Original Research Article

SERO-SURVEILLANCE OF HEPATITIS B VIRUS AMONG PREGNANT WOMEN IN SOKOTO, NORTH-WESTERN NIGERIA.

ABSTRACT

Background: Maternal infection with Hepatitis B virus (HBV) can lead to chronic infection in the neonate and the risk depends on her status of Hepatitis B surface antigen (HBsAg) and Hepatitis B e antigen (HBeAg).This study aimed at determining the sero-surveillance of HBV among pregnant women in Sokoto, Nigeria. Blood samples from 93 pregnant women were tested for the HBV using Immunochromatographic Methods (Aria, USA) and seropositive samples evaluated for TNF-alpha and ALT. Questionnaires were administered to get socio-demographic information. Statistical Analysis: SPSS ® statistical software. Results: The HBV seroprevalence was17.2% for HBsAg. Conclusions: The seroprevalence of HBsAg was 17.2%. Elevated TNF-alpha level and ALT level were associated with HBV infection. Therefore, screening of pregnant women for Hepatitis B is necessary to identify the neonates at risk.

Keywords: Hepatitis B Virus, Hepatitis B Surface Antigen, Hepatitis B Surface Antibody

INTRODUCTION

HBV belongs to the family Hepadnaviridae and along with HCV are the only oncogenic hepatitis viruses. It is a common cause of acute and chronic liver disease worldwide (WHO, 2015). It is transmitted through contact with the blood or other body fluids of an infected person (WHO, 2015). It is endemic in Sub-Saharan Africa including Nigeria (Saidu, et.al., 2015) and vertical transmission is a major mode of transmission (Akani, et.al., 2005): therefore, prevention of mother to child transmission is an essential step in reducing the global burden of chronic HBV (Poustchi et.al.,2011).

The liver is an important site of synthesis of cytokines and major clearance organ for various cytokines (Saleh and Hadi, 2016). Hepatitis causes an inflammatory hepatic illness characterized by mononuclear and polymorphonuclear cellular infiltrates with evidence of hepatic macrophage activation (Castillo, et.al.,2002). Tumor necrosis factor (TNF-α) is a principal mediator of inflammation and cellular immune responses with multiple biological functions, including cytotoxicity towards tumor cells and virus-infected cells, several immunomodulatory actions and initiation of the inflammatory response (Saleh and Hadi, 2016). It is a proinflammatory cytokine released mainly from monocytes and lymphocytes in response to inflammatory stimuli. TNF-α is an important proinflammatory cytokine in the host defense mechanism against many intracellular pathogens (Fabrizio, et.al., 2014). It suppresses HBV replication and promotes its eradication by stimulating HBV-specific cytotoxic T-cell response (Fabrizio, et.al., 2014). The production of TNF-α by mononuclear cells is thought to be increased in patients with chronic liver disease (Saleh and Hadi, 2016). Persistent viral hepatitis infection results in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).

Liver cell injury or necrosis is measured by determining alanine aminotransferase (ALT) levels (Hunt and Sharara, 1999). During normal pregnancy serum alanine aminotransferase activity level remains within the normal limits established for non-pregnant women (Bacq, 2013; Everson, 1998), therefore, measurement of serum aminotransferase levels thus remains the most useful test for the routine diagnosis of liver diseases during pregnancy (Hunt and Sharara, 1999). Measurement of serum alanine aminotransferase (ALT) activity level is the most useful test for the diagnosis of liver diseases such as viral hepatitis. Serum ALT activity values above the upper normal limit should be considered pathologic and should lead to further investigations (Everson, 1998). Thus, when viral hepatitis or liver disease is clinically suspected in a pregnant woman, any increase in serum ALT activity levels should be considered pathologic, and should prompt further evaluation.

Viral hepatitis is the most common cause of jaundice in pregnancy (Hunt and Sharara, 1999). Viral hepatitis B in pregnancy is associated with high risk of maternal, fetal, and neonatal complications (Oladeinde, et.al., 2013). Acute hepatitis in pregnancy has been shown to induce premature labor and prematurity with its attendant effects (Oladeinde, et.al., 2013; Eke, et.al., 2011). According to Eke, et.al. (2011), studies have shown an increase in the incidence of prematurity over that seen in the general population with a higher risk of intra-ventricular hemorrhage as well, which was probably related to the higher incidence of preterm delivery. Maternal infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) can expose the fetus/newborn to a subsequent chronic hepatitis infection (CDC, 2006). However, perinatally acquired HBV is a largely preventable condition (Vu-Lam, et.al., 2010). The risk of vertical transmission depends on the time at which the pregnant woman acquired HBV infection, and on her statuses of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg). Without prophylaxis, the risk of perinatal HBV infection in an infant with an HBsAg positive mother is less than 10% if the mother's HBeAg status is negative.

