

### LYMPHOCYTE TRANSFORMATION OF HEPATITIS B VIRUS INFECTED PREGNANT WOMEN, ATTENDING SPECIALIST HOSPITAL SOKOTO.

#### ABSTRACT

Hepatitis B virus (HBV) is a major public health problem in sub-Saharan Africa with high morbidity and mortality. Vertical transmission is a significant contributor to new cases. This study aimed to determine the prevalence of HBV infection, to assess the immune competence of Hepatitis B (HB) viral infected pregnant women using lymphocyte transformation. It was a cross-sectional comparative observational study. Simple random sampling technique was applied. One hundred HB infected pregnant women and one hundred controls were recruited. Data were analysed using SPSS (version 23) software. A P-value  $\leq 0.05$  was considered statistically significant. The results recorded showed a prevalence of 6.6 %. The percentage of lymphocyte transformation was significantly lower ( $p < 0.05$ ) for HBV infected subjects compared with control. The rate of lymphocyte transformation with Phytohaemagglutinin was significantly lower ( $p < 0.05$ ) when compared with Concanavalin A. Conclusively HB infection affects the adaptive immune response. Pregnant women should be screened for Hepatitis B surface Antigen (HBsAg) during routine Antenatal clinic and Concanavalin A-based drugs should be recommended for HB infected pregnant women.

**Keywords:** Hepatitis B, lymphocyte transformation, Phytohaemagglutinin, Concanavalin A

#### INTRODUCTION

Hepatitis B virus (HBV) is a DNA virus of the family *Hepadnaviridae* and the causative agent of hepatitis B (HB) infection (Pungpapong *et al.*, 2007). It is 50-100 times more infectious than the Human Immunodeficiency Virus (HIV) and 10 times more infectious than hepatitis C virus (HCV). Many carriers do not realize they are infected with the virus, thus it is referred to as a “silent killer” (Samuel *et al.*, 2004). The minimum infectious dose is so low that practices like sharing a toothbrush or a razor blade can lead to transmission of the infection (Chang, 2008). HBV also shares similar routes of transmission with HIV (Willey *et al.*, 2008). Approximately 350 million people are infected with HBV worldwide with Nigeria classified among the group of countries endemic for HBV infection (Eke *et al.*, 2011). Vertical transmission is a significant contributor of new HBV cases each year, with 35–50% of transmission from mother to neonate in endemic countries (ACOG, 2016; Cunningham *et al.*, 2013). Vertical transmission occurs largely via a neonate's exposure to maternal blood and vaginal secretions during birth. (Cunningham *et al.*, 2013). While the risk of progression to chronic infection is approximately 5% among infected adults, it is as high as 95% among neonates subject to vertical

transmission. The risk of viral transmission is approximately 10–20% when maternal blood is positive for HBsAg, and up to 90% when also positive for HBeAg (ACOG, 2016).

In Nigeria the following prevalence has been documented; Saidu *et al.* (2015) reported 6.51% among pregnant women in Sokoto, Nigeria. Yakasai *et al.* (2012) reported a prevalence of 7.9% among pregnant women in Kano, Nigeria. While in the South East, Ezeani *et al.* (2008) reported 7% among pregnant women and Oluboyo *et al.* (2014) reported 6% in pregnant women in Nnewi, South-East Nigeria.

The immune responses of HB viral infection are in two phases; the innate which acts in the early acute phase, where natural killer (NK) cells are the first lines of defence, and the activation of these cells helps to reduce the viral load through the secretion of cytokines, such as IFN-gamma (Lanier, 2008). The crucial role in the pathogenesis of HBV infection and the control of HBV replication is played by the adaptive immune response primed after the initial response by the innate immunity. These responses involve B lymphocytes and plasma cells, which produce antibodies, CD4+ T lymphocytes (helper T cells), CD8+ T lymphocytes (cytotoxic T cells), and antigen-presenting cells (APC) such as dendritic cells and macrophages (Rosenberg, 1999).

The competence of the immune system of HBV infected pregnant women could be evaluated by determining the lymphocyte transformation under the influence of some mitogens or antigen since lymphocyte function is a central mechanism involved in coordinated immune responses. Therefore, the present study is designed to assess the lymphocyte function in HBV infected pregnant women using the lymphocyte transformation test.

