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Original Research Article

Seroprevalence and Risk Factors of Toxoplasmosis among Pregnant Women Attending Antenatal Clinic in Kaduna Metropolis and Environs

ABSTRACT

Toxoplasmosis due to infection by *Toxoplasma gondii is* estimated to affect one third of the global population. In pregnancy, *T. gondii* infection represents the risk to induce miscarriage and congenital transmission. This study was therefore conducted to determine the seroprevalence of anti-*T.gondii*IgM antibodies among pregnant women attending antenatal clinics in Kaduna south, Nigeria. Enzyme-linked imuunosorbent assays (ELISA) was used and structured questionnaire to obtain information on risk factors associated with infection among pregnant women attending antenatal clinics at GwamnaAwam General Hospital Kakuri and Yusuf Dantsoho Memorial Hospital Tudun Wada in Kaduna State. A total of One hundred and ninety two (192) blood samples (96 from each of the selected hospital) were collected from the pregnant women and screened using specific anti-*Toxoplasma gondii*IgM antibodies kit (IBL- International GMBH a Tecan Group Company). Result obtained from the 192 women serum analysed showed an overall seroprevalence of 31.3% using *T. gondii* specific IgM antibodies. Seropositivity was found to be statistically associated with age ($P<0.05$) while Relative Risk (RR) analysis showed that women aged between 31-35 yrs. were found to have higher risk of contracting toxoplasmosis than all other age groups (RR 4.620). Chi-Square test showed that pregnancy stages and previous history of miscarriage of pregnant women were found to significantly influence prevalence of *T. gondii*infection ($P<0.05$). Women with previous history of miscarriage was found to be more likely to be infected with toxoplasmosis than women with no history of miscarriage (RR= 1.278). The results of this study justifies the need to include laboratory testing for *T. gondii* infection during antenatal investigations and to educate women about the parasite in order to prevent maternal and subsequent congenital infections that might have been provoked *in-utero*.

Keywords*: Toxoplasma gondii; Seroprevalence; antibodies;risk factors; pregnant women*

1. INTRODUCTION

Toxoplasma gondii is an obligate intracellular protozoan parasite that causes toxoplasmosis in man and other warm blooded animals. The coccidium parasitizes member of the cat family as its definitive host and has a wild range of intermediate hosts including humans [1]. Globally, about one third of the world's human population is estimated to carry *Toxoplasma gondii* parasite [2]. Toxoplasmosis is one

UNDER PEER REVIEW

of the most prevalent and widespread parasitic infections, it is often neglected especially in developing countries. Although it has long been reported to be widespread in many countries in West Africa [3]. The infection is usually mild and asymptomatic causing cold and flu in immunocompetent individuals but can rise to cervical lymphadenopathy, mononucleosis-like syndrome or inflammation of the choroid in up to 20% of infected persons; and in immune deficient individuals, such as acquired immune deficiency syndrome (AIDS) patients. Primary or reactivated infection can also result in encephalitis, pneumonia and inflammation of the heart muscles [4]. Toxoplasmosis has been reported as the commonest opportunistic infection in HIV/AIDS patient in developed and developing countries[5]. Worldwide, toxoplasmosis is reported to occur in all countries with seropositivity rates ranging from less than 10% to over 90% [6].

Association of toxoplasmosis to congenital impairment has recently been affirmed [7]. Consequences of primary congenital infection in pregnant women with normal immunity include miscarriages, still birth, blindness, deafness, mental retardation, microcephalus, hydrocephalus and other neurological diseases in the fetus[8]. Toxoplasmosis also enhances mother-child transmission of Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV) [9].

Approximately, half a billion humans have antibodies to *Toxoplasma gondii*)[10]. Authors such as [11, 12,13], reported that about 30-40% adult humans in the United States have antibodies to *T. gondii*. Apparently, a healthy immune system may keep the parasite in check [14]. Considerable variations in seroprevalence of toxoplasmosis have been reported in diverse regions and in different individuals which are mainly related to factors that expose the population to the infected cysts. Age, occupation, sanitary conditions, educational status, contact with cat, soil, ruminate animals and location have been the main determinant risk factors of infection by *T. gondii*15].

