[a]
21-25	113	2(0.6)	111(31.1)	
26-30	106	3(0.8)	103(28.9)	
31-35	63	2(0.6)	61(17.1)	
36-40	22	0(0.0)	22(6.2)	
41-45	6	0(0.0)	6(1.7)	
Total	**357**	**10(2.8)**	**347(97.2)**	

KEY: a = Pearson Chi-square test, Pos = Positive, Neg= Negative, % = Percentage

Table 2. Prevalence of *Toxoplasma gondii* (IgM) in pregnant women based on senatorial zones of Kaduna state

Senatorial zone	No. examined	IgM pos(%)	IgM Neg(%)	*P-value*
Northern zone	119	8(2.2)	111(31.1)	0.003[a]
Central zone	119	2(0.6)	117(32.8)	
Southern zone	119	0(0.0)	119(33.3)	
Total	**357**	**10(2.8)**	**347(97.2)**	

KEY: a = Pearson Chi-square test, Pos = Positive, Neg= Negative, % = Percentage

Table 3. Prevalence of *Toxoplasma gondii* (IgM) in pregnant women based on trimesters

Trimesters	No. examined	IgM (pos%)	IgM neg(%)	*P-value*
First trimester	33	2(0.6)	31(8.7)	0.218[a]
Second trimester	216	7(2.0)	209(58.5)	
Third trimester	108	1(0.2)	107(30.0)	
Total	**357**	**10(2.8)**	**347(97.2)**	

KEY: a = Pearson Chi-square test, Pos = Positive, Neg= Negative, % = Percentage

Toxoplasmosis infection during the first trimester could be very harmful to the fetus because after maternal acquisition of *T*. *gondii* for the first time during gestation the parasite might enter the fetal circulation by infection through the placenta. This may result in severe congenital toxoplasmosis and can result in death of the fetus and can also result in spontaneous abortion [56].

4. CONCLUSION

A prevalence of 2.80% of toxoplasmosis was observed in the study with pregnant women in the Northern Senatorial district have a high prevalence of toxoplasmosis relative to the other senatorial districts. Pregnant women aged 16-20 years have high prevalence of toxoplasmosis , in the same vein there was high prevalence of toxoplasmosis during the second trimester.Therefore the chance of acquiring acute infection of *T. gondii* is obvious during pregnancy having potential tragic outcomes for the mother, and new-born despite the fact that it can be prevented.

ETHICAL CONSIDERATION

The ethical permission was obtained from the Kaduna State Ministry of Health Review Ehical Committee in a letter with reference number: MOH/ADM/744/VOL. 1/527 before sample was collected from the hospitals.

CONSENT

The study was however explained to the patients and informed consent obtained before administering the questionnaire

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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