Once an individual is infected by the HBV, the acute phase of the disease is followed by an initial immunologic response that tries to limit the multiplication of the viral particles within the host. Immediately this effort to clear the virus fails, the infected host immune system will be compromised owing to the exponential increase of the virus (Ishikawa, 2012). This may lead to vertical transmission of HB before or at delivery (Cunningham *et al.*, 2013; Behrouz *et al.*, 2011).

The yoke of morbidity and mortality due to HBV infection are high in developing countries (WHO, 2016; GBDS, 2015; Zampino *et al.*, 2015). The infectivity spreads through the different population strata. Children, adults and pregnant women are all prone to this viral infection (Ray and Bradley, 2011). However, of great concern is infectivity amongst pregnant women due to the state of the immune response during pregnancy and possible transmission to the baby (Behrouz *et al.*, 2011). Immunity to HB infections is the function of both the innate and adaptive immune response. There is the paucity of data as regards lymphocyte transformation in HB infected pregnant women in the study area which informed the current study. The results from this study may have potential benefit for continuous management of HB viral infected pregnant women.

The study aimed to assess the immune competence of HB viral infected pregnant women using the lymphocyte transformation

The objectives are to:

1. Determine the prevalence of HBV infection in pregnant women in Specialist Hospital Sokoto.
2. Assess the lymphocyte transformation in HB viral infected pregnant women.
3. Evaluate the rate of lymphocyte transformation when induced with mitogens such as phytohaemagglutinin and concanavalin A.
4. Compare the rate of lymphocyte transformation in HB viral infected pregnant women with non-infected pregnant women.

## **MATERIALS AND METHODS**

### **STUDY AREA**

The study was carried out in the Department of Obstetrics and Gynaecology, Specialist Hospital Sokoto in Sokoto State. It is a tertiary hospital and a referral centre for all the General Hospitals in the state. It has a 120-bed capacity and the Department of Obstetrics and Gynaecology runs an Ante-Natal clinic with the patient size of about 70-90 weekly. Sokoto state shares boundary with the Republic of Niger to the North, Kebbi State to the West and South, and Zamfara to the South and East. The total population and annual growth rate stood at 3.7 million and 3.0% respectively in the 2014 national population census (NPC, 2014).

## **STUDY POPULATION**

In this study, pregnant women attending ante-natal clinic from March to October were screened for HBV using Onsite HBsAg Rapid Test Strip manufactured by CTKBiotech, California USA. The pregnant women were sub-grouped into HBV infected group (Test) and HBV non-infected group (control). The pregnant women were further grouped according to pregnancy trimester / gestational age. However none in the first trimester was reported because they start reporting for antenatal in their second trimester. The pregnant women were also sub-grouped according to their age brackets. Samples obtained from participants were evaluated for their lymphocyte transformation assay.

## **STUDY DESIGN AND SAMPLING TECHNIQUE**

It is a cross-sectional comparative observational study. Simple random sampling technique was applied for recruiting pregnant women until the desired sample size was obtained.

### **Sample Size Determination**

The sample size for the study was determined using the standard formula for calculation of minimum sample size (Taofeek, 2009), using a previous prevalence of 6.51% (0.065) (Saidu *et al.*, 2015). A minimum sample size of 98 was obtained including 5% attrition rate. However, the sample size was rounded up to 100 for convenience.

### **Ethical Considerations and Informed Consent**

The ethical approval for this research was obtained from the Ethics and Research Committee of the Sokoto State Ministry of Health (SKHREC/024)016) dated 1/08/16 and from the Specialist Hospital Sokoto (SHS/SUB/133/VOL.1) dated 9/6/16.

Informed consent for inclusion into the study was obtained from each participant using a standard informed consent form. At the enrolment, a structured interviewer-administered questionnaire was used to elicit data on subjects socio-economic and demographic characteristics including age, marital status, occupation, educational level, the status of Hepatitis A and B, and clinical stage of infection whether acute or chronic, whether on treatment or not.