Infection due to *Toxoplasma gondii* is a worldwide zoonosis. The organism infects herbivorous, omnivorous and carnivorous animals, including birds [16, 17]. Two main routes of transmission have been described in humans viz: oral-ingestion of the parasite (cyst) and through placental transmission to the fetus in pregnant women. The oral routes involve the ingestion of raw or undercooked meat that contains cysts or the ingestion of water or food contaminated with oocysts[18, 19, 20]. Infection of some women with the habit of tasting raw meat such as paste from chopped pork for meat balls

UNDER PEER REVIEW

during cooking has been reported [21]. Transmission through contaminated soil is also possible in humans by accidental ingestion of soil contaminated with *T. gondii*oocysts.

In human infection, *t. gondii* elicit immune response which can be detected by several serological tests including enzyme linked immunosorbent assay (ELISA). Immunoglobulin G (IgG) antibodies usually appear within 1–2 weeks of acquisition of the infection, peak within 1–2 months, decline at various rates, and usually persist for life. It has been observed that the functional affinity of specific IgG antibodies is initially low after primary antigenic challenge and that it increases during subsequent weeks and months by antigen-driven B cell selection. Protein-denaturing reagents including urea are used to dissociate the antibody-antigen complex.

Immunoglobulin M (IgM) antibodies may appear earlier and decline more rapidly than IgG antibodies. An IgM test is still used by most laboratories to determine if a patient has been infected recently or in the distant past. In people with recently acquired primary infection, *T. gondii*– specific IgM antibodies are detected initially, and in most cases, these titers become negative within a few months. However, in some people, positive *T. gondii*–specific IgMtiters can still be observed during the chronic phase of infection [22]. Some investigators have reported that IgM antibodies can be detected as long as 12 years after the acute infection [23]. The persistence of these IgM antibodies does not appear to have any clinical relevance, and these patients should be considered chronically infected.

Immunoglobulin A (IgA) antibodies may be detected in sera of acutely infected adults and congenitally infected infants and may persist for many months or more than a year [12]. Immunoglobulin E (IgE) antibodies are detectable in sera of acutely infected adults, congenitally infected infants, and children with congenital toxoplasmicchorioretinitis[24, 25]. The duration of IgEseropositivity is shorter than IgM or IgA antibodies and hence appears useful for identifying recently acquired infections[26,25]

2. MATERIALS AND METHODS

2.1 Study Area

The research was conducted in Kaduna metropolis,Kaduna metropolis is the capital of Kaduna State. It is located between latitudes10^0 22^I 00^{II} - 10^0 40^I 00^{II} N and longitudes 70^0 20^I 00^{II} – 70^0 28^I 00^{II} E

UNDER PEER REVIEW

[27]. The metropolis occupies an area of about 260 km^2, and the distance between the eastern and western limits of the city is approximately 13.7 km.

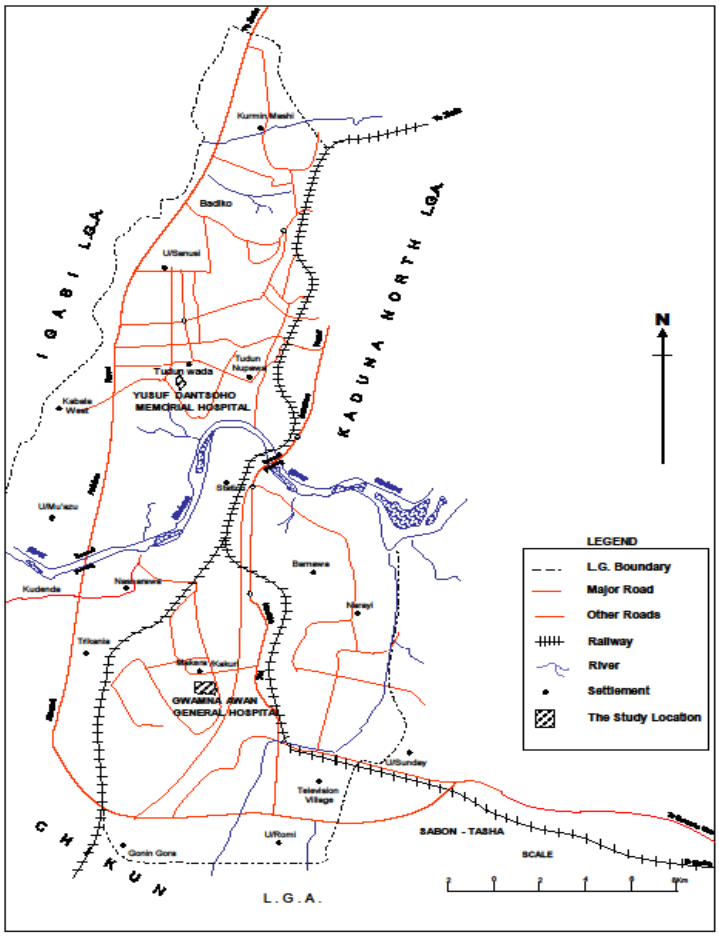

FIG. : KADUNA SOUTH LOCAL GOVERNMENT AREA SHOWING THE STUDY LOCATION.
Source : Geo. Dept. NDA, Kaduna (2017)

Figure 1: Map of Kaduna South LGA showing the study location.