Screening for HBV is routine in pregnancy in most developed countries of the world. It is a recommendation by most of the 122 Colleges of Obstetrics and Gynecology all over the world to screen pregnant women for HBV routinely in pregnancy (Eke, et.al., 2011), however, in Nigeria, screening of antenatal women for HBV and HCV is not a routine practice (Eke, et.al., 2011). Also, routine vaccination of newborns is not widely available in low resource settings in Nigeria. Even though Nigeria approved the inclusion of HBV in its National Program on Immunization (NPI) in 1995, the vaccine only became widely available in 2004 (Sadoh and Eregie, 2008). Despite the fact that the vaccine had been made available via the NPI, immunization coverage for Hepatitis B is still not optimal

HBV infection leads to liver cell injury, resulting in elevated TNF-alpha and ALT levels which also results in complications in both mother and foetus and it has also been shown that women with a history of preeclampsia are at an increased risk for developing cardiovascular diseases (Vitoratos, et.al., 2010; Moreli, et.al., 2012). Screening pregnant women can give a reliable prevalence of the disease in a population and provide an avenue for preventing MTCT virus (Eke, et.al., 2011).

OBJECTIVE OF THE STUDY

The objective of this study is to determine the prevalence of Hepatitis B virus infection in pregnant women.

Specific Objectives of the Study

1. Determine the prevalence of hepatitis B among pregnant women in Sokoto, North-Western Nigeria.

2. To detemine TNF-alpha and Alanine transaminase (ALT) concentration among infected and non infected pregnant women.

3. To Evaluate the risk factors associated with HBV infection among pregnant women in Sokoto.

MATERIALS AND METHODS

STUDY AREA

The study was conducted in Specialist Hospital, Sokoto metropolis, Sokoto State, North- western Nigeria. This hospital services rural communities from all parts of the state, including neighbouring states. Sokoto state lies between longitude 11030´ to 13050´ East and latitude 40 to 6´ North and is bound with the Republic of Niger to the North, Kebbi State to the West and South, and Zamfara to the South and East. Sokoto is in the dry Sahel geographical region of Nigeria, surrounded by sandy savannah and isolated hills with an annual average temperature of 28.3 °C, on the whole, a very hot area. The warmest months are February to April when daytime temperatures can exceed 45 °C (Federal Republic of Nigeria Official Gazette, 2007). The rainy season is from June to October during which showers are a daily occurrence. The showers rarely

last long and are a far cry from the regular torrential rain known in wet tropical regions. From late October to February, during the cold season, the climate is dominated by the Harmattan wind blowing, Sahara dust over the land, the dust dims the sunlight thereby lowering temperatures significantly and also leading to the inconvenience of dust. 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 DESIGN

The study was a purposive study for detection of serum HBV among pregnant women, and TNF-alpha and ALT determination in infected patients. Consecutive random sampling technique was used to select participants, and semi-structured close/open-ended questionnaire administered prior to sample collection until the desired sample size was obtained.

SAMPLING METHOD

The study was a cross-sectional survey among pregnant women attending ANC in the specialist hospital out from September to October 2017. A random sampling technique was adopted and the subjects recruited until the desired sample size was obtained. This sampling technique provided the opportunity for the realistic generalization of the research population (Goodman, 2007). A questionnaire based on WHO generic protocol (Appendix I) was adopted and administered to generate the primary data where adequate information on every participant was obtained. Patient information such as identification number, address, socio-economic status was collected. The validity of the questionnaire was determined by the critique of research experts. The modification of the questionnaire was based on the experts' comments and advice. The reliability of the questionnaire was determined through the administration of the modified copy to some pregnant women in the hospital selected for study. The results provided the basis for the final modification of the questionnaire.

SAMPLE COLLECTION

Blood samples were collected from consenting pregnant women attending ANC in Specialist Hospital, Sokoto. Three milliliter (3 ml) of venous blood samples were collected aseptically using a sterile vacutainer, blood specimen bottle, holder, and needle, from each subject into a sterile plain vacutainer blood specimen bottles, adequately labelled (patient ID and date of collection) and allowed to clot at room temperature (220C). After clotting, it was centrifuged at 3000 rpm for 5 minutes to obtain a clear unhaemolyzed serum. The serum samples were separated using clean Pasteur pipettes into sterile Eppendorf tubes and stored at –20°C in Central Research Laboratory, City Campus, Usmanu Danfodiyo University, Sokoto until analysed. Haemolytic, icteric or lipaemic specimens were not used. A serum specimen logbook was kept in the laboratory where information on all pregnant women were checked regularly and matched with the information in the questionnaire to ensure proper entry of information.

ETHICAL CONSIDERATIONS

Due ethical approval was obtained from the State Ministry of Health and from the ethical committee of the Specialist Hospital, Sokoto.

Inclusion Criteria

Pregnant women attending ANC in Sokoto metropolis who have given their informed consent will be eligible to participate.

Exclusion Criteria

Pregnant women who did not give their consent will be excluded from this study

Questionnaire administration

A semi-structured close/open-ended questionnaire will be will be developed and tested for validity and reliability and subsequently administered to subjects.