### **Inclusion Criteria**

All consenting pregnant women aged 16-45 years, visiting the Ante-natal clinic of Specialist Hospital Sokoto were eligible to participate in the study.

## **Exclusion Criteria**

HB positive pregnant women were also screened for Hepatitis A, Hepatitis C and HIV and positive ones were excluded from the study.

## **BLOOD SAMPLE COLLECTION**

Using a sterile vacutainer, holder and needle, 6 ml of venous blood was aseptically collected from each subject (2 ml in a sterile plain and 4 ml in heparinized vacutainers). The blood in the plain vacutainer was allowed to clot at room temperature, after which it was centrifuged at 3000 rpm for 5 minutes to obtain a clear un-haemolyzed serum. The serum was transferred into a clean, dried serum vial and was rapidly used to determine HB status.

The 4 ml of the blood sample was used for the lymphocyte transformation assay using the method adopted by Onyenekwe *et al.*, (2011). The procedure is in three different steps (i) Lymphocyte isolation (ii) Lymphocyte viability test (iii) Lymphocyte culture techniques using mitogens stimulation in the culture medium.

## **PROCEDURE**

The test was performed at room temperature 25°C. The strip was immersed into the specimen, with the arrow pointing towards the specimen. It was taken out after 10 seconds and was laid on a flat, clean, dry, non-absorbed surface. The result was read after 15 minutes. The result was interpreted as described by the manufacturer (CTKBiotech, California USA).

## **LYMPHOCYTE TRANSFORMATION ASSAY**

Lymphocyte transformation assay was performed using the method adopted by Onyenekwe *et al.* (2011). The procedure is in three different steps

1. Lymphocyte isolation
2. Lymphocyte viability test
3. Lymphocyte transformation by culture techniques

### **Lymphocyte Isolation**

Lymphocyte isolation was performed using the method adopted by Boyum (1968).

### **Lymphocyte Viability Test**

The test was performed as described by Arinola (2005).

### **Lymphocyte Culture**

The technique was carried out as described by Onyenekwe *et al.* (2009)

## DATA ANALYSIS

Data analysis was carried out using SPSS (version 23) software. Descriptive analysis of percentages, mean, standard deviation and standard error of mean were carried out. The results of percentage lymphocyte transformed cells obtained from HBV positive pregnant subjects were compared with values of controls using independent sample T-test, while one-way analysis of variance (ANOVA) was used for comparisons of three or more groups. A posthoc analysis was carried out using LSD multiple comparisons test following recorded significance. A probability value less than or equal to 0.05 ( $P \leq 0.05$ ) was considered statistically significant.

## RESULTS

One hundred (100) HBV infected pregnant women were gotten after the screening of one thousand five hundred and eighteen (1,518) pregnant women, giving a prevalence rate of 6.6% (100/1518).

The result in Table 1 shows the percentage distribution of HB infection among pregnant women based on the socio-demographic characteristics of the study subjects. In this study, majority of the HBV-infected pregnant women were between 21- 25 years age group (34%), followed by 31-35 years (22%), then 16-20 years and 26-30 years with (18%) and (17%) respectively. Majority of the infected subjects were predominantly students (43%), followed by businesswomen with (31%), civil servants with (15%) and housewives with (11%). Based on their educational qualification, the majority were Secondary with (40%), followed by Islamic education with (30%), then Tertiary with (19%), and Primary with (11%). Finally, 55% were in their third trimester while 45% were in their second trimester.

**Table 1: Percentage Distribution of HBV-infected Pregnant Women based on Sociodemographic Characteristics of the Study Subjects**

<b>Characteristics</b>	<b>Number of subjects</b>	<b>Percentage (%)</b>
<b>Age(Years)</b>	100	100
16-20	18	18
21-25	34	34
26-30	17	17
31-35	22	22
36-above	9	9
<b>Tribe</b>	100	100
Hausa	88	88
Igbo	4	4
Yoruba	8	8
Ibera	0	0
<b>Occupation</b>	100	100
Students	43	43

Business	31	31
Civil servants	15	15
Housewives	11	11
<b>Educational Level</b>	100	100
Islamic education	30	30
Primary	11	11
Secondary	40	40
Tertiary	19	19
<b>Trimester</b>	100	100
Second trimester	45	45
Third trimester	55	55

Majority of the HBV-infected pregnant women are Hausa's (88%), and are predominantly students (43%) and fall within the age group of 21-25years with secondary educational level.

