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T-Cell Mediated Immune Responses in Obstetric Population Acutely Infected With Toxoplasmosis in Kano, Nigeria

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Abstract
Toxoplasmosis is a disease caused by an obligate intracellular protozoan parasite *Toxoplasma gondii* which is endemic worldwide.. There is paucity of reports on the immunological responses of pregnant women to toxoplasmosis in Kano. This study was carried to determine T-cell mediated immune responses in pregnant women with acute toxoplasmosis in Kano and factors associated with seropositivity. A total of 320 blood samples were collected and assayed for anti-*Toxoplasma gondii* Immunoglobulin M (IgM) antibody using ELISA test kit. Samples were assayed for CD4, CD8, using BDFACSCount and the cytokines interferon-gamma (IFN-ϒ), tumour necrosis factor-alpha (TNF-α) using ELISA test kits while differential leucocytes count was carried out microscopically by Leishman staining technique. The IgM seroprevalence was 24(7.5%) and 296(92.5%) were negative. Associated risk factors were cat ownership and consumption of unwashed vegetables (*p* values = 0.0014 and 0.001 respectively). Mean CD4, CD8, CD4:CD8 and differential count were within normal range for the study subjects with no statistically significant relationship across trimesters. IFN-ϒ and TNF-α were elevated. Moderate eosinophilia appeared with statistically significant relationship between lymphocyte and neutrophil counts across trimesters (*p value* = 0.0278). The immunomodulatory effect of pregnancy does not classically suppress T-cell mediated immune responses as our findings revealed normal but slightly reduced distribution of CD4 and CD8 cell counts with elevated IFN-ϒ and TNF-α. The findings revealed the immune status of the study subjects, as slightly modulated, nonetheless, it is urgent to offer adequate health education programs on toxoplasmosis to pregnant women yet to be infected on how to prevent the infection so as to avoid occurrence of congenital infection.
Key Words: Toxoplasmosis, IgM, CD4, Cytokines, Kano..

INTRODUCTION

Toxoplasmosis is a disease caused by an obligate intracellular protozoan parasite *Toxoplasma gondii* (*T. gondii*). It belongs to the domain - *Eucaryota*, superphylum – *Alveolata*, phylum – *Apicomplexa,* class- *Conoidasida*, order- *Eucoccidiorida*, family- *Sarcocystidae*, subfamily- *Toxoplasmatinae,* genus- Toxoplasma and *Toxoplasma gondii* type specie . It is one of the most successful intracellular protozoan parasites on earth and highly prevalent in most warm blooded vertebrate (El – Malky *et al.*, 2014). Humans can become infected with toxoplasmosis by drinking water or eating food contaminated with oocysts released in cat feces or by accidental ingestion of raw or undercooked meat, such as pork and sheep meat containing *T. gondii* tissue cysts (Dubey *et al.*, 2012). The direct detection of *T. gondii* is difficult, therefore, most clinical laboratories use serological tests to detect antibodies against *T. gondii*, such as the latex agglutination test, enzyme-linked immunosorbent assay (ELISA), and indirect fluorescent antibody test (IFAT) (Murat *et al.*, 2013). Toxoplasmosis among women of childbearing and postmenopausal age should be considered a major public health concern. In addition, when *T. gondii* encysts in the tissues, there is no drug available to eliminate the parasite (Suzuki *et al.*, 2010). It has a life cycle which consists of three forms: tachyzoites, bradyzoites (in tissues) and sporozoites.

The parasite resides in the brain, heart, lungs and most frequently in the lymphnodes. It is a disease affecting 500 million people worldwide and seroprevalence varies (from 5% to 90), depending on geographical location, age, habit of eating raw meat, unwashed fruit and vegetables and general level of hygiene (Al-Jebouri *et al.*, 2013). Researchers have hypothesized that ingesting inadequately treated drinking water may be a significant source for human infection (Krueger *et al.*, 2014).

Resistance to infection with obligate intracellular *Toxoplasma* parasites largely depends on T helper-1 (Th-1) type cell mediated immune response and interferon-gamma (INF-γ) released from CD4+, CD8+ T lymphocyte and is the most critical mediator of immunity against *T. gondii* (Takacs *et al.*, 2012). A study by Debierre –Grockiego, *et al.* (2003) identifies *T. gondii* glycosyl phosphatidyl inositols (GPI), and their core glycans as molecules that induce tumour necrosis factor-alpha (TNF-α) production in macrophages. The combination of IFN-γ and TNF-α mediate killing of tachyzoites by macrophages (Khan *et al.*, 1994).

This research work aimed at determining T-cell mediated immune responses in pregnant women acutely infected with toxoplasmosis and the targeted goals were determining the seroprevalence of toxoplasmosis, relating CD4+/CD8+ T-cell counts, IFN-ϒ and TNF-α levels and differential leucocyte counts with stages of pregnancy.

MATERIALS AND METHODS

Study Area

The study areas covered were Sabo Bakin Zuwo Maternity Hospital (SBZMH), Bamalli Nuhu Maternity Hospital (BNMH) and Murtala Muhammad Specialist Hospital (MMSH), all in Kano Municipal Local Government Area, Kano Central. Others were Wudil General Hospital (WGH) located in Wudil Local Government Area and Gaya General Hospital (GGH) located in Gaya Local Government Area, Kano North.

Kano State lies approximately between latitudes 10^0 33'N and 12^0 23'N and longitudes 7^0 45'E and 9^0 29'E, with a population of 9,401,288 during the 2006 census. It has an estimated land size of 21,276.872 km^2 with 1,754,200 hectares agricultural and 75,000 hectares forest vegetation and grazing land (Abaje *et al.*, 2014). The State is bordered by Jigawa State in the north-east, Katsina State in the north-west and Kaduna State on the southern boundary.

Study Population

Study participants were apparently healthy pregnant women attending the aforementioned selected Hospitals for antenatal care, who gave consent to be included in the study and are within the age range of 18 to 45 years. A structured questionnaire was used to obtain biodata of the participants such as age, stage of pregnancy

Study Design

The study is a cross sectional study in which seroprevalence of toxoplasmosis was determined in pregnant women and associated risk factors in relation to gestational age and T-Cell immune responses profile of the study subjects were also determined.

