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Sero-epidemiological assessment of toxoplasmosis among antenatal attendees of university of Port Harcourt teaching hospital, rivers state, Nigeria

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Abstract

Toxoplasmosis is a disease that can infect pregnant mothers and women of child bearing age and is transmitted to the unborn baby. The sero-prevalence of toxoplasmosis with its associated risk factors in two hundred and seventy-seven pregnant subjects attending University of Port Harcourt Teaching Hospital was investigated to ascertain the current sero-prevalence status of toxoplasmosis in River State. Blood samples were collected from the subjects through a veno-puncture and the blood tests were performed using the rapid test kits for *Toxoplasma gondii* IgG and IgM antibodies following manufactures instructions. Commercial pregnancy test kits and HIV test kits were used to confirm their pregnancy and HIV status. Out of the 277 pregnant subjects examined, the overall prevalence was 1.8% (5/277) positive for *T. gondii* IgG antibody and 6.9% (19/277) positive for HIV. None of the HIV pregnant subjects were positive for toxoplasmosis. All data were presented in frequency and percentages. The chi-square analysis was used to assess the distribution of toxoplasmosis and other variables. All the analysis was done with the Statistical Package for Social Sciences (SPSS) version 25 and a p-value of less than 0.05 was considered significant. The age group of 20 -29 years and 30-39 years had the highest sero- prevalence rates of 3(60%) and 2(40%) respectively for IgG while age group 40-49 years recorded no sero-prevalence. Exposure related sero-prevalence showed that eating of raw vegetables, undercooked meat, and exposure to contaminated soil through farming were more sensitive risk factors associated with toxoplasmosis than others. There was no statistical significance using cat as pets at P>0.05. A Statistical significance difference of toxoplasmosis was higher among the subjects that ate raw vegetables and drank contaminated water (P<0.05) compared to those who ate cooked vegetables and drank treated water. The need for health campaign to enlighten the society and pregnant mothers on the dangers of acquiring toxoplasmosis and how to avoid it is advocated.

Keywords: *Toxoplasma gondii*, toxo-IgG, toxo-IgM, pregnancy, sero-prevalence, Nigeria

Introduction

Toxoplasmosis is one of the most important human diseases and represents considerable clinical and public health problems that vary widely in epidemiology and clinical manifestations in different parts of the world.[1,2] Toxoplasmosis is a human disease caused by the parasitic protozoa *Toxoplasma gondii*, a coccidian zoonotic intra-cellular parasite that has several life stages and a complex life cycle.[3,4] In the history of the occurrence of HIV infection, toxoplasmosis is identified as one of the most significant adaptable infections associated with AIDS patients.[5] Humans may acquire *T. gondii* infections by the oral, trans placental route, from blood transfusion or organ transplantation. Infection via oral route is caused by the ingestion of *T. gondii* tissue cysts in under cooked food or the ingestion of *T. gondii* oocysts in water contaminated with cat faeces.[6] Studies have shown that primary infection during pregnancy can lead to abortion, still birth, and other serious congenital consequences.[2] In neonate of infected pregnant mother, the spectrum of clinical symptoms may include foetal death, neurological damage (cerebral calcification, seizure, retardation), hydrocephalus or microcephaly,[7–9] chorioretinitis, fever, hepatosplenomegaly and rash.[10] Infection acquired during the second month through the end of the sixth month of pregnancy is more likely to cause severe disease than are third trimester infection. Several studies have shown close association of chronic toxoplasmosis and behavioural changes and schizophrenia.[11]