The result in Table 2 shows a comparison of Mean ( $\pm$  SD) Lymphocyte Transformation among HBV infected pregnant women and HBV non-infected pregnant women.

The mean ( $\pm$  SD) percentage lymphocyte transformation with PHA was significantly lower ( $P < 0.05$ ) in HBV infected pregnant women ( $4.78 \pm 2.51\%$ ), compared with the corresponding value of control participants ( $6.33 \pm 2.92\%$ ).

Likewise, the mean ( $\pm$  SD) percentage lymphocyte transformation with Con A was significantly lower ( $P < 0.05$ ) in HBV infected pregnant women ( $9.09 \pm 4.16\%$ ), compared with the corresponding value of control participants ( $12.69 \pm 5.38\%$ ).

Also, the mean ( $\pm$  SD) percentage lymphocyte transformation in the Test group and Control group with PHA was significantly lower ( $P < 0.05$ ) than that of Con A.

**Table 2: Mean Lymphocyte Transformation among Hepatitis B Infected Pregnant Women and Non-Infected Pregnant Women.**

Groups	Lymphocyte Transformation Con A (%)	Lymphocyte Transformation PHA (%)	P-value
Test (n=100)	$9.09 \pm 4.16$	$4.78 \pm 2.51$	0.005
Control (n=100)	$12.69 \pm 5.38$	$6.33 \pm 2.92$	0.003
P-Value	0.003	0.001	

Values are Mean  $\pm$  SD, n= Number of subjects, % = percentage, Con A= Concanavallin A, PHA = Phytohaemagglutinin, Test = HBV infected pregnant women, Control = HBV non-infected pregnant women.

The result in Table 3 shows Mean ( $\pm$  SD) Percentage Lymphocyte Transformation in HBV infected pregnant women of different age ranges.

The mean ( $\pm$  SD) percentage lymphocyte transformation with Con A between the different age groups showed no significant difference ( $P > 0.05$ ) in each case, while the mean ( $\pm$  SD) percentage lymphocyte transformation with PHA between the different age groups showed a significantly lower difference ( $P < 0.05$ ).

The mean ( $\pm$  SD) percentage lymphocyte transformation in the different Age groups (16-20), (21-25), (26-30), (31-35) and (36-above) with PHA was significantly lower ( $P < 0.05$ ) than that of Con A within the same age group in each case.

In the Post hoc using LSD, the mean ( $\pm$  SD) percentage lymphocyte transformation with Con A between age (16-20) and (36 and above), showed a statistically non-significant difference ( $P > 0.05$ ), while the mean ( $\pm$  SD) percentage lymphocyte transformation with PHA between age (16-20) and (36 and above) showed a significantly lower difference ( $P < 0.05$ ).

Similarly, the mean ( $\pm$  SD) percentage lymphocyte transformation with Con A and PHA between age (21-25) and (36 and above) showed a significantly lower difference ( $P < 0.05$ ) in each case.

Also, the mean ( $\pm$  SD) percentage lymphocyte transformation with Con A and PHA between age (31-35) and (36 and above) showed a significantly lower difference ( $P < 0.05$ ) in each case.

**Table 3: Mean Lymphocyte Transformation among Hepatitis B Infected Pregnant Women at different Age Ranges.**

Age (years)	Lymphocyte Transformation Con A (%)	Lymphocyte Transformation PHA (%)	P-value
16-20 (n=18)	8.25 $\pm$ 3.30	4.83 $\pm$ 2.38	0.010
21-25 (n=34)	9.69 $\pm$ 3.83	5.50 $\pm$ 2.81	0.001
26-30 (n=17)	8.74 $\pm$ 4.60	4.12 $\pm$ 2.42	0.005
31-35 (n=22)	10.36 $\pm$ 4.68	4.91 $\pm$ 2.15	0.003
36-above (n=9)	6.06 $\pm$ 3.52	2.83 $\pm$ 1.46	0.000
F-Value	2.209	2.507	
P-Value	0.074	0.047	
Post hoc Test Using LSD			
(16-20) vs (36-above)	0.189	0.047	
(16-20) vs (36-above)	0.019	0.004	
(31-35) vs (36-above)	0.009	0.034	



Values are Mean  $\pm$  SD, n=Number of subjects, Con A = Concanavalin A, PHA = Phytohaemagglutinin, % = percentage.