Inclusion Criteria

HIV negative pregnant women, apparently healthy, at the time of sampling were included in this study.

Collection of Sample

A total of 320 blood samples from pregnant women were collected for the study using Vacutainer needles and holder into a serum separator and EDTA containers. Each blood sample was properly labeled. Samples from the serum separator containers were allowed to settle so that serum can be separated. Serum samples and the whole blood samples in the EDTA containers were then transported to Molecular Biology Laboratory of the Aminu Kano Teaching Hospital in an ice packed container where they were kept at -20^{o}C until used.

Anti *Toxoplasma gondii* IgM

Assay Procedure: according to VIRO-IMMUN Labor-diagnostika GmbH, Manufacturer of ANTI - TOXO IgM ELISA test kits.

Enumeration of CD4/CD8 Cells

Assay Procedure: The sample was placed in the work station and reagent tubes were labeled with sample identification number (Each reagent has twin tubes; one CD4 and the other CD8). Each reagent pair was vortexed upside down for 5 seconds, then upright for 5 seconds using the vortex mixer, the reagent tube were opened with the coring station. The Samples were mixed adequately by inversion and 50 µl of the sample was pipetted and dispensed into each of the twin reagent tubes changing tips between tubes. The tubes were capped and vortexed upright for 5 seconds after which the reagents pairs were placed in the work station and closed to protect from light, they were then incubated for 1 hour at room temperature, after which 50 µl of fixative solution was dispensed into each reagent tube. They were recapped with new caps and vortexed upright for 5 seconds then ran by placement of the CD4 tube on the machine's sample holder and pressing run.

When the analysis for CD4 was completed, the other tube for CD8 was placed also on the sample holder and assayed.

INTERFERON GAMMA (IFN-Y) ASSAY

Assay Procedure: The ABC working solution and TMB color developing agent were kept warm at 37°C for 30 minutes. before use. When diluting samples and reagents, they were mixed completely and evenly. Standard curve was prepared. 0.1ml per well of 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2 pg/ml, 15.6pg/ml human IFN-γ standard solutions were aliqouted into the precoated 96-well plate. 0.1ml of the sample diluent buffer was added into the control well (Zero well). 0.1ml of each properly diluted sample of human serum, plasma was added to each empty well. The plates were then sealed with the cover and incubated at 37°C for 90 min. The cover was then removed, plate content was discarded, and the plate was blotted onto paper towels. 0.1ml of biotinylated antibody working solution was added into each well and the plate was incubated at 37°C for 60 min. It was then washed 3 times with 0.01M PBS, and each time the buffer was allowed to stay in the wells for 1 min after which it was discarded and the plate was blotted onto paper towels. 0.1ml of prepared ABC working solution was added into each well and the plate was further incubated at 37°C for 30 min. It was further washed 5 times with 0.01M PBS, and each time the washing buffer was left to stay in the wells for 1 min. The washing buffer was then discarded and the plate was blotted onto paper towels. 90µl of the prepared TMB was added into each well and incubated at 37°C in dark for 15 min. 0.1ml of prepared TMB stop solution into each well. The absorbance was read at 450nm.

TUMOUR NECROSIS FACTOR ALPHA (TNF-α) ASSAY

Assay Procedure: The ABC working solution and TMB color developing agent were kept warm at 37°C for 30 minutes. before use. When diluting samples and reagents, they were mixed completely and evenly. Standard curve was prepared. 0.1ml per well of 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.2 pg/ml, 15.6pg/ml human TNF-α standard solutions were aliqouted into the precoated 96-well plate. 0.1ml of the sample diluent buffer was added into the control well (Zero well). 0.1ml of each properly diluted sample of human serum, plasma was added to each empty well. The plates were then sealed with the cover and incubated at 37°C for 90 min. The cover was then removed, plate content was discarded, and the plate was blotted onto paper towels. 0.1ml of biotinylated antibody working solution was added into each well and the plate was incubated at 37°C for 60 min. It was then washed 3 times with 0.01M PBS, and each time the buffer was allowed to stay in the wells for 1 min after which it was discarded and the plate was blotted onto paper towels. 0.1ml of prepared ABC working solution was added into each well and the plate was further incubated at 37°C for 30 min. It was further washed 5 times with 0.01M PBS, and each time the washing buffer was left to stay in the wells for 1 min. The washing buffer was then discarded and the plate was blotted onto paper towels. 90µl of the prepared TMB was added into each well and incubated at 37°C in dark for 15 min. 0.1ml of prepared TMB stop solution into each well. The absorbance was read at 450nm.

DIFFERENTIAL LEUCOCYTES COUNT

Making and staining blood films

Thin blood films were made according to the method described by Chessbrough, (2006), by placing a drop of blood on the end of a clean dry slide and a clean smooth edged glass slide was used to spread the drop of blood to make a thin film. The film was allowed to air dry. The film was then placed on a staining rack and covered with leishman stain. It was then diluted with buffered distilled water pH 6.8 (see appendix v for composition and preparation) for 8 minutes after which the diluted stain was washed off with tap water and allowed to air dry. The film was examined microscopically and the different leucocytes were counted using a mechanical differential cell counter, taking into consideration their morphology and staining reaction as described by Chessbrough, (2006).

Statistical analysis of the Data

Data entry and analysis was performed using SPSS version 20.0 software. Summaries were presented in terms of counts and percentages. Differences in proportions were evaluated by Pearsons- chi- square test and correlation. A statistical result was considered significant whenever a p-value was ‹ 0.05 at 95% confidence limit.

RESULTS

The questionnaire data revealed that all of participants have never heard or seen information about toxoplasmosis prior to interview. A total of 320 pregnant women were included in the study, the mean age of the study participants was 22.7 years with age range 17-40 years.