In Rivers State, one of the major states in the South-South geo-political zone of Nigeria little has been done to ascertain the sero-prevalence of *T. gondii* in humans among HIV positive pregnant women. The reported overall seroprevalence of human toxoplasmosis in Nigeria is estimated to be 32% with the following reports for North-West 32%, North-East 22%, North-Central 24% and South-West 37%.[12] About 2% to 3% of all inherited disorders results from perinatal contaminations. *Toxoplasma gondii* infection is one of the most frequently encountered infections that have a lot to do with congenital abnormalities.[13] Most often it causes mild maternal disorder but with serious consequences on the foetus.[7,8] To the extent that the treatment on the mother has no obvious positive effect on the foetus. Therefore, awareness of this disease is of utmost importance to the clinicians for appropriately guiding their maternal patients on

preventive measures against the infections.[13] Toxoplasmosis has been reported to be often asymptomatic in both maternal and congenital infection but can be severe when the infection occurs during pregnancy. Studies have also shown that only about 10% of the infected pregnant women presented with generalized signs and symptoms especially in developed countries like United States of America, whereas countries like Brazil the prevalence of toxoplasmosis among pregnant women is between 50% and 80%.[14,15] A wide range of *T. gondii* seroprevalence rates from 4% to 85% has been reported in adolescent women and/ or pregnant women from diverse countries of the world, both tropical and subtropical parts. These sero-prevalence variations detected in the populations from diverse countries, or even within the same state, echo disparities in geographical and climatic physiognomies, cultural and dietary lifestyles of the populace of these regions with such other factors as sampling size, distinct immunoassays adopted for diagnosis.[16] The study was aimed at investigating toxoplasmosis among two subpopulations of pregnant subjects, the healthy and the immune compromised HIV positive pregnant subjects; and the influencing risk factors among the study population.

Materials and method

This study was carried out at the University of Port Harcourt Teaching Hospital (UPTH), a tertiary Health Care Institution owned by the Federal Government of Nigeria. It is one of the five-star Federal Government owned Hospitals that carters for the health need of the larger populace within Rivers State and the neighboring states. The study population consisted of apparently healthy-looking pregnant subjects irrespective of their trimesters. A total of 304 subjects were enrolled in this research. At the end of the questionnaire distribution and collection only 277 subjects volunteered to participate in the blood sample. Ethical approval was obtained from University of Port Harcourt Teaching Hospital (UPTH) Ethical Committee. After the approval, the proposal was further presented to the Department of Obstetrics and Gynecology (O&G), thereafter, informed and written consents were obtained from the volunteers after due consultation. The research was a descriptive cross-sectional study of pregnant women with or without HIV attending antenatal clinics in the University of Port Harcourt Teaching Hospital (UPTH). Questionnaires were administered to get information on some variables such as age, stage of pregnancy, occupation, residential area, previous abortions, contact with pets (cats, dogs) and educational background of the women, those practicing gardening or farming and sources of their water supply.

Sample collection

Blood samples were collected from all the pregnant women attending antenatal clinic at UPTH. Using EDTA bottles, 5ml syringe and needle, sterile cotton swab, a toniquette, 4mls of blood was collected from the cubital vein and aseptically dispensed into the EDTA bottle; screw capped, gently rotated to mix well and allowed to stand for the serum to separate. The separated serum was stored at -20ºc after immediate use.

Sample analysis

Serological test–test for toxoplasma immunoglobulins

The *Toxoplasma gondii* kits for "IgG" and "IgM" were purchased. All tests were performed following the instructions of the manufacturer. Blood samples were collected through a venipuncture

from the subjects. The buffer, the pouch, and the controls were allowed to attain room temperature and the blood samples were tested immediately after collection as whole blood was used. The test device was placed on a clean level surface and a drop of the specimen (approximately 10µl) was dropped vertically into the specimen wells of the test device. Two drops of buffer which was approximately (80µl) were then added to the test wells. The test was allowed to run while waiting for the color lines to develop. The results were read off based on the lines development.

Pregnancy test

Pregnancy test was carried out on those coming for the first time without showing physical signs of pregnancy. Commercial Pregnancy Test Kits by ASSURANCE were used following manufacturer's instructions. The pouch was brought to room temperature before opening it, the test strip was removed from the sealed pouch and it was used immediately. The blood samples were allowed to separate by standing for 10 minutes and the plasma collected. The test strips were immersed vertically in the plasma specimen for at least 15 seconds putting into the consideration the maximum level as indicated on the test strip.

