



## 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- $\gamma$ ), tumour necrosis factor-alpha (TNF- $\alpha$ ) 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- $\gamma$  and TNF- $\alpha$  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- $\gamma$  and TNF- $\alpha$ . 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- $\gamma$ ) 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- $\alpha$ ) production in macrophages. The combination of IFN- $\gamma$  and TNF- $\alpha$  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- $\gamma$  and TNF- $\alpha$  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° 33'N and 12° 23'N and longitudes 7° 45'E and 9° 29'E, with a population of 9,401,288 during the 2006 census. It has an estimated land size of 21,276.872 km<sup>2</sup> 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°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  $\mu$ l 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  $\mu$ l 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- $\gamma$ ) 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- $\gamma$  standard solutions were aliquoted 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. 90 $\mu$ l 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- $\alpha$ ) 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- $\alpha$  standard solutions were aliquoted 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. 90 $\mu$ l 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 2<sup>nd</sup> and 3<sup>rd</sup> 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	Immuglobulin M (IgM)	Total	<i>p values</i>
<b>Age group (Years)</b>	No. positive		
17-20	03(3.8%)	79	0.2402
21-24	06 (7.4%)	81	
25-28	09 (12%)	75	
29-32	02 (4.4%)	45	
33-36	04 (13%)	31	
37-40	0 (%)	09	
<b>Total</b>	<b>24(7.5%)</b>	<b>320</b>	
$\chi^2$	5.495		
<b>Gestational Age (Trimester)</b>			
1 <sup>st</sup>	0 (%)	07	0.3027
2 <sup>nd</sup>	03(3%)	102	
3 <sup>rd</sup>	21 (10%)	211	
<b>Total</b>	<b>24 (7.5%)</b>	<b>320</b>	
$\chi^2$	4.775		
<b>Educational Status</b>			
Illiterate	2 (5%)	40	0.9362
Primary	4 (10%)	39	
High School	16 (7%)	217	
Tertiary	2 (8%)	24	
<b>Total</b>	<b>24 (7.5%)</b>	<b>320</b>	
$\chi^2$	08165		
<b>Cat Ownership</b>			
Yes	7 (27%)	26	0.0014
No	17 (6%)	294	
<b>Total</b>	<b>24 (7.5%)</b>	<b>320</b>	
<b>Consumption of Unwashed Vegetables/ Fruits</b>			
Yes	14 (47%)	30	0.0001
No	10 (3%)	290	
<b>Total</b>	<b>24 (7.5%)</b>	<b>320</b>	

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

Trimester	N	CD4 cells/ $\mu$ l	p. value	CD8 cells/ $\mu$ l	p. value	CD4:CD8	p. value
1 <sup>st</sup>	0	-		-		-	
2 <sup>nd</sup>	3	679	0.5058	620	0.2093	1.168	0.2466
3 <sup>rd</sup>	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 3<sup>rd</sup> trimester. The mean CD4 cell counts were 985 cells/ $\mu$ l, 756 cells/ $\mu$ l, and 730.94 cells/ $\mu$ l, 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/ $\mu$ l, 548.25 cells/ $\mu$ l and 497.7 cells/ $\mu$ l for 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> 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 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimesters respectively, so also there is no significant difference, p value= 0.2978 (Table 3).

Mean IFN- $\gamma$  for IgM positive study subjects was 18.5 for 2<sup>nd</sup> trimester and 132.5 for 3<sup>rd</sup> trimester with no significant association, p. value = 0.596 (Table 4), while Mean IFN- $\gamma$  values IgM negatives were 3.31pg, 44.27pg, and 23.75pg for 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimesters respectively with no significant association

across the trimesters, p value= 0.6674 (Table 4).