Eighty one (25%) of the pregnant women were in the Age group of 21 – 24 and 9 (2.8%) were in the age group of 37 – 40 (Table 1). The distribution of educational status showed that 217 (67.8%) of women had completed high school education, only 24 (7.5%) had attained tertiary education level while 40 (12.5%) had never attended any school (Table 1). Seven (2.2%) were in their first trimester, 102 (31.8%) in second trimester and 211 (66%) in the third trimester pregnancy (Table 1). Twenty six (8.1%) of the participants owned cat and 30 (9.4%) had the habit of consuming unwashed vegetables/fruits (Table 1).

Out of the 320 pregnant women examined for anti- *T. gondii* IgM antibody, 24 (7.5%) were positive (Table1). Seroprevalence of Toxoplasma IgM antibody was 0%, 0.9%, 6.5% for first, second and third trimester respectively. The seroprevalence of *T. gondii* IgM antibody was highest among primary school leavers 4/39 (10%) and lowest among illiterates 2/40 (5%) (Table1).

Pregnant women who owned domestic cats were 26 (8.1%) compared to 294 (91.8%) that do not keep cats. There was significant association in Toxoplasma seropositivity among pregnant women reported to have domestic cats (Table 1). Thirty (9.4%) of the study participants have the habit of consuming unwashed vegetables/fruits. There was significant association between habit of eating of unwashed vegetables/fruit and *T. gondii* IgM.

Mean CD4 values were 679.67cells/µl and 702.53cells/µl for second and third trimesters respectively in pregnant women that were positive for IgM *T. gondii* antibody. None among the 24 study participants that appeared positive for IgM antibody was in first trimester as shown in Table 1. There was no significant association between CD4 and trimesters. Mean CD8 values were 620 cells/µl and 619 cells/µl for 2nd and 3rd trimesters respectively with no statistical significance across the trimesters, (Table 2), while CD4:CD8 was 1.168 and 1.158, for 2nd and 3rd trimesters respectively, with no significant association with the trimesters.

Table 1: Seroprevalence of Toxoplasmosis based on age, trimester and some other associated risk factors

Risk Factors	Immunoglobulin M (IgM)	Total	*p values*
Age group (Years)	No. positive		
17-20	03(3.8%)	79	
21-24	06 (7.4%)	81	
25-28	09 (12%)	75	0.2402
29-32	02 (4.4%)	45	
33-36	04 (13%)	31	
37-40	0 (%)	09	
Total	**24(7.5%)**	**320**	
x^2	5.495		
Gestational Age (Trimester)			
1st	0 (%)	07	
2nd	03(3%)	102	0.3027
3rd	21 (10%)	211	
Total	**24 (7.5%)**	**320**	
x^2	4.775		
Educational Status			
Illiterate	2 (5%)	40	
Primary	4 (10%)	39	0.9362
High School	16 (7%)	217	
Tertiary	2 (8%)	24	
Total	**24 (7.5%)**	**320**	
x^2	08165		
Cat Ownership			
Yes	7 (27%)	26	
No	17 (6%)	294	0.0014
Total	**24 (7.5%)**	**320**	
Consumption of Unwashed Vegetables/ Fruits			
Yes	14 (47%)	30	
No	10 (3%)	290	0.0001
Total	**24 (7.5%)**	**320**	

Table 2: CD4, CD8 and CD4: CD8 based on Trimester for IgM positive in the study population.

Trimester	N	CD4 cells/µl	*p. value*	CD8 cells/µl	*p. value*	CD4:CD8	*p. value*
1st	0	-		-		-	
2nd	3	679	0.5058	620	0.2093	1.168	0.2466
3rd	15	702		619		1.158	

Key- N=no of pregnant women, *p. value*< 0.05 indicates significant statistical relationship. IgM- Immunoglobulin M, N= Number of pregnant women.

Table 3 shows CD4, CD8 and CD4: CD8 based on gestational age for pregnant women that appeared negative for IgM *T. gondii* antibodies. Only a participant was in her first trimester, 12 were in their second trimesters while 25 were in their 3rd trimester. The mean CD4 cell counts were 985 cells/µl, 756 cells/µl, and 730.94 cells/µl, for first, second and third trimesters respectively, no significant association was observed between mean CD4 cell count and trimesters, *p value* = 0.192 (table 3). Mean CD8 values were 531.0 cells/µl, 548.25 cells/µl and 497.7 cells/µl for 1st, 2nd and 3rd trimesters respectively with no significant association across the trimesters, p = 0.5479 (Table 3). Mean CD4: CD8 values were 1.17, 1.47 and 1.49 for 1st, 2nd and 3rd trimesters respectively, so also there is no significant difference, *p value*= 0.2978 (Table 3).

Mean IFN-**γ** for IgM positive study subjects was 18.5 for 2nd trimester and 132.5 for 3rd trimester with no significant association, *p. value* = 0.596 (Table 4), while Mean IFN-γ values IgM negatives were 3.31pg, 44.27pg, and 23.75pg for 1st, 2nd and 3rd trimesters respectively with no significant association across the trimesters, *p value*= 0.6674 (Table 4).

TNF – α values were 89.1pg for 2nd trimester and 55.6 for 3rd trimester for the IgM positive study subjects, with significant association (*p. value* = 0.001). The mean TNF-α values for the IgM negatives for 1st 2nd and 3rd trimesters were 0.60pg, 8.40pg, and 3.90pg respectively with no significant difference (*p. value* = 0.440) Table 4.

Lymphocyte count based on trimesters for 2nd and 3rd trimester were 22.5% and 33.1%, with no participant appearing under 1st trimester. Statistical significance was observed in the lymphocytes count across 2nd and 3rd trimester (*p value*= 0.022). Neutrophil count was 68% in the 2nd trimester and 57.5% in the 3rd trimester, however, there was no significant association between neutrophil count and trimesters (*p value* = 0.162). Eosinophils were 7% in 2nd trimester and 6.06% in the 3rd trimester, and no statistical association was observed (*p value* = 0.750). Monocytes count was 2.5% and 3.93% for 2nd and 3rd trimesters respectively, also no statistical significance, (*p value* = 0.613)(Table 5).