The effect of Trimester on Mean ( $\pm$  SD) Percentage Lymphocyte Transformation between HBV infected pregnant women (Test), HBV non-infected pregnant women (Control) is presented in Table 4.

The results indicate that the mean ( $\pm$  SD) percentage lymphocyte transformation with Con A and PHA, between 2nd-trimester test group compared with a 3rd-trimester test group and between 2nd-trimester control group compared with 3rd-trimester control group shows no significant difference ( $P > 0.05$ ) in each case.

On the other hand, mean ( $\pm$  SD) percentage lymphocyte transformation with Con A and PHA, and between 2nd-trimester test group and 2nd-trimester control group shows a significantly lower difference ( $P < 0.05$ ) in each case.

Also, the mean ( $\pm$  SD) percentage lymphocyte transformation with Con A and PHA between 3rd-trimester test group and 3rd-trimester control group shows a significantly lower difference ( $P < 0.05$ ) in both cases, between 3rd-trimester test group and 3rd-trimester control group shows no significant difference ( $P > 0.05$ ).

Finally, the mean ( $\pm$  SD) percentage lymphocyte transformation in the second-trimester test group, third-trimester test group, second-trimester control group and third-trimester control group with PHA was significantly lower ( $P < 0.05$ ) than that of Con A in each case.

**Table 4: Mean Lymphocyte Transformation among Hepatitis B Infected Pregnant Women and Non-infected Pregnant Women at different Trimesters.**

Trimester	Lymphocyte Transformation Con A (%)	Lymphocyte Transformation PHA (%)	P-value
2nd TT (n=45)	8.59 $\pm$ 4.77	4.62 $\pm$ 3.06	0.005
3rd TT (n=55)	9.50 $\pm$ 3.58	4.90 $\pm$ 1.97	0.001
2nd TC (n=50)	12.50 $\pm$ 6.02	6.13 $\pm$ 3.05	0.004
3rd TC (n=50)	12.87 $\pm$ 4.70	6.53 $\pm$ 2.80	0.005
F-Value	9.623	5.669	

P-Value	0.000	0.001
Post hoc Test Using LSD		
2nd TT vs 3rd TT	0.348	0.613
2nd TC vs 3rdTC	0.701	0.465
2nd TT vs 2nd TC	0.002	0.008
3rd TT vs 3rd TC	0.001	0.003

Values are Mean ± SD, n = Number of subjects, Con A = Concanavalin A, PHA = Phytohaemagglutinin, 2nd TT = second trimester test, 3rd TT = third trimester test, 2nd TC = second trimester control, 3rd TC = third trimester control

## DISCUSSION

This study revealed a prevalence of 6.6% of HB amongst pregnant women in Sokoto which is close to values of 6.51% previously reported by Saidu *et al.* (2015), Yakasai *et al.* (2012) reported a prevalence of 7.9% among pregnant women in Kano, Nigeria. This was also similar to work done in South-East Nigeria by Ezeani *et al.* (2008) who reported 7% among pregnant women in Awka and Oluboyo *et al.* (2014) who reported 6% among pregnant women in Nnewi, South-East Nigeria.

However, the study was at variance with the report by Jatau and Yabaya (2009) who recorded 13.3% and that of Luka *et al.* (2008) who recorded 8.3% respectively in Zaria. The variations in the reported seroprevalence of HBV in the pregnant women may be due to geographical variation, differences in cultural practices, sexual behaviour and practices, and differences in the test methods employed to detect HBV infection (Olokoba *et al.*, 2011). With greater awareness to HIV infection leading to a drastic drop from 6.4% in 2012 (FRN, 2014) to 2.4% in 2015 (Buseri and Okonkwo, 2015), this could also contribute to the low prevalence rate recorded since both HB and HIV share a similar route of transmission (Willey *et al.*, 2008).

