Journal of Advances in Medicine and Medical Research

32(5): 97-107, 2020; Article no.JAMMR.55855
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Lymphocyte Transformation of Hepatitis B Virus Infected Pregnant Women, Attending Specialist Hospital Sokoto

C. O. Onwuliri[1*], C. C. Onyenekwe[2], M. H. Yeldu[3], A. A. Panti[4], A. B. Shuaibu[5] and M. Kalgo[3]

[1]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, P.M.B. 2346 Sokoto, Sokoto State, Nigeria.
[2]Department of Medical Laboratory Science, College of Health Sciences, Nnamdi Azikiwe University, P.M.B. 5025 Awka, Anambara State, Nigeria.
[3]Department of Immunology, School of Medical Laboratory Science, Usmanu Danfodiyo University Sokoto, P.M.B. 2346 Sokoto, Sokoto State, Nigeria.
[4]Department of Obstetrics and Gynaecology, Usmanu Danfodiyo University Teaching Hospital Sokoto, P.M.B. 2370 Sokoto, Sokoto State, Nigeria.
[5]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University Sokoto, P.M.B. 2346 Sokoto, Sokoto State, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors COO and CCO designed the study. Authors COO, CCO, MHY and AAP managed the analyses of the study. Authors COO, CCO, MHY, AAP and MK managed the literature searches and wrote the protocols. Authors COO and ABS performed the statistical analysis and wrote the first draft manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2020/v32i530419
Editor(s):
(1) Dr. Ione Maria R. Soares Lopes, Federal University of Piaui (UFPI), Brazil.
Reviewers:
(1) Sevede Daouda, Côte d'Ivoire.
(2) Syed Naqui Kazim, Jamia Millia Islamia University, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/55855

Original Research Article

Received 10 February 2020
Accepted 16 April 2020
Published 23 April 2020

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 of new cases. The aim of this study was 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

**Corresponding author: E-mail: christopher.onwuliri@udusok.edu.ng;*

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 ≤ 0.05 was considered statistically significant. The results recorded showed a prevalence of 6.6%. The percentage 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.

1. INTRODUCTION

Hepatitis B virus (HBV) is a DNA virus of the family *Hepadnaviridae* and the causative agent of hepatitis B (HB) infection [1]. It is 50-100 times more infectious than 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" [2]. The minimum infectious dose is so low that practices like sharing a tooth brush or a razor blade can lead to transmission of the infection [3]. HBV also shares similar routes of transmission with HIV [4]. Approximately 350 million people are infected with HBV worldwide with Nigeria classified among the group of countries endemic for HBV infection [5]. Vertical transmission is a significant contributor of new HBV cases each year, with 35–50% of transmission from mother to neonate in endemic countries [6,7]. Vertical transmission occurs largely via a neonate's exposure to maternal blood and vaginal secretions during birth [7]. 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 [6].

In Nigeria the following prevalence has been documented; Saidu et al. [8] reported 6.51% among pregnant women in Sokoto, Nigeria. Yakasai et al. [9] reported a prevalence of 7.9% among pregnant women in Kano, Nigeria. While in the South East, Ezeani et al. [10] reported 7% among pregnant women and Oluboyo et al. [11] 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 defense, and the activation of these cells helps to reduce the viral load through the secretion of cytokines, such as IFN-gama [12]. 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 [13].

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 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 exponential increase of the virus [14]. This may lead to vertical transmission of HB before or at delivery [7,15].

The yoke of morbidity and mortality due to HBV infection are high in developing countries [16,17,18]. The infectivity spreads through the different population strata. Children, adults and pregnant women are all prone to this viral infection [19]. However, of great concern is infectivity amongst pregnant women due to the

state of immune response during pregnancy and possible transmission to the baby [15]. Immunity to HB infections is the function of both the innate and adaptive immune response. There is 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 aim of the study was to assess the immune competence of HB viral infected pregnant women using the lymphocyte transformation. The objectives are to:

Determine the prevalence of HBV infection in pregnant women in Specialist Hospital Sokoto and assess the lymphocyte transformation in HB viral infected pregnant women.

Evaluate the rate of lymphocyte transformation when induced with mitogens such as phytohaemagglutin and concanavalin A and compare the rate of lymphocyte transformation in HB viral infected pregnant women with non infected pregnant women.