Table 3: Mean CD4, CD8 counts and CD4:CD8 based on Trimesters for IgM Negative in the study population

Trimester	N	CD4 cells/µl	*p. value*	CD8 cells/µl	*p. value*	CD4: CD8	*p. value*
1st	1	985	**0.192**	531	**0.5479**	1.17	**0.2978**
2nd	12	756		548		1.47	
3rd	24	730		497		1.49	

Key: N= number of pregnant women, *p. value*< 0.05 indicates significant statistical relationship, IgM= Immunoglobulin M

Table 4: Determination of TNF – α and IFN –γ levels in the study population that were *T. gondii* IgM negative and positive based on Trimester of pregnancy

	IgM Negative.					IgM Positive				
Trimester	N	IFN-ϒ (pg)	*p. value*	TNF - α (pg)	p. value	N	IFN-ϒ (pg)	*p. value*	TNF-α (pg)	*p. value*
1st	01	3.31	0.6674	0.60	0.440	0.0	0.0	0.596	0.0	0.001
2nd	12	44.27		8.40		03	18.5		89.1	
3rd	25	23.75		3.90		15	132.5		55.6	

Key- N= number of pregnant women, *p. value*< 0.05 indicates significant statistical relationship; IFN-ϒ= Interferon-gamma, TNF-α= Tumour necrosis factor alpha, IgM= Immunoglobulin M

Table 5: Differential Leucocytes Count based on trimesters for IgM Positive in the study population

Trim	N	Lymph	*p*. value	Neutrop	*p*. value	Eosinop	*p*. value	Monocyt	*p*. value
1st	0	0		0	0.0076	0	0.0278	0	0.4986
2nd	03	22.50	0.0415	68.0		7.00		2.50	
3rd	15	33.10		57.5		6.06		3.93	

Key- N=no of pregnant women, *p*. value<0.05 indicates significant statistical relationship; IgM=Immunoglobulin M, Lymph=Lymphocyte, Neutroph.=Neutrophil, Eosinoph=Eosinophil, Monocyt=Monocyte.

Participants that appeared IgM negative have mean differential lymphocyte count of 27.9% in 1st trimester, 25.2% in the second trimester and 31.54% in the 3rd trimester with no significant difference (*p value* = 0.6123), Neutrophils were 62.73%, 64.08% and 63.45% for 1st, 2nd and 3rd trimester respectively with p. value of 0.7774, signifying no statistical significance. Eosinophils mean differential counts for 1st, 2nd, and 3rd trimesters were 1.85%, 1.75%, and 2.08% respectively (*p* value=0.5243), while mean differential Monocytes counts were 2%, 2.3% and 2.4% for 1st, 2nd and 3rd trimesters respectively (*p value*= 0.1789). No statistical association between leucocytes and gestational age (Table 6).

Table 6: Differential Leucocytes Count for IgM Negative based on Trimester in the study population

Trimester	N	Lympho	*p*. value	Neutroph	*p*. value	Eosinop	*p*.value	Monocyt	*p*. value
1st	01	27.90	0.6123	62.70	0.7774	1.85	0.5243	02	0.1789
2nd	12	25.20		64.08		1.75		2.3	
3rd	25	31.54		63.45		2.08		2.4	

Key: N=no of pregnant women, *p*. value< 0.05 indicates significant statistical relationship; IgM=Immunoglobulin M, Neg.=negative, Lymph=Lymphocyte, Neutroph.=Neutrophil, Eosinoph=Eosinophil, Monocyt=Monocyte.

Discussion

This current study is one of the few studies carried out in Kano that explores the seroprevalence of *Toxoplasma gondii* infection among HIV negative pregnant women in some health facilities and demonstrates Th1- cells responses and some cytokines response among pregnant women with primary and chronic Toxoplasmosis to the best of our knowledge:

An overall *Toxoplasma gondii* IgM antibody seroprevalence of 7.5% (24/320) was recorded. The 7.5% seroprevalence of *T. gondii* IgM antibody in this study is similar to 8.8% obtained by Al-Mohammad *et al*. (2010) in Saudi Arabian Maternity Hospital, 7.6% reported from Lagos, Nigeria (Deji-Agboola *et al.*, 2011) and 8.4% obtained by Mousa *et al*. (2011) in Libya and differs from 4.6% and 0.8% from Zaria, Northern Nigeria reported by Ogoina *et al*. (2013) and Ishaku *et al*. (2009) respectively, and 3.4% by Goncalves *et al*. (2010) in Brazil, it is however lower than 58.1% reported in Baghdad, Iraq (Aziz and Drueish, 2011) and 50% obtained by Al-Ani, (2012) in a study. The 7.5% IgM antibody seroprevalence obtained in this study suggests a high prevalence of recent *T. gondii* infection among the pregnant women with more occurring in the third trimester (87.5%) than the second trimester while none occurred in the first trimester. The risk of fetal infection via transplacental transmission and severity in the case of *T. gondii* recent infection is greater in late maternal infection (3rd trimester) and can typically results in normal appearance newborns whose infection symptoms are initially unnoticed; however, if left untreated, it can lead to mental and psychomotor retardation, microcephaly, hydrocephalus, seizures, blindness, and death when effected individuals reach the age of 20-30 years (Montoya and Liesenfeld, 2004).

This study also found no significant association existing between seroprevalence of *T. gondii* IgM antibodies and age. The highest prevalence rates of IgM (12%) was detected in the age group of 25 – 28. It is highly risky, as it is the most fertile period of child bearing age and this also highlights the need to continue to educate women of child-bearing age on prevention of toxoplasmosis. However, different studies

reported an increase in seropositivity of anti *T. gondii* antibodies with increasing age. Elichilia *et al*. (2015) showed that younger women were more infected compared to elderly ones. This might be explained by the fact that young people are more adventurous than elderly ones and thus prefer outing compared to elderly, this outing exposes them to grilled meat (which might be under cooked), fruit and saladies which may be contaminated with the parasite hence increased risk of infection (Makiko *et al.,* 2011).