Statistical analysis

All statistical data were presented in frequency and percentage. The chi-square analysis was used to assess the distribution of the toxoplasmosis and other variables such as family history, medical history and other environmental factors. All statistical analysis was done with the Statistical Package for Social Sciences (SPSS) version 25 and a p-value of less than 0.05 was considered significant.

Results

Out of the 277 subjects investigated for *Toxoplasma gondii* antibodies, five 5(1.8%) were positive for toxoplasmosis. IgG antibody was reactive, indicating latent or chronic infection. Therefore, the prevalence rate of toxoplasmosis among pregnant subjects in Port Harcourt Rivers State, Nigeria as at the time of the study was 1.8% as seen in Figure 1. Table 1 shows the socio-demographic data of the studied population which showed the mean age to be 30.4±4.8. Again 60% of the subjects that tested positive for *Toxoplasma gondii* anti-bodies belonged to the age group 20-29 years and 40% were within 30-39 years. Based on their educational status, all the positive subjects for *T. gondii* anti-bodies had secondary and tertiary educational back ground. Occupation related sero-prevalence shows the highest infection for *T. gondii* among civil servants (60%), entrepreneurs (20%) and the unemployed (20%). Table 2 shows that of all pregnant subjects in their first and second trimesters positive for *T. gondii* IgG antibody, 3(60%) had 1-2 children, 2(40%) had history of abortion and 5(100%) had no previous blood transfusion. The Table 2, below shows that the occurrence of toxoplasmosis was not statistically dependent on there is no trimesters, number of children, previous abortions and previous blood transfusion. Table 3 shows that 5(100%) subjects that were positive for toxoplasmosis had no contact with cats/pets. Eating of suya was incriminated with 4(80%) of them being positive. Consumption of raw vegetables and milk and contaminated water were equally demonstrated as major risk factors in the transmission of toxoplasmosis. Table 3, showed that the occurrence of toxoplasmosis was statistically highly significant among subjects that eat raw vegetables (p=0.036) only.
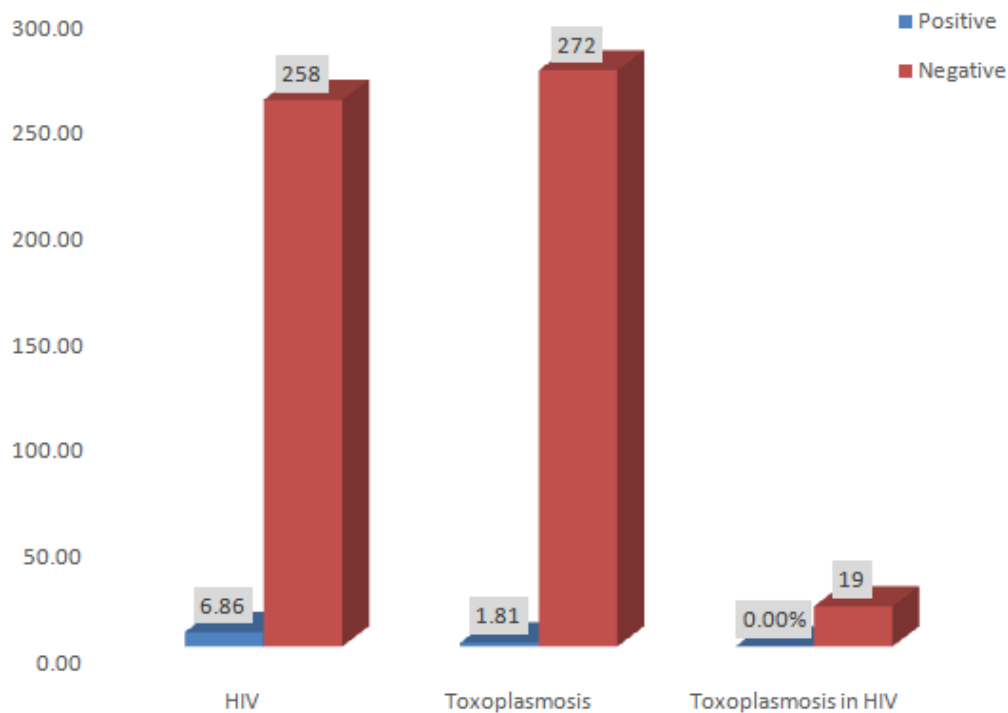
Figure 1 Overview of the prevalence of toxoplasmosis and HIV in the study population.