2. MATERIALS AND METHODS

2.1 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 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 [20].

2.2 Study Population

In this study pregnant women attending ante-natal clinic within the period of October, 2017 to March, 2018 were screened for HBV using Onsite HBsAg Rapid Test Strip manufactured by CTKBiotech, Carlifonia USA. The pregnant women were sub-grouped into HBV infected group (Test) and HBV non infected group (control). The pregnant women where further grouped according to pregnancy trimester / gestational age. However non in the first trimester was reported due to the fact that 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.

2.3 Study Design and Sampling Technique

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

2.4 Sample Size Determination

The sample size for the study was determined using the standard formula for calculation of minimum sample size [21], using a previous prevalence of 6.51% (0.065) [8]. A minimum sample size of 98 was obtained including 5% attrition rate. However, the sample size was rounded up to 100 for convenience.

2.5 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.

2.6 Exclusion Criteria

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

2.7 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 blood sample was used for the lymphocyte transformation assay using the method adopted by Onyenekwe et al. [22].

2.8 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, Carlifonia USA).

2.9 Lymphocyte Transformation Assay

Lymphocyte transformation assay was performed using the method adopted by Onyenekwe et al. [22]. The procedure is in three different steps

- Lymphocyte isolation.
- Lymphocyte viability test.
- Lymphocyte transformation by culture techniques.

2.9.1 Lymphocyte isolation

Lymphocyte isolation was performed using the method adopted by Boyum [23].

2.9.2 Lymphocyte viability test

The test was performed as described by Arinola [24].

2.9.3 Lymphocyte culture

The technique was carried out as described by Onyenekwe et al. [25].

2.10 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 post-hoc analysis was carried out using LSD multiple comparisons test following recorded significance. Probability value less than or equal to 0.05 ($P \leq 0.05$) was considered statistically significant.

3. RESULTS

One hundred (100) HBV infected pregnant women were gotten after 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 the pregnant women based on 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 business women with (31%), civil servants with (15%) and housewives with (11%). Based on their educational qualification, 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.

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

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

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

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

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

The mean (± 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 (± SD) percentage lymphocyte transformation with PHA between the different age groups showed a significantly lower difference ($P < 0.05$).

Table 1. Percentage distribution of HBV-infected pregnant women based on sociodemographic characteristics of the study subjects

Characteristics	Number of subjects	Percentage (%)
Age(Years)	100	100
16-20	18	18
21-25	34	34
26-30	17	17
31-35	22	22
36-above	9	9
Tribe	100	100
Hausa	88	88
Igbo	4	4
Yoruba	8	8
Ibera	0	0
Occupation	100	100
Students	43	43
Business	31	31
Civil servants	15	15
Housewives	11	11
Educational Level	100	100
Islamic education	30	30
Primary	11	11
Secondary	40	40
Tetiary	19	19
Trimester	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

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 ± 4.16	4.78 ± 2.51	0.005
Control (n=100)	12.69 ± 5.38	6.33 ± 2.92	0.003
P-Value	0.003	0.001	

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

The mean (± 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 (± SD) percentage lymphocyte transformation with Con A between age (16-20) and (36 and above), showed a statistically non significant differences ($P > 0.05$), while the mean (± 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 (± 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 (± 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.

The effect of Trimester on Mean (± 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 (± SD) percentage lymphocyte transformation with Con A and PHA, between 2nd trimester test group compared with 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 (± 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 (± SD) percentage lymphocyte transformation with Con A and PHA between 3rd trimester test group and 3rd trimester control group shows a significantly lower differences ($P < 0.05$) in both cases, between 3rd trimester test group and 3rd trimester control group shows no significant difference ($P > 0.05$).

Finally, the mean (± 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 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 ± 3.30	4.83 ± 2.38	0.010
21-25 (n=34)	9.69 ± 3.83	5.50 ± 2.81	0.001
26-30 (n=17)	8.74 ± 4.60	4.12 ± 2.42	0.005
31-35 (n=22)	10.36 ± 4.68	4.91 ± 2.15	0.003
36-above (n=9)	6.06 ± 3.52	2.83 ± 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 ± SD, n=Number of subjects, Con A = Concanavalin A, PHA = Phytohaemagglutinin, % = percentage

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 ± 4.77	4.62 ± 3.06	0.005
3rd TT (n=55)	9.50 ± 3.58	4.90 ± 1.97	0.001
2nd TC (n=50)	12.50 ± 6.02	6.13 ± 3.05	0.004
3rd TC (n=50)	12.87± 4.70	6.53 ± 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

4. 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. [8], Yakasai et al. [9] 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. [10] who reported 7% among pregnant women in Awka and Oluboyo et al. [11] who reported 6% among pregnant women in Nnewi, South East Nigeria.