Results from this study showed the highest prevalence of 34 % of the positive subject within (21-25years) age bracket. This also agrees with the findings of several authors like Jatau and Yabaya (2009) that recorded the highest prevalence (19%) within the same age bracket of (21-25years). This outcome is similar to reports by Olokoba *et al.* (2011) who recorded the highest prevalence 39.8% rates among women in age groups (25–29years). This may be associated with higher sexual activities within these age groups as 21-25years range in this study are within sexually active age groups (Olokoba *et al.*, 2011). Occupationally students recorded the highest percentage of 43% as the majority of those within 21-25years of age are students. Also, the third trimester recorded 55% while the second trimester had 45%. There is a strong indication that their babies may be exposed to this infectious agents and once exposed, have a higher risk (90%) of developing chronic infection compared to adolescents (50%) and adults (<10%) as the vertical transmission is thought to be a major mode of transmission of HBV in endemic areas such as Nigeria (Wright, 2006).

In this study, concanavalin A and phytohaemagglutinin were used to stimulate lymphocyte for blast formation. The percentage blast formation due to concanavalin A and phytohaemagglutinin were significantly lowered in HB infected pregnant women. Only the T cells that have not been pre-stimulated by the virus would be in a state to respond to stimulation. The observation in this study showed a significantly lower ( $P < 0.05$ ) lymphocyte transformation in HB infected pregnant women subjects compared with control. This is in agreement with the previous study done by Nahmais and O'Reilly (2012) that did not only show depression on non-specific responses but also recorded depression of cell-mediated immunity to HB antigens as demonstrated by lymphocyte transformation. The study is also in agreement with work done by Sodomann *et al.* (1979) that demonstrated that lymphocytes from patients with viral hepatitis are hypo-responsive to phytohaemagglutinin during the acute phase of the disease, but responsiveness returned to normal before recovery in all but chronic cases. The implication is that there is lowered blast formation (T cell function) in HB infection that leads to induced cellular immune disturbance since the adaptive immune response (T cell) play a crucial role in the pathogenesis and control of HBV infection.

The findings of this study revealed a significantly lower ( $P < 0.05$ ) stimulation rate for phytohaemagglutinin compared to concanavalin A for test and control group respectively, which signifies a higher proliferative response by concanavalin A stimulation to phytohaemagglutinin as observed for both groups. The result was in agreement with Faruk *et al.* (2005) and Barbora *et al.* (2016) who observed that concanavalin A is a stronger mitogen to phytohaemagglutinin invitro and in-vivo respectively. The difference in their stimulation property could be linked to the fact that concanavalin A can stimulate both the T cell and some B cell while phytohaemagglutinin is solely a T cell mitogen.

The observations in this study disclosed a significantly lower ( $P < 0.05$ ) lymphocyte function with phytohaemagglutinin concerning the different age groups. When ages (16-20), (21-25) and (31-35) were compared with (36-above) a significantly lowered ( $P < 0.05$ ) percentage mean lymphocyte transformation with phytohaemagglutinin was observed in each case. These findings also uncovered that a significantly lowered ( $P < 0.05$ ) percentage mean lymphocyte transformation with concanavalin A was observed when age groups (21-25) and (31-35) were compared with (36-above). This was in agreement with Kruisbeek (1976), and Yan *et al.* (2010) that demonstrated a decrease in lymphocyte transformation in ageing rats and with an increase in age of humans respectively. This could be attributed to the fact that T cell activation and proliferation decreases with age (Miller, 2000; Jiang *et al.*, 2007).

The findings of this study unveiled a significantly lower ( $P < 0.05$ ) stimulation rate for phytohaemagglutinin compared to concanavalin A in each age group, which signifies a higher proliferative response by concanavalin A stimulation to phytohaemagglutinin. The result was in agreement with Faruk *et al.* (2005) and Barbora *et al.* (2016), who observed that concanavalin A is a better mitogen to phytohaemagglutinin invitro and in-vivo respectively. The difference in their stimulation property could be linked to the fact that concanavalin A can stimulate

both the T cell and some B cell while phytohaemagglutinin is solely a T cell mitogen.