Table 1 Sero-prevalence of toxoplasmosis in relation to socio-demographic distributions of the subjects in the study population

Variable	Value	No of subjects examined	No Positive (%)	Chi-square-(p-value)
Age Group	20–29years	120	3 (60.0)	0.65 (0.721)**
	30–39years	149	2 (40.0)	
	40–49 years	8	0 (0.0)	
Education	No formal education	3	0 (0.0)	0.70 (0.873)**
	Primary	13	0 (0.0)	
	Secondary	85	1 (20.0)	
	Tertiary	176	4 (80.0)	
Occupation	Housewife	17	0 (0.0)	9.48 (0.148)**
	Entrepreneur/Businesswoman	151	1 (20.0)	
	Farmer	2	0 (0.0)	
	Student	17	0 (0.0)	
	Civil servant	83	3 (60.0)	
	Unemployed	7	1 (20.0)	

**Distribution of toxoplasmosis was not statistically significant (p>0.05)

Table 2 Sero- Prevalence of toxoplasmosis in relation to family history and parity

Variables	Value	No of subjects examined	Positive n (%)	Chi-square (p-value)
Trimester	First	108	3 (60.0)	2.46 (0.291)**
	Second	79	2 (40.0)	
	Third	90	0 (0.0)	

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Table Continued...

Variables	Value	No of subjects examined	Positive n (%)	Chi-square (p-value)
No. of children	1–2	116	3 (60.0)	1.15 (0.765)**
	3–4	35	0 (0.0)	
	5–6	5	0 (0.0)	
	None	12	2 (40.0)	
Previous abortions	Yes	109	2 (40.0)	0.01 (0.654)**
	No	168	3 (60.0)	
Previous transfusion	Yes	20	0 (0.0)	0.39 (0.686)**
	No	257	5 (100.0)	

**Distribution was not statistically significant (p>0)

Table 3 Exposure related sero-prevalence of toxoplasmosis in the study population

Variable	Value	Number of subjects examined	Positive n (%)	Chi-square (p-value)
Contact with Pets	Yes	8 (2.9)	0 (0.0)	0.15 (0.697)**
	No	269 (97.1)	5 (100.0)	
Use of gloves when cleaning pet litters	Yes	141 (51.8)	0 (0.0)	2.0 (3.67)**
	No	136 (48.2)	5 (100.0)	
Exposure to soil	Yes	97 (35.3)	1 (20.0)	0.50 (0.477)**
	No	180 (64.7)	4 (80.0)	
Preferred Types of Meat	Beef	115 (41.2)	3 (60.0)	4.69 (0.911)**
	Goat	63 (22.8)	1 (20.0)	
	Chicken	60 (21.7)	1 (20.0)	
	Pork	39 (14.3)	0 (0.0)	
Preferred Way of Cooking Meat	Well done	261 (94.1)	5 (100.0)	0.31(0.855)**
	Undercooked	2 (0.7)	0 (0.0)	
	Don't eat meat	14 (5.1)	0 (0.0)	
Do you eat Suya	Yes	227 (82.0)	4 (80.0)	0.01(0.909)**
	No	50 (18.0)	1 (20.0)	
Consume Raw Milk	Yes	94 (33.5)	3 (60.0)	1.85 (0.356)**
	No	183 (66.5)	2 (40.0)	
Eat Raw Vegetables	Yes	149 (52.9)	5 (100.0)	4.37(0.036)*
	No	128 (47.1)	0 (0.0)	
Washing of Hands before preparing a meal	Yes	265 (95.6)	5 (100.0)	0.23 (0.631)**
	No	12 (4.4)	0 (0.0)	
Do you treat or boil water before drinking	Always	78 ((28.3)	1 (20.0)	1.37 (0.504)**
	Sometimes	98 (34.9)	3 (60.0)	
	Not at all	101 (36.8)	1 (20.0)	