The highest seroprevalence of *T. gondii* IgM antibody found was 9.9% in pregnant women at their third trimester. However, there was no statistical significant between gestational age of the fetus and seropositivity. This result is in agreement with a study by Hussein *et al*. (2014) who observed a non significant difference between trimesters and prevalence among pregnant women where a 0% sero prevalence was obtained for the first trimester. It is also similar to results obtained by Gelaye *et al*. (2015) that showed no significant association between seroprevalence of infection and gestational period of the participants. None of the participants (in their first trimester) was seropositive to IgM antibody which

indicated a very low transmission rate through the placenta- risk and minimal foetal implication of the *T. gondii* oocyst infection-risk, hence the unlikelihood of deformation or death as the first trimesters is the formation stage of the foetus. However, contrary to this, a significant association was found to exist between *T. gondii* oocyst infection and trimester in which a higher prevalence was recorded for pregnant women in the first and third trimester (Alayande *et al.,* 2013). The attending of the Kano pregnant women to antenatal clinics in the first trimesters is less than the second and third trimesters, which could be a cause for low frequency of the subject in the first trimester in this study.

This study showed no significant association between seroprevalence in pregnant women and level of education. There are similar reports in Iran (Fallah *et al.,* 2008) and Ethiopia (Endris *et al.,* 2014). The present study demonstrated that illiterate women tend to have decreased seropositivity of anti *T. gondii* by virtue of their literacy. This can be due to higher economic status of literate women compared to illiterates and their different habits in eating poultry and junk food which are major source of *T. gondii* transmission. Nevertheless, literate pregnant women might have over looked the importance of prevention measures to avoid being accidentally infected with toxoplasmosis or their knowledge of toxoplasmosis might have been incorrect, leading to a condition of frequent exposure. A national survey in the United States showed that pregnant women's level of knowledge of toxoplasmosis symptoms and measures was relatively low to prevent infection among those with a senior high school education level (Jones *et al.,* 2001).

The findings in this study showed significant association between *T. gondii* infection and presence of domestic cats at home. It corroborates with studies reported from Taiwan (Lin *et al.,* 2008) and Ethiopia (Zemene *et al.,* 2012). In contrast Ishaku *et al*. (2009), Nijan and Al-Amleh (2009) and Saki *et al*. (2015) reported absence of statistical association between *T. gondii* infection and presence of domestic cats in the house hold. The way the cat's litter box is cleaned rather than the simple presence of cats account for exposure of individuals to the parasite. Moreover, the prevalence of the parasite among the domestic cats may depends on the type of cats (stray vs. pet cats) in different countries in that stray cats were reported to be more exposed to the parasite as compared to pet cats (Lee *et al.,* 2010). Furthermore, oocysts of *T. gondii* are usually not found on cat fur, but are found hidden in soil along with cat feces. Since contact with soil is difficult to avoid especially in Kano which is characterized by dusty winds during harmattan, this might explain the high prevalence of toxoplasmosis in Kano. To prevent cats from becoming infected, they most be fed on well cooked meat and if possible, kept indoors to prevent them from hunting or scavenging. This is not practiced in Kano as cats are not fed well or kept strictly indoors.

As IgM is considered an indicator for recent/active infection, the present study reveals a strong statistical significance between consumption of unwashed fruits/vegetables with recent infection. This is in agreement with the study conducted by Alvarado – Esquivel *et al*. (2011). This study's results strengthen the evidence that acute *T. gondii* infection may result from consumption of unwashed vegetables and fruits and indicate that this is a major factor contributing to maternal infection in Kano. Even though the percentage of unwashed fruits consumers is small, the frequency of seropositive in those that use to consume unwashed vegetables and fruits is appreciable and alarming to link acute toxoplasmosis with the consumption.

However, eating unwashed fruits and vegetables showed no significant association with *T. gondii* infection studies conducted by Ayi *et al*. (2009), Alvarado – Esquivel *et al*. (2011) and Benson *et al*. (2013) in Ghana, Mexico and Tanzania respectively.

However, the absence of a statistically significant relationship between the prevalence of *T. gondii* infection among the investigated population and many of the factors explored in this study does not confirm that these factors have no influence on the transmission of Toxoplasmosis.

Immunity in pregnancy is physiologically compromised and may affect the CD4 cell count, as lower CD4 + cell count was reported in pregnancy compared with non pregnant females (Oladepo *et al.,* 2009). The documented relationship between gestational age and $CD4^+$ cell levels in pregnant woman varies in the literature. While Akinbami *et al*. (2014) and Chama *et al*. (2009) found no relationship between gestational age and $CD4^+$ cell counts in HIV negative pregnant women in Nigeria and Kenya respectively.

This study found the mean CD4 cell counts for 1^{st} 2^{nd} and 3^{rd} trimesters in the acute *T. gondii* infection to be slightly lower than the mean normal CD4 T-lymphocyte baseline for healthy HIV-negative pregnant women in a study conducted in Maiduguri by Chama *et al*. (2009) who reported 751.41 cells/µl and Aina *et al*. (2005) that obtained 771 cells/µl. This is expected, given the relatively critical essence of CD4 T-lymphocyte in controlling such intra-cellular pathogens like *Toxoplasma gondii*. Interlaboratory variability and the use of different methods in the measurement of absolute CD4 cell counts could possibly account for the observed differences. In contrast, pregnant women that appeared seronegative to IgM anti-*T. gondii* antibodies in the present study have mean CD4 cell counts for 1^{st}, 2^{nd} and 3^{rd} trimesters consistent to the aforementioned values found by Chama *et al*. (2009) and Aina *et al*. (2005).

This study also reported an insignificant association between CD4 cell count and gestational age just like Akinbami *et al*. (2014) and Temmerman *et al*. (1995). However, Gomo *et al*. (2004) showed that gestational age influence CD4 cell count. The distribution of CD8 cell counts and CD4:CD8 for the acutely infected in the present study falls within the reference ranges established by Oladepo *et al*. (2009) and Rungta *et al*. (2008) respectively, nonetheless they tend to have a higher CD8 T cells count as it is essential for control of *Toxoplasma gondii* infection. However there is no significant statistical relationship between both mean CD8 cell counts and CD4:CD8 with trimester for both the infected and the uninfected.