2.2 Study Design

The research is a descriptive cross sectional study. Women attending antenatal clinics of GwamnaAwam General Hospital Kakuri and Yusuf Dantsoho Memorial Hospital Tudun Wada were recruited for the study. All women were interviewed using a structured questionnaire with relevant information recorded for each woman. The questionnaires were administered to obtain information on important variables such as age, stage of pregnancy, occupation, and educational status of the women

UNDER PEER REVIEW

2.3Selection of Hospitals

GwamnaAwam General Hospital, Kakuri and Yusuf Dantsoho Memorial Hospital, Tudun Wada Kaduna located in Kaduna South LGA of Kaduna State were selected by simple random techniques. The 2 hospitals provide medical and antenatal services to inhabitants of Kaduna metropolis and environs.

2.4Target Population

The targeted population for the study were pregnant women of all ages attending antenatal clinic at GwamnaAwam General Hospital, Kakuri and Yusuf Dantsoho Memorial Hospital, Tudun Wada within Kaduna metropolis and environs.

2.5 Sample Size

One hundred and ninety two (192) samples (96 from each of the selected hospital) from pregnant women attending antenatal who have voluntarily given their consent to participate in the study was used.

2.6 Ethical Permission

Approval to conduct the research was obtained from the Kaduna State Ministry of Health Ethical Committee before sample collection commenced in the 2 State hospitals. The aim of the study was also clearly explained to pregnant women attending antenatal clinic in the selected health facilities in order to get their informed consent. Women who do not consent were excluded from the study. To ensure confidentiality of subjects, names of respondents were not recorded during sample collection. Code of ethics on the use of human subjects in research as outlined in the NDA Research and Development Policy, 2016 was also adhere to strictly.

2.7 Questionnaire Administration

Information on the patients' age, previous abortion, and stage of pregnancy were collected at the point of sample collection. The questionnaire was interpreted in local language for those who could not understand English.

2.8 Blood Sample Collection

UNDER PEER REVIEW

Blood samples were collected from participants at the public health facilities with the help of medical personnel of the hospitals by venupuncture. Samples collected from each subject was transferred into a sterile labelled specimen bottle. Samples collected were then centrifuged at 1000rpm for 3 minutes to separate serum from blood cells. Serum sample obtained and stored in a thermox box were separately transferred to labelled fresh sample bottles, and later transported to Veterinary Teaching Hospital Laboratory (VTH/LAB), Faculty of Veterinary Medicine, Ahmadu Bello University Zaria for analysis. Serum samples were kept at – 200°C until analysed.

2.9Enzyme-Linked Immunosorbent Assay

Commercial Kits for specific anti-*Toxoplasma gondii*IgM antibodies were purchased and was used according to the Manufactures instruction (IBL- International GMBH a Tecan Group Company).

2.10Detection of *Toxoplasma gondii* Antibody

Antibody ELISA technique was employed, strictly as instructed by manufacturer. One in forty (1:40) dilutions of specimen, negative control, positive control and calibrator were prepared by adding 5µl of the aforementioned separately to 200µl of sample diluent and mixed well. One hundred microliter (100µl) of diluted sera, calibrator and controls were dispensed into appropriate wells. One hundred microliter (100µl) of absorbent solution was dispensed in 1A well position for reagent blank. The holder was tapped to remove air bubbles from the liquid and was mixed well and incubated for 30 min at room temperature. Liquid from each of the wells was removed and washed three times repeatedly with washing buffer. One hundred microliter (100µl) of enzyme conjugate was dispensed into each well and incubated for 30 min at room temperature.The enzyme conjugate was then removed from all the wells and washed repeated three timeswith washing buffer. One hundred microliter (100µl) of TMB Chromogenic Substrate was then dispensed to each well and again incubated for 15 minutes at room temperature. To stop the reaction, 100µl of 2NHCl was added and results was read using an automated ELISA machine.