*Distribution of toxoplasmosis was statistically significant (p<0.05**). Distribution of toxoplasmosis was not statistically significant (p>0.05)

Discussion

The sero-prevalence of anti *T. gondii* IgG found among the pregnant women in this study was 1.8% which is much lower than the work reported[17] in Kaduna which had a sero-prevalence of 31.3% and another[2] work among pregnant women in Port Harcourt with a sero-prevalence of 27.2%. Increased health enlightenment campaigns and transmission dynamic awareness creation would have contributed to the decline in infection rate observed. The widened gap of sero-prevalence between northern and southern zones of Nigeria may be attributed to the different climatic conditions between the North and South as this work was carried out during the rainy season hence

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Toxoplasma gondii oocyst is favored by hotter temperature which is prevalent in the north all most all through the year.[18] The HIV positive pregnant subjects in this study were all toxoplasma sero-negative, probably due to the smallness of their sample size comparatively, besides their being on antiretroviral therapy as regular antenatal attendees in UPTH. Age status analysis showed 60% highest sero-prevalence among age group 20-29 years followed by 40% for age group 30-39 years in the present study. This result agrees with the work done in Rivers State[2] which recorded highest sero-prevalence of *T. gondii* antibody IgG among the age groups 8.9% (25-29) and 7.5% (30-34) years, though with lower infection rates respectively. It has been observed that the respondents' level of knowledge of *Toxoplasma gondii* infection is still very low since only 4.4% indicated of having heard of *T. gondii* infection; not withstanding that 80% of them had tertiary education; a neglected tropical disease indeed. The finding is similar to those of Oboro,[19] suggesting the need to intensify effort on the disease awareness creation in Rivers State. Consumption of raw vegetables was statistically significant as a major risk factor in this study, as 100% sero-positivity was found among those that were in the habit of eating raw vegetables. This may be attributed to the fact that these vegetables may have been contaminated with the oocyst from the cat litters disposed in the farmland. Eating of beef like Suya, use of untreated water and participating in garden work or exposure to soil were also implicated as risk factors, although, not statistically significant in this study. It is clear from the results of this research that having cats as pet is not a major risk factor since none of the infected subjects have ever possessed cats or were in close association with cats.

Contrary to the observations of earlier reporters in other parts of the globe that having cats as pets, was a major risk factor in the transmission dynamics of toxoplasmosis in Nigeria especially in the southern part, cats are believed to be used in wizardry; so very few people would prefer to domesticate cats as pets to dogs, goats and other animals. This also seemed to affect the sero-epidemiology of toxoplasmosis in Nigeria and in the South in particular. There may be other domesticated mammals involved in the life cycle of this protozoa parasite yet to be known in this part of the globe. Again, the implication of detecting only *Toxoplasma* IgG specific anti immunoglobulin in a low-level suggested latent toxoplasmosis in the population. Ordinarily toxo-IgG antibody is more reactive by all diagnostic techniques available than toxo-IgM[20] which indicates current infections in any given population when detected. The absence of IgM in a population with such a low level of avid IgG antibody therefore is in agreement with earlier researchers[20] reports in developed world.

Conclusion

From this current work toxoplasmosis does exist in the study population but at a lower level of 1.8% sero-prevalence when compared to earlier reports which ranged from 10% to 70%.Among the HIV positive pregnant subjects, there was no cross infection between the two debilitating infections. Some of the risk factors and variables engaged in the cause of this research were at variance and inconsistent with previous works, inconsistent in the sense that those who tested positive for *T. gondii* antibodies did not have cats as pets neither did they wear hand gloves while cleaning litters or gardening. The fact still remains that toxoplasmosis is a zoonotic disease and that unhygienic environments, eating of raw or undercooked meat are of high risk. Eating of raw vegetables particularly has been implicated in this work as a major risk factor in *T. gondii* transmission. Scanty data and information availability on this topic within the country could be attributed to high cost of the reagents and high exchange rate, nonetheless, more research work is advocated for, on this all important neglected tropical disease.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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