An analysis of IFN-γ and TNF-α using sera from infected and uninfected pregnant women yielded elevated values relative to uninfected pregnant women, with highest elevation of IFN-γ for the infected pregnant women occurring in the 3rd trimester which corresponds with the values of CD4 and CD8 cell counts for the trimester as these lymphocytes subsets are responsible for the production of the cytokines (IFN-γ and TNF-α), more so, they have been reported to be often elevated during pregnancy. It may also be due to the nature of the infecting *T. gondii* strains or to the genetic susceptibility of the pregnant women.

A study in animal models has shown that protective and inflammatory immune responses to *T. gondii* are mediated by the elevation of IFN-γ and TNF-α in pregnant mice (Dupont *et al.,* 2012). de-la-Torre *et al*. (2013) showed that IFN- γ levels were elevated in asymptomatic individuals infected with *T. gondii*. In contrast, Pernas *et al*. (2014) observed that IFN-γ and TNF-α were among 23 cytokines assayed that were lower in serum of acutely infected pregnant USA patients relative to uninfected pregnant patients. The mechanistic basis for the depressed cytokine could be the result of down-regulatory pathways activated in an attempt to neutralize potential immunopathology in various organs including the placenta.

The present study observed no statistical association between IFN-γ, TNF-α and trimesters for both the infected and uninfected study subjects, however, in TNF-α, a noticeable elevation was observed in the infected pregnant women and a statistical correlation exists across the trimesters. In toxoplasmosis, TNF-α appears to be indispensable for macrophage activation, this could be a reason for its high expression and relative significance in acute infection.

The mean differential leucocytes count results from this study showed significant difference in the infected subjects and slight deviation from what was obtained by Miri-Dashe *et al*. (2014) as reference range for normal pregnant women in Nigeria. There was a moderate eosinophilia in the acutely infected pregnant women.

This is related to the elevated cytokine TNF-α which activates eosinophil cytotoxicity toward protozoa and in general, protozoan infections are not associated with eosinophilia.

However, case reports do exist suggesting toxoplasmosis can cause eosinophilia (Hoffman *et al.*, 2013).

Conclusion

The data obtained from this study showed low seroprevalence of acute toxoplasmosis among pregnant women. The findings also revealed the immune status of the study subjects, across the infected and uninfected as slightly lowered but not classically suppressed, with CD4, CD8 cell counts and CD4:CD8 within the normal range and not statistically related to the gestational age of the pregnancies. The cytokines, IFN-γ and TNF-α, assayed in this study were elevated and not statistically significant across trimesters other than TNF-α that was statistically significant across the trimesters in the study participants.

Recommendations

The high level of ignorance about the disease calls for intensive education of the populace, especially, women of child-bearing age including pregnant women with risk factors associated with toxoplasmosis so that preventive measures could be taken. It is urgent for the Kano state Ministry of Health to offer adequate health education programs on toxoplasmosis to pregnant women not yet infected with toxoplasmosis to avoid the occurrence of congenital toxoplasmosis during pregnancy.

REFERENCES

Abaje, I. B., Ndabula, C. and Garba, A. H. (2014). Is the changing rainfall patterns of Kano State and its adverse impacts an indication of climate change?. *European Scientific Journal*, **10**(2): 192-206.

Aina, O., Dadik, J., Charurat, M., Amangaman. P., Gurumdi. S., Mang, E., Guyit, R., Lar, N., Datong, P., Daniyam, C., Kanki, P. and Abimiku, A. (2005). Reference values of CD4 T lymphocytes in human immunodeficiency virus–negative adults. *Clinical and diagnostic Laboratory Immunology,* **12**(4): 525 – 530.

Akinbami, A,A., Adewumi, A.A., Rabiu, K..A., Wright, K.O., Dosumu, A.O., Dada, M.O and Adeyemo,T.A. (2010). Seroprevalance of *Toxoplasma gondii* antibodies amongst pregnant women at the Lagos State University Teaching Hospital, Nigeria. *Nigerian Postgraduate Medical Journal,* **17**(2): 164 – 167.

Al-Ani, R.T.(2012):Study of Toxoplasma infection in women recurrent abortion in first trimester of pregnancy by Indirect immunofluorescent antibody test(IFAT). *Diyala Journal of Pure Science*, **8**: 24-30

Alayande, M.O., Edungbola, L.D., Fabiyi, J.P. and Awosan, K.J.(2013). Occurrence of antibody to Toxoplasma infection among pregnant women with obstetric histories and at different trimesters in Sokoto, Northwest, Nigeria. *American Journal of Research Communication*, **1**(9): 240-247

Al-Jebouri, M., Al-Janabi, M. and Ismail, M. (2013). The prevalence of toxoplasmosis among female patients in Al-Hawija and Al-Baiji Districs in Iraq, *Open Journal of Epidemiology*. **3**(2). 1-4.

Al-Mohammad, H.I., Amin, T.T., Balaha, M.H. and Al-Moghannun, M.S. (2010). Toxoplasmosis among the pregnant women attending a Saudi maternity hospital: seroprevalence and risk factors. *Annals of Tropical Medicine and Parasitology,* **104**(6)**:** 493 – 504..

Alvarado–Esquivel, C., Estradu–Martinez, S. and LiesenFeld, O. (2011). *Toxoplasma gondii* infection in workers occupationally exposed to unwashed raw fruits and vegetables: a case control seroprevalence study, *Parasites and vectors,* **4**:234 – 235.

Ayi, I., Edu, S.A.A., Apea–Kubi, K.A., Boamah, D., Bosompen, K.M. and Edoh, D. (2009). Sero-Epidemiology of Toxoplasmosis amongst pregnant women in the greater Accra region of Ghana, *Ghana medical Journal*. **43** (3). 107-114.