However, the study was at variance with the report by Jatau and Yabaya [26] who recorded 13.3% and that of Luka et al. [27] who recorded 8.3% respectively in Zaria. The variations in the reported sero-prevalence of HBV in the pregnant women may be due to geographical variation, differences in cultural practices, sexual behavior and practices, and differences in the test methods employed to detect HBV infection [28]. With greater awareness to HIV infection leading to a drastic drop from 6.4% in 2012 [29] to 2.4% in 2015 [30], this could also contribute to the low prevalence rate recorded since both HB and HIV share a similar route of transmission [4].

Results from this study showed the highest prevalence of 34% of the positive subject within (21-25 years) age bracket. This also agrees with the findings of several authors like Jatau and Yabaya [26] that recorded the highest prevalence (19%) within the same age bracket of (21-25 years). This outcome is similar with reports by Olokoba et al. [28] who recorded the highest prevalence 39.8% rates among women in age groups (25–29 years). This may be associated with higher sexual activities within these age groups as 21-25 years range in this study are within sexually active age groups [28]. Occupationally students recorded the highest percentage of 43% as majority of those within 21-25 years of age are students. Also the third trimester recorded 55% while the second trimester had 45%. There is strong indication that their babies may be exposed to this infectious agents and once exposed, have higher risk (90%) of developing chronic infection compared to adolescents (50%) and adults (<10%) as vertical transmission is thought to be a major mode of transmission of HBV in endemic areas such as Nigeria [31].

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 [32] 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. [33] that demonstrated that lymphocytes from patients with viral hepatitis have been found to be 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. [34] and Barbora et al. [35] who observed that concanavalin A is a stronger mitogen to phytohaemagglutinin *in vitro* and *in vivo* respectively. The difference in their stimulation property could be link 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 with respect to 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 [36], and Yan et al. [37] that demonstrated decrease in lymphocyte transformation in ageing rats and with increase in age of humans respectively. This could be attributed to the fact that T cell activation and proliferation decreases with age [38,39].

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. [34] and Barbora et al. [35], who observed that concanavalin A is a better mitogen to phytohaemagglutinin *in vitro* and *in vivo* respectively. The difference in their stimulation property could be link 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 [40] 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. [41] and Yamamoto et al. [42] that showed a significant diminishing difference of lymphocyte transformation between the trimesters. However, Saleem et al. [41] recorded that mutigravidae pregnant women has little or no change in their lymphocyte transformation while primigravidae pregnant women and pregnant women who had 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 second trimester test, third trimester test, second trimester control and third trimester control. This signifies a higher proliferative response by concanavalin A stimulation to phytohaemagglutinatin as observed for all the trimesters. The result was in agreement with Faruk et al. [34] and Barbora et al. [35], who observed that concanavalin A is a better mitogen to phytohaemagglutinin *in vitro* and *in vivo* respectively. The difference in their stimulation property could be link 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 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 [40], 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 [32], 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 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 [40], 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 [32], that show depression on non specific responses and depression of cell-mediated immunity to HB antigens using lymphocyte transformation.

5. CONCLUSION

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

The percentage blast formation (functional activity of lymphocyte) significantly reduced in HBV infected pregnant women compared to the uninfected pregnant women. This indicated that cell mediated immune system was negatively affected in HB infection. The rate of stimulation with Con A was almost twice compared to PHA.