2.11 Statistical Analysis

Descriptive statistical analysis was performed using SPSS 17.0. All statistical analyses were carried out using a 95% confidence interval, a significance level of $P < 0.05$ and Chi-Square test of

UNDER PEER REVIEW

independence was carried out for all contingency table. Relative risk (RR) was evaluated for significant risk factors. Questionnaires administered were evaluated to determine the correlation between seroprevalence and several risk factors using Two-tailed test of significance.

3. RESULTS

The overall prevalence of anti-toxoplasma antibodies detected by ELISA is presented in Table 1. Out of 192 pregnant women sampled from GwamnaAwam General Hospital Kakuri and Yusuf Dantsoho Memorial Hospital T/Wada, 31.3% showed evidence of toxoplasma infection. The highest prevalence of 39.6% was recorded among pregnant women at Yusuf Dantsoho Memorial Hospital T/Wada while relatively lower prevalence (22.9%) was recorded among women that attended antenatal clinic at GwamnaAwam General Hospital Kakuri. Statistical analysis indicated significant differences in seroprevalence of toxoplasmosis among pregnant women in the 2 health facilities ($P < 0.05$).

Table 2 showed the seroprevalence of toxoplasma infection based on age of subjects sampled. The highest evidence of toxoplasma infection was recorded in 31-35yr. age group with a seroprevalence of 67.7%. This was followed by women within 21-25, > 40 and 36-40 yr. age groups with seroprevalences of 34.6%, 28.5% and 27.8% respectively. The least prevalence (17.9%) of toxoplasma infection was recorded in pregnant women aged below 20 yrs. Among the different age groups, women aged between 31-35yr. age group were found to have higher risk of contracting toxoplasmosis than all other age groups (RR 4.620).

Out of the 192 pregnant women tested, 64 were in their 1st trimester, 96 in 2nd trimester and 32 in 3rd trimester (Table 3). Pregnant women in their 2nd trimester showed the highest evidence of toxoplasma infection with seroprevalence of 35.4%, followed by women in their 3rd and 1st trimesters with seroprevalence of 31.3% and 25.0%. Statistical analysis showed a significant difference between trimesters with respect to anti-toxoplasma antibodies ($P < 0.05$).

From a total of 192 pregnant women examined, 149 had no history of miscarriage, 33 had 01 case of miscarriage while 10 have had more than 1 case of miscarriage (Table 4). The highest seroprevalence of toxoplasma infection was recorded in women who had one (1) history of miscarriage once (48.5%), followed by women that had more than 1 miscarriages in their life time with

UNDER PEER REVIEW

178 seropositivity of 40%. However, pregnant women with no history of miscarriage had significantly lower
179 seroprevalence of 26.8%. Chi-Square analysis indicated a strong association between previous
180 history of abortion and the presence of antibodies to toxoplasma infection ($P < 0.05$). Women with
181 previous history of miscarriages are about 2x more likely to be infected with toxoplasmosis than
182 women with no history of miscarriage (RR= 1.278)

Table1: Overall Prevalence of Toxoplasmosis

Health Facility	Number of Subject Examined	Number of Subject Positive	Percentage (%) of Subjects Positive
GwamnaAwam General Hospital Kakuri	96	22	**22.9**
Yusuf Dantsoho Memorial Hospital Tudun Wada	96	38	**39.6**
Total	**192**	**60**	**31.3**

X^2 = 14.267 Df = 1 *P*-value = 0.039

UNDER PEER REVIEW

Table 2: Seroprevalence of *T. gondii* in Relation to Age of Sampled Women

Age	Number of Subject Examined	Number of Subject Positive	Percentage (%) of Subjects Positive	Relative Risk (RR)
<20	39	7	17.9	0.481
21-25	26	9	34.6	1.165
26-30	71	16	22.5	0.640
31-35	31	21	67.7	4.620
36-40	18	5	27.8	0.846
>40	7	2	28.5	0.880
Total	**192**	**60**	**31.3**	

$X^2 = 25.600$ Df = 5 *P*-value = 0.00

UNDER PEER REVIEW

UNDER PEER REVIEW

Table 3: Seroprevalence of *T. gondii* among Pregnant Women Based on Pregnancy Stages

Pregnancy stage	Number of Individuals Examined	Number of Individuals Positive	Percentage (%) of Individuals Positive
1st trimester	64	16	25.0
2nd trimester	96	34	35.4
3rd trimester	32	10	31.3
Total	**192**	**60**	**31.3**