Aziz, F. M. and Druesh, M. J. (2011). Toxoplasmosis: serious disease during pregnancy. *Baghdad Science Journal,* **8:** 91 – 5.

Benson, R.K., Bemo, M., Mshan, S.E., Massinde A,N., Mazigo, H.D., Micheal, D., Majina, C. and Grob, U. (2013). Sero prevalence and factors associated with *Toxoplasma gondii* infection among pregnant women attending antenatal care in Mwanza Tanzania. *Parasites and vector,* **6:** 222

Chama, C.M., Morrupa, J.Y., Abja, U.A. and Kayode, A.(2009). Normal CD4-T lymphocyte baseline in healthy HIV negative pregnant women. *Journal of obstetrics and Gynaecology*, **29**(8): 702-704.

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries Part II. 2nd edition. *Cambridge University Press*,**2**: 320,387,396.

Debierre-Grockiego, F., Azzouz, N., Schmidt, J., Dubremetz, J., Geyer, H., Geyer, R., Weingart, R., Schmidt, R.R. and Schwarz, R.T. (2003). Roles of glycosylphosphatidyl inositol of *Toxoplasma gondii*: Induction of tumour necrosis factor-alpha production in macrophage. *Journal of Biological Chemistry*, **278** (35): 32987-32993.

Deji-Agboola, A.M., Busari, O.S., Osinupebi, O.A. and Amoo, A.O.J. (2011). Seroprevalence of *Toxoplasma gondii* antibodies among pregnant women attending antenatal clinic of federal medical centre, Lagos Nigeria. *International Journal of Biological and Medical Research,* **2**(4):1135 – 1139.

De-la-Torre, A., Sauer, A., ptaff, A.W., Bourcier, T. and Brunet, J., (2013). Severe South American Occular Toxoplasmosis associated with decreased IFN-ϒ. *PLoS Neglected tropical diseases*, **25**(4)

Dubey, J. P., Tiao, N., Gebreyes, W.A. and Jones, J.L. (2012). A review of toxoplasmosis in humans and animals in Ethiopia. *Epidemiology and Infection,* **140**(11):1935 – 1938.

Dupont, C.D., Christian, D.A. and Hunters, C.A. (2012). Immune response and Immunopathology during toxoplasmosis. *Seminars in Immunopathology*, **34**: 793-813.

Elichilia, R.S., Stanley, G.N., Wilfred, C., Gileard, M., Sonda, T., Dominic, M., Emanuel, G.K. and Bailthazar, M.N. (2015). Seroprevanece and factors associated with *Toxoplasma gondii* infection among pregnant women attending antenatal care in the referral hospital in Tanzania: cross sectional study. *Annals of clinical and laboratory research*, **3**(2):17.

El-Malky, M.A., Al-Harithi, S.A., Mohamed, R.T., EL-Bali, M.A. and Saudy, N.S. (2014). Vaccination with Toxoplasma lysate antigen and CpG oligodeoxynucleotides: comparison of immune responses in intranasal versus intramuscular administrations *Parasitology Research,***113**(6): 2277-2284.

Endris, M., Belyhum, Y., Moges, F., Adefiris, M., Tekeste, Z., Mulu, A. and Kassu A. (2014). Seroprevalence and associated risk factors of *Toxoplasma gondii* in pregnant women attending in northwest Ethiopia. *Iranian Journal of parasitolagy,* **9**(3):407 – 414.

Fallah, M., Rabiee, S., Matini, M. and Taherkhani, H. (2008). Seroepidemiology of toxoplasmosis in primigravida women in Hamadan Islamic Republic of Iran. *Eastern Mediterranean health Journal,* **14**(1**):** 163 – 71.

Gelaye W, Kebede T, and Hailu A, (2015). High prevalence of anti-toxoplasma antibodies and absence of *Toxoplasma gondii* infection risk factors among pregnant woman attending routine antenatal care in two hospitals of Addis Ababa, Ethiopia. *Internal Journal if Infectious Disease*, **34:** 41-45.

Gomo, E., Vennerval, B.J., Ndhlovu, P., Kaetel, P., Nyazema, N. and Friis, H. (2004). Predicts and reference values of CD_4 and CD_8 T-lymphocyte counts in pregnancy a cross sectional study among HIV negative women in Zimbabwe. *Central African Journal of Medicine,* **50**(1-2): 10 -9.

Goncalves, M.C., de Matos, C.C., Franco-Spegiorin, L..J., Vaz-Oliani, D.C., Oliani, A.H. and Matos, L.C. (2010). Seropositivity rates for toxoplasmosis, rubella, syphilis, cytomegalovirus, hepatitis and HIV among pregnant women receiving care at a public health service, Saopaulo State, Brazil. *Brazillian Journal of infectious Diseases*, **14**(6)**:** 601 – 5.

Hoffman, R., Benz, E. J., Silberstein, L. E., Heslop, H. E., Weitz, J. I. and Anastasi, J. (2013). *Hematology: Basic Principles and Practice*, **2**: 2231.

Hussein, A., Aman, K.E., Darakhshan, P.K., Abdullah, N., Ayesha, A., Ibrahim,.B., Mohamed, S.M., Saif, E.A. and Ibrahim, M.E. (2014). Seroepidemiology of *Toxoplasma gondii* amongst pregnant woman in Jazan province. Saudi Arabia. *Journal of Tropical Medicine,* **2014**: 1-6.

Ishaku, B.S., Ajogi, I., Umoh, J.U., Lawal, I. and Randawa, A.J. (2009). Seroprevalence and risk factors for *Toxoplasma gondii* infection among antenatal women in Zaria. *Nigerian Research Journal of Medicine and Medical Science,* **4**(2):483 – 488.

Jones, J.L., Kruszon-Muran, D., Wilson, M., Mcqullan, G., Navin, T. and McAuley, J.B. (2001). Toxoplasma gondii infection in the United States: Seroprevalence and risk factors. *American Journal of Epidemiology*, **154**(4): 357-365.