6. RECOMMENDATIONS

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

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

CONSENT AND ETHICAL APPROVAL

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pungpapong S, Kim WR, Poterucha JJ. Natural history of hepatitis B virus infection: An update for clinicians. Mayo Clinical Procedures. 2007;82:967-975.
2. Samuel D, Muller R, Alexande G. Educational Research, National Hepatitis B Virus Programme. Infectious Diseases. 2004;234:221-332.
3. Chang MH. Hepatitis B virus infection. Foetal, Neonatal Medicine. 2008;12(3): 160-167.
4. Willey JM, Sherwood LM, Woolverton CJ. Presscott, Harley and Kleins microbiology 4th Ed. McGraw Hill Publishers, New York, USA. 2008;936-972.
5. Eke AC, Eke UA, Okafor CI, Ezebielu IU, Ogbuagu C. Prevalence correlates and pattern of hepatitis B surface antigen in a low resource setting. Virology Journal. 2011;8:12.
6. American Congress of Obstetrician and Gynaecologists. Clinical Management Guidelines for Obstetrician-Gynecologists: “Viral Hepatitis in Pregnancy". Practice Bulletin; 2016.
   Available:www.acog.org
7. Cunningham FG, Leveno KJMD, Bloom SL, Spong SY, Dashe JS, Hoffman BL, Casey BM, Sheffield JS. Hepatic, biliary and pancreatic disorders. Williams Obstetrics, Twenty-Fourth Edition. New York, NY: McGraw-Hill; 2013.
8. Saidu AY, Salihu Y, Umar AA, Muhammad BS, Abdullahi I. Seroprevalence of hepatitis B surface antigen among pregnant women attending Ante-Natal Clinics in Sokoto Metropolis. Journal of Nursing and Health Science. 2015;4:46-50.
9. Yakasai IA, Ayyuba R, Abubakar IS, Ibrahim SA. Sero-prevalence of hepatitis B virus infection and its risk factors among pregnant women attending Antenatal Clinic at Aminu Kano Teaching Hospital, Kano, Nigeria. Journal of Basic and Clinical Reproductive Sciences. 2012;1:1-2.
10. Ezeani MC, Onyenekwe CC, Meludu SC, Okonkwo JEN, Igwegbe AO, Anyiam DCD. Prevalence of malaria parasites, hepatitis B and C viral infections in pregnant women attending antenatal clinic in Nnewi Nigeria. Journal of Biomedical Investigation. 2008;6(1):1-6.

11. Oluboyo BO, Ugochukwu VI, Oluboyo AO, Ihim AC, Chukwuma GO, Ogenyi SI, Onyemelukwe A. Prevalence of hepatitis B and C viral infections in pregnant women attending antenatal clinic in Nnewi, Nigeria. European Scientific Journal. 2014;10(3):1857–1861.
12. Lanier LL. Evolutionary struggles between NK cells and viruses. Nature Review Immunology. 2008;8:259–268.
13. Rosenberg W. Mechanisms of immune escape in viral hepatitis. Gut. 1999;44:759-764.
14. Ishikawa T. Immunoregulation of hepatitis B virus infection–rationale and clinical application. Nagoya Journal of Medical Science. 2012;74:217-232.
15. Behrouz N, Narges M, Arezoo E, Mehdi M, Hossein P. Hepatitis B virus infection during pregnancy: Transmission and prevention. Middle East Journal of Digestive Disease. 2011;3(2):92–102.
16. World Health Organisation. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection; 2016.
17. Global Burden of Disease Study 2013, Collaborators. Global, regional and national incidence, prevalence and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;386(9995):743–800.
18. Zampino R, Boemio A, Sagnelli C, Alessio L, Adinolfi LE, Sagnelli E, Coppo N. Hepatitis B virus buden in developing countries. World Journal of Gastroenterology. 2015;21(14):11941-11953.
19. Ray MM, Bradley DH. Seroprevalence of markers for hepatitis B viral infection. International Journal of Infectious Diseases. 2011;15:78-121.
20. National Population Commission. 2006 National Population Census: Federal Republic of Nigeria, Official Gazette. 2014;94(24):96-97.
21. Taofeek I. Research methodology and dissertation for health and allied health professionals. 1st Edition. Cress Global Link Limited, Abuja, Nigeria. 2009;70-74.
22. Onyenekwe CC, Ifeanyi MI, Ele PU, Ukibe NR, Meludu SC, Ezeani MC, Ezeachukwu CC, Amilo GI, Umeanaeto PU. Evaluation of some cellular immune index in HIV infected participants. International Journal of Biochemical Sciences. 2011;5(3):1311-1313.
23. Boyum A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scandinavian Journal of Clinical and Laboratory Investigation. 1968;97:77-89.
24. Arinola OG. Leucocyte phagocytosis in children with urinary schistosomiasis and asymptomatic malaria parasitemia. African Journal of Clinical and Experimental Microbiology. 2005;6(2):81-86.
25. Onyenekwe CC, Ukibe NR, Meludu SC, Igwegbe AO, Ifeanyi MI, Ezeugwunne I, Ezeani M, Onochie A, Ofiaeli N, Aboh N, Ilika A. Neutrophil ingestion rate of nitroblue tetrazolium in subjects with malaria-HIV co-morbidity. Tropical Journal of Medical Research. 2009;12(1):1-5.
26. Jatau ED, Yabaya A. Sero-prevalance of hepatisis B virus in pregnant women attending a clinic in Zaria, Nigeria. Science World Journal. 2009;4(2):1-3.
27. Luka SA, Ibrahim MB, Iliya SN. Seroprevalence of hepatitis B surface antigen among pregnant women attending Ahmadu Bello University Teaching Hospital Zaria. Nigerian Journal of Parasitology. 2008;29(1):38-41.
28. Olokoba AB, Salawu FK, Danburam A, Olokoba LB, Midala JK, Badung LH, Olatinwo A. Hepatitis B virus infection amongst pregnant women in North-Eastern Nigeria – A call for action. Nigerian Journal of Clinical Practice. 2011;14:10-13.
29. Federal Republic of Nigeria. The 2014 Global AIDS Response Program Report (GARPR); 2014.
30. Buseri FI, Okonkwo CN. Population-based survey of HIV sero-status and vertical transmission among naïve pregnant women in Sokoto, Nigeria. Asian Journal of Medical Sciences. 2015;6(3):49-57.
31. Wright TL. Introduction to chronic hepatitis B infection. American Journal of Gastroenterology. 2006;101(1):1-6.
32. Nahmais AJ, O'Reilly RJ. Immunology of human infection: Viruses and parasites. Springer Science and Business Media. 2012;212-213.
33. Sodomann CP, Rother M, Havemann K, Martini GA. Lymphocyte transformation to