X^2 = 15.600 Df = 2 *P*-value = 0.00

Table 4: Relationship of *T. gondii* antibodies and previous history of miscarriage among sampled pregnant women

History of Miscarriage	Number of Subjects Examined	Number of Subjects Positive	Percentage (%) of Subjects Positive	RR
None	149	40	26.8	0.553
Once	33	16	48.5	1.213
Twice	10	4	40	1.278
Total	**192**	**60**	**31.3**	

X^2 = 33.600, Df = 2, *P*-value = 0.00

UNDER PEER REVIEW

4. DISCUSSION

This study revealed the presence of IgM to *T. gondii* in pregnant women attending antenatal clinic at some health facilities in Kaduna metropolis and environs. In *T. gondii* infection IgM and IgG are the 2 main immune response exhibited by the host. The presence of IgM detected among the pregnant women sampled is indicative of either a recent *T. gondii* infections or reactivated dormant toxoplasmosis [28].

The overall seroprevalence of 31.3% recorded in this study is relatively higher than that reported by [29] who recorded a prevalence rate of 22.2% in Maiduguri municipal council, Brono state Nigerian in a study of toxoplasmosis carried out among pregnant women. However, [28] in a similar study recorded 29.9% prevalence in Zaria, Kaduna state. Relatively lower prevalence of *T. gondii* infection (18.5%) reported by[30] among pregnant women attending antenatal care at FelegeHiwotReferral Hospital, Northwest Ethiopia despite a higher seroprevalence (83.6%) was earlier reported by [31] among pregnant women. Conversely, higher seroprevalence of 63.5% was reported in Colombia (32] and 77.5% in Brazil [33]. Higher prevalence were also reported in some neighbouring African countries from previous studies conducted with prevalence of 63.1% in São Tome and Principe [34], 34.1% in Sudan [35], 36.3% in Madagascar [36], 37.5% in Somalia [37]and in Ethiopia; 90% from HIV infected and HIV uninfected individuals [38]; 75% from a survey carried out in general population [39].The detection of antibodies to *T. gondii* in many African countries including Nigeria underscore the need for continuous education of women of child-bearing age on the consequences of the infection on their unborn babies and ways of preventing toxoplasmosis.

The variation in the prevalence of *T. gondii* with age of women tested has been affirmed by many author [40,41, 42,43].The present also demonstrate the effect of age on the prevalence of *T. gondii*infection among antenatal women, with the risk of exposure to *T. gondii*infection increasing with age. In the present study the highest percentage seropositivity (67.7%) was recorded among the 31-35yrs age group with a Relative Risk (RR) of 4.620 and lowest percentage positive (17.9%) per age group found among women <20 yrs (RR= 0.481).[44] Recorded 81.4% among women of child bearing age. Similarly, to the study of Zemene*et al* [31] that observed a significant association between seroprevalence of toxoplasmosis and age of individuals studied. Several intrinsic and extrinsic factors

UNDER PEER REVIEW

could have been responsible for variation among the different age groups. Some of which may include the level of personal hygiene and socio-economic status of the family, thus highlighting the need to continue to educate women of child-bearing age on prevention of toxoplasmosis.

The relatively high seroprevalence (35.4%) of toxoplasma antibodies found in pregnant women at their second trimester of gestation period in the present study is in agreement with a study in Ethiopia by [45]. The authors recorded the highest seroprevalence of toxoplasma antibodies in pregnant women in their second trimester.

5. CONCLUSION

The seroprevalence of recorded toxoplasmosis among pregnant women surveyed in the study area is relatively high compared to similar studies carried out in other parts of Nigeria. The detection of IgM to toxoplasmosis in 31.3% of pregnant women in the study area is indicative of the endemicity of the infection among the populace in the state. Women at the age of 31-35yrs are found to be at higher risk of contracting toxoplasmosis than any other age group. Pregnancy stage (trimester) and history of miscarriage are found to be highly associated with toxoplasmosis.

6. RECOMMENDATIONS

Serological screening for toxoplasma infection should be conducted during antenatal clinics to enable early detection of infection.Public awareness should be embarked upon by relevant government agencies and non-governmental organisation (NGOs) to enlighten the populace on the consequences of toxoplasmosis and the need for prevention.

CONSENT

All authors declared that written informed consent was obtained from the patient (or other approved parties) for publication of this paper and accompanying images.

UNDER PEER REVIEW

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UNDER PEER REVIEW

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UNDER PEER REVIEW

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