Khan, I.A., Matsurra, T. and Kasper L.H. (1994). IL-12 enhances murine survival against acute toxoplasmosis. *Infection and Immunity,* **62**(5): 1639-1642.

Krueger, W.S., Hilborn, E.D., Converse, R.R. and Wade, T.J. (2014). Drinking water source and human *Toxoplasma gondii* infection in the United State. A cross sectional analysis of National Health and Nutrition Examination Survey (NHANES) data. *Biomed central public health*, **14**(1)**:** 711.

Lee, S., Kim, J., Kim, Y., Cho, S., Ahn, H., Woo, H., Lee, W. and Nam, H. (2010). Prevalence of *Toxoplasma gondii* infection in stray and house hold cats in regions of Seoul, Korea. *Korean Journal of Parasitology,* **48**(3): 267 – 270.

Lin, Y. L., Liao, Y. S. and Liao, LR. (2008). Seroprevalence and sources of Toxoplasma infection among indigenous and immigrant pregnant women in Taiwan. *Parasitology Research,***103**: 67-74.

Makiko, S., Shunichi, N., Masachi, H., Hirotoshi, N. and Satoshi, H. (2011).

Anti-Toxoplasma Antibody Prevalence, Primary Infection Rate, and Risk Factors in a Study of Toxoplasmosis in 4,466 Pregnant Womenin Japan. *Journal of clinical and vaccine immunology*, **19**(3): 365-367.

Miri-Dashe, T., Osawe, S., Tokdung, M., Daniel, N., Choji, R.P., Mamman, I., Dene, K., Damulak, D. and Abimuku, A. (2014). Comprehensive Reference Ranges for Haematology and Clinical Chemistry Laboratory Parameters derived from normal Nigerian Adults. *PLoS ONE Neglected Tropical Diseases,* **9**(5): doi:10.1371.

Montoya, J.G. and Liesenfeld O. (2004). Toxoplasmosis. *Lancet*, **363**(9425)**:** 1965-1976.

Mousa, D.A., Mohammad, M.A. and Toboli, A.B. (2011). *Toxoplasma gondii* infection in pregnant women with previous adverse pregnancy outcomes. *Medical Journal of Islamic World Academy of Sciences,* **19:** 95 – 102.

Murat, J. B., Hidalgo, H. F., Brenier-Pinchart, M. P. and Pelloux, H. (2013). Human toxoplasmosis: which biological diagnostic tests are best suited to which clinical situations?. *Expert Review on Anti Infective Therapy,* **11**(9):943-56.

Nijan, K. and Al- Amleh S. (2009). Seroprevalence and associated risk factors of toxoplasmosis in pregnant women in hebron district, Palestine. *East Mediterranean Health Journal,* **15:**1279 – 1284.

Ogoina, D., Onyemelukwe, G.C., Musa, B.O. and Obiako, R.O. (2013). Seroprevalence of IgM and IgG antibodies to toxoplasma infection in healthy and HIC – positive adults from Northern Nigeria. *Journal of infection in developing Countries,* **7**(5): 398 – 403.

Oladepo, D.K., Idigbe, E.O., Audu, R.A., Inyang, U.S., Imade, U.E., Philip, A.O., Okafor, G.O., Olalaye, D., Mohammed, S.B., Odunukwe, N.N., Harry, T.O., Edyong-Ekpa, M., Idoko, J., Musa, A.Z., Adedeji, A., Nasidi, A., Ya'aba, Y. and Ibrahim, K. (2009). Establishment of reference values of CD4 and CD8 lymphocyte subsets in healthy Nigerian adults. *Clinical and Vaccine Immunology,* **16**(9): 1374 – 1377.

Pernas, L., Ramirez, R., Holmes, T H., Montoya, J. G. and Boothroyd, J. C. (2014). Immune profiling of pregnant Toxoplasma-infected US and Columbia Patients reveals surprising impacts of infection on peripheral blood cytokines. *Journal of infectious diseases,* **210**(6): 923-931.

Robert-Gangneux, F. and Darde M. L. (2012). Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical microbiology reviews,* **25**(2): 264 – 276

Rungta, A., Hooja, S., Vyas, N., Rishi, S., Rao, A. and Gupta, S. (2008). Enumeration of CD4 and CD8 T lymphocytes in healthy HIV seronegative adults of northwest India: A preliminary study. *Indian Journal of Pathology and Microbiology*, **51**(1): 127-129.

Saki, J., Mohammadpour N., Moramezi, F. and Khademvatan, S. (2015). Seroprevalence of *Toxoplasma gondii* in women who have aborted in comparison with the women with normal delivery in Ahuaz southwest of Iran. *The scientific world journal,* **(2015)**, doi: 10.1155/2015/764369

Sarmukaddam, S.B. and Gerad, S.G. (2006). On validity of assumption while determining sample size. *Indian Journal of Community Medicine*, **29**(2):2004 – 2006.

Suzuki, Y., Wang, X., Jortner, B.S., Payne, L., Ni, Y., Michie, S.A., Xu, B.m Kudo, T. and Perkins, S. (2010). Removal of *Toxoplasma gondii* cysts from the brain by perforin medialed activity of CD8 + T cells. *American Journal of pathology,* **176**(4):1607-1613.

Takács, A. C., Swierzy, I. J. and Lüder, C. G. (2012). Interferon-restricts *Toxoplasma gondii* development in marine skeletal muscle cells via nitric oxide production and immunity related GTPases. *PLos ONE,* **7**(9).

Temmerman, M., Nagelkerke, N., Bwayo, J., Chomba, EN., Ndinya-Achola, J. and Plot, P. (1995). HIV 1 and immunological changes during pregnancy: A comparison between HIV 1 seropositive and HIV 1 seronegative women in Nairobi kenya. *AIDS* **9**(9): 1057-1060

Zemene, E., Yewhalaw, D., Abera, S., Belay, T., Samuel, A., and Zeynudin, A. (2012): Seroprevalence of *Toxoplasma gondii* and associated risk factors among pregnant women in Jimma town, Southwestern Ethiopia. *BMC Infectious Diseases*. **12**:337.