phytohaemagglentinin (PHA) in hepatitis B antigen-positive and negative hepatitis. Research in Experimental Medicine. 1979;175:95-107.
34. Faruk S, Bayram K, Gunnur D, Osman C. Concanavalin A and phytohaemagglutinin stimulated lymphoproliferative responses in cord blood mononuclear cells. Turkish Journal of Immunology. 2005;10(1):13-18.
35. Barbora B, Tomáš A, Milada C, Vladimír H, Jaroslav P, Michal V. Application of concanavalin A during immune responsiveness skin-swelling tests facilitates measurement interpretation in mammalian ecology. Ecology Evolution. 2016;6(13):4551-4564.
36. Kruisbeek AM. Age-related changes in ConA- and LPS-induced lymphocyte transformation. I. Effect of culture conditions on mitogen responses of blood and spleen lymphocytes from young and aged rats. Mechanism of Ageing and Development. 1976;5:125-138.
37. Yan J, Greer JM, Hull R, O'Sullivan JD, Henderson RD, Read SJ, McCombe PA. Ageing on human lymphocyte subsets: Comparison of males and females. Immunity and Ageing. 2010;7:4.
38. Miller RA. Effect of ageing on T lymphocyte activation. Vaccine. 2000;18:1654-1660.
39. Jiang J, Gross D, Elbaum P, Murasko DM. Aging affects initiation and continuation of T cell proliferation. Mechanism of Ageing and Development. 2007;128:332-339.
40. Birkeland SA, Kristoffersen K. Lymphocyte transformation with mitogens and antigens during normal human pregnancy: A longitudinal study. Scandinavian Journal Immunology. 2006;11(3):321–325.
41. Saleem MA, Jha P, Buckshee K, Farooq A. Studies on mitogen-Induced lymphocyte transformation and the effect of pregnancy serum on mitogen-Induced normal lymphocyte culture. Journal of Gynecology and Obstetrics Investigation. 1992;33:9–14.
42. Yamamoto T, Hirata H, Taniguchi H, Kawai Y, Uematsu A, Sugiyama Y. Lymphocyte transformation during pregnancy: An analysis using whole-blood culture. Obstetrics and Gynaecology. 1980;55(2): 215-219.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/55855