TNF -  $\alpha$  values were 89.1pg for 2<sup>nd</sup> trimester and 55.6 for 3<sup>rd</sup> trimester for the IgM positive study subjects, with significant association (p. value = 0.001). The mean TNF- $\alpha$  values for the IgM negatives for 1<sup>st</sup> 2<sup>nd</sup> and 3<sup>rd</sup> 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 2<sup>nd</sup> and 3<sup>rd</sup> trimester were 22.5% and 33.1%, with no participant appearing under 1<sup>st</sup> trimester. Statistical significance was observed in the lymphocytes count across 2<sup>nd</sup> and 3<sup>rd</sup> trimester (p value= 0.022). Neutrophil count was 68% in the 2<sup>nd</sup> trimester and 57.5% in the 3<sup>rd</sup> trimester, however, there was no significant association between neutrophil count and trimesters (p value = 0.162). Eosinophils were 7% in 2<sup>nd</sup> trimester and 6.06% in the 3<sup>rd</sup> trimester, and no statistical association was observed (p value = 0.750). Monocytes count was 2.5% and 3.93% for 2<sup>nd</sup> and 3<sup>rd</sup> 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/ $\mu$ l	p. value	CD8 cells/ $\mu$ l	p. value	CD4: CD8	p. value
1 <sup>st</sup>	1	985	0.192	531	0.5479	1.17	0.2978
2 <sup>nd</sup>	12	756		548		1.47	
3 <sup>rd</sup>	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 -  $\alpha$  and IFN -  $\gamma$  levels in the study population that were *T. gondii* IgM negative and positive based on Trimester of pregnancy**

Trimester	N	IgM Negative.				IgM Positive				
		IFN- $\gamma$ (pg)	p. value	TNF - $\alpha$ (pg)	p. value	N	IFN- $\gamma$ (pg)	p. value	TNF- $\alpha$ (pg)	p. value
1 <sup>st</sup>	01	3.31	0.6674	0.60	0.440	0.0	0.0	0.596	0.0	0.001
2 <sup>nd</sup>	12	44.27		8.40		03	18.5		89.1	
3 <sup>rd</sup>	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- $\gamma$ = Interferon-gamma, TNF- $\alpha$ = 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
1 <sup>st</sup>	0	0		0	0.0076	0	0.0278	0	0.4986
2 <sup>nd</sup>	03	22.50	0.0415	68.0		7.00		2.50	
3 <sup>rd</sup>	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 1<sup>st</sup> trimester, 25.2% in the second trimester and 31.54% in the 3<sup>rd</sup> trimester with no significant difference (p value = 0.6123), Neutrophils were 62.73%, 64.08% and 63.45% for 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> trimester respectively with p. value of 0.7774, signifying no statistical significance. Eosinophils

mean differential counts for 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> 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 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> 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
1 <sup>st</sup>	01	27.90	0.6123	62.70	0.7774	1.85	0.5243	02	0.1789
2 <sup>nd</sup>	12	25.20		64.08		1.75		2.3	
3 <sup>rd</sup>	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 Druish, 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 (3<sup>rd</sup> 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 salads 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 must 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<sup>+</sup> 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<sup>+</sup> cell counts in HIV negative pregnant women in Nigeria and Kenya respectively.

This study found the mean CD4 cell counts for 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> 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/ $\mu$ l and Aina *et al.* (2005) that obtained 771 cells/ $\mu$ l. This is expected, given the relatively critical essence of CD4 T-lymphocyte in controlling such intracellular 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<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> 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- $\gamma$  and TNF- $\alpha$  using sera from infected and uninfected pregnant women yielded elevated values relative to uninfected pregnant women, with highest elevation of IFN- $\gamma$  for the infected pregnant women occurring in the 3<sup>rd</sup> 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- $\gamma$  and TNF- $\alpha$ ), 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- $\gamma$  and TNF- $\alpha$  in pregnant mice (Dupont *et al.*, 2012). de-la-Torre *et al.* (2013) showed that IFN- $\gamma$  levels were elevated in asymptomatic individuals infected with *T. gondii*. In contrast, Pernas *et al.* (2014) observed that IFN- $\gamma$  and TNF- $\alpha$  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- $\gamma$ , TNF- $\alpha$  and trimesters for both the infected and uninfected study subjects, however, in TNF- $\alpha$ , a noticeable elevation was observed in the infected pregnant women and a statistical correlation exists across the trimesters. In toxoplasmosis, TNF- $\alpha$  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- $\alpha$  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- $\gamma$  and TNF- $\alpha$ , assayed in this study were elevated and not statistically significant across trimesters other than TNF- $\alpha$

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.

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