The observation in this study showed no significant difference ( $P > 0.05$ ) in each case for Concanavalin A and Phytohaemagglutinin lymphocyte function between second trimesters HB infected pregnant women compared with third trimester HB infected pregnant women. Also, there was no significant difference ( $P > 0.05$ ), for Con A and PHA lymphocyte function between second-trimester control compared with third-trimester control. This study is in agreement with Birkeland and Kristoffersen (2006) that demonstrated no change in lymphocyte transformation using phytohaemagglutinin in the different stages in pregnancy; however, this study is in variance with Saleem *et al.* (1992) and Yamamoto *et al.* (1980) that showed a significant diminishing difference of lymphocyte transformation between the trimesters. However, Saleem *et al.* (1992) recorded that multigravidae pregnant women have little or no change in their lymphocyte transformation while primigravidae pregnant women and pregnant women who had repeated abortion may show a drastic difference in their lymphocyte transformation in their different stages. The findings of this study also revealed a significantly lowered ( $P < 0.05$ ) stimulation rate in each case for phytohaemagglutinin compared to concanavalin A in the second-trimester test, third-trimester test, second-trimester control and third-trimester control. This signifies a higher proliferative response by concanavalin A stimulation to phytohaemagglutinin as observed for all the trimesters. The result was in agreement with Faruk *et al.* (2005) and Barбора *et al.* (2016), who observed that concanavalin A is a better mitogen to phytohaemagglutinin invitro and in-vivo respectively. The difference in their stimulation property could be linked to the fact that concanavalin A can stimulate both the T cell and B cell while phytohaemagglutinin is solely a T cell mitogen.

This study uncovered a significantly lower ( $P < 0.05$ ) lymphocyte transformation in each case for concanavalin A and phytohaemagglutinin between the second-trimester HB infected pregnant women compared with second trimester HB non-infected pregnant women that served as control subjects. This work also agrees with the fact that the reduced lymphocyte transformation was not brought about by the stages of pregnancy thereby in agreement with work done by Birkeland and Kristoffersen (2006), that demonstrated no change in lymphocyte transformation using phytohaemagglutinin in the different stages in pregnancy but that the reduced lymphocyte function was brought about by the activity of the virus thereby in agreement with Nahmais and O'Reilly (2012), that did not only show depression on non-specific responses but also recorded depression of cell-mediated immunity to HB antigens as demonstrated by lymphocyte transformation.

This study also revealed that there was a significantly lower ( $P < 0.05$ ) lymphocyte function in each case for concanavalin A and phytohaemagglutinin between the third-trimester HB infected pregnant women compared with third trimester HB non-infected pregnant women that served as control. It shows that the reduced lymphocyte transformation was not brought about by the stages of pregnancy thereby in agreement with work done by Birkeland and Kristoffersen (2006), that demonstrated no change in lymphocyte transformation using PHA in the different

stages in pregnancy but buttress the fact that the reduced lymphocyte function was brought about by the activity of the virus thereby in agreement with Nahmais and O'Reilly (2012), that show depression on non-specific responses and depression of cell-mediated immunity to HB antigens using lymphocyte transformation.

### **CONCLUSION**

The prevalence of HBV among pregnant women was observed as 6.6% in Sokoto Specialist Hospital.

The percentage blast formation (the functional activity of lymphocyte) significantly reduced in HBV infected pregnant women compared to the uninfected pregnant women. This indicated that the cell-mediated immune system was negatively affected by HB infection.

The rate of stimulation with Con A was almost twice compared to PHA

### **RECOMMENDATIONS**

The following recommendations were made from the findings of this study:

1. Pregnant women should be consistently screened for HBsAg during routine ANC.
2. Drugs should be recommended for HB infected pregnant women.
3. Further studies may focus on stimulating B cells, To have more understanding of the contribution of B cells immunity to Hepatitis infection.
4. Future research should focus on the determination of cytokine levels of cultured lymphocytes isolated from Hepatitis infected patients.
5. Future research should be conducted to include those on treatment in the study to assess the impact of the drugs on the immune cells.

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