SAMPLE SIZE ESTIMATION

The sample size was calculated at 6.51% prevalence, (Saidu et.al., 2015) at 95% confidence interval, with desired precision of 5%, using the formula.

$n = (Z^2Pq) / d2$ (Thrushfield, 2007),

The calculated sample size was 93.

DETERMINATION OF HBV PROFILE USING IMMUNOCHROMATOGRAPHIC METHOD (ARIA, USA)

A commercial Aria *HBV*-5 Panel Rapid Test Kit was used to detect the HB profile algorithm (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb) in serum samples collected according to manufacturer's instructions. The *Aria* HBV 5-Parameter Rapid Test for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb in human serum or plasma.

The serum samples were analyzed for the in-vitro quantitative determination of Human Tumor Necrotic Factor concentrations using a commercially obtainable Enzyme Immunoassay (ELISA) kit from WKEA™, China.

Alanine transaminase (ALT) is a transaminase enzyme. It is also called serum glutamic pyruvic transaminase (SGPT) or alanine aminotransferase. (ALT) is found in serum and in various bodily tissues, but is usually associated with the liver. The serum samples were estimated for the level of ALT using colorimetric method (Endpoint) (Randox Switzerland™), obtained from the manufacturer. The assay was carried out according to the manufacturer's instructions, and it was ensured that the same incubation time was maintained for all samples.

DATA ANALYSIS

Data obtained and recorded during sampling and laboratory findings was entered and stored in MS-Excel. The data was subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 20 (Inc., Chicago, IL, USA). Data were represented using descriptive statistics and presented using percentages. Chi square analysis was used to determine the association between the infections and variables socio demographic variables. Independent sample t-test was used to make comparison between the mean ALT levels and TNF-alpha level of HEV Infected and non-infected patients. P-value of less than or equal to ($P \leq 0.05$) was regarded as statistically significant.

RESULTS

Sero-surveillance studies showed that pregnant women had a high positive rate of HBV at 17.2% (16/93) as shown in Table 1.

Table 1: Sero-surveillance of HBV Infection among Pregnant Women in Sokoto, North-Western Nigeria.

Viral Hepatitis	Positive	Negative	Total	Prevalence (Percentage %)
HBV	**16**	**77**	**93**	**17.2**

TNF-ALPHA AND ALT LEVELS IN HBV INFECTION AMONG PREGNANT WOMEN IN SOKOTO, NORTH-WESTERN NIGERIA.

TNF-alpha levels and ALT levels were evaluated among seropositive samples and it was observed that samples that tested positive had significantly high TNF-alpha level compared to uninfected control. Independent sample t test showed statistical significance with p-value less than 0.05 at 95% CI as shown in Table 2 below

Table 2: Mean ALT and TNF-alpha levels among HBV Infected Pregnant women and Uninfected Pregnant Women in Sokoto, North-Western Nigeria

		TNF-alpha	**ALT**
Groups:	Non-infected Pregnant women	6.08 ± 0.558	6.23 ± 0.414
	HBV Infected Pregnant women	30.21 ± 3.088	14.34 ± 1.017
P-value:		0.0 26	0.00

Values are Mean ± SEM, n= Number of subjects, ALT= Alanine Transaminase, TNF-ALPHA= Tumor Necrotic Factor - Alpha, P value < 0.05 is considered as statistically significant.

Table 3: Socio-demographic Pattern of HBV Among Pregnant Women in Sokoto, North-Western Nigeria (n=93)

Parameter	Level	HBV status				Df	Chi-square	P-value at 95%CI
		Negative		Positive				
		Count	%	Count	%			
Age group (years)	18–25	59	79.7	15	20.3			1.000
	26–35	13	100	0	0	2	0.00	
	36–54	5	83.3	1	16.7			
Type of family	Monogamous	65	87.3	9	12.7	1	0.00	1.0000
	Polygamous	12	63.2	7	36.8			
Location	Rural	23	63.9	13	36.1			
	Urban	33	91.7	3	8.3	1	0.00	1.0000
Educational	Non-Formal	40	81.7	9	18.3			
	Primary	18	85.7	3	14.3			
	Tertiary	6	85.7	1	14.3			
Employment Status	House wife	62	80.2	15	19.8	2	0.00	1.0000
	Civil Servant	6	100	0	0	3	0.25	0.9696
	Business	10	90.9	1	9.1			
Gestational Stage	1st Trimester	0	0	0	0			
	2nd Trimester	22	75.9	7	24.1	2	0.00	1.0000
	3rd Trimester	55	85.9	9	14.1			
Parity	Primiparous	10	90.9	1	9.1	1	0.00	1.0000
	Multiparous	67	81.7	15	18.3			

Table 4: HBV Knowledge Attitude and Practice Among Pregnant Women in Sokoto, North-Western Nigeria

Variables	HBV Status		Df	Chi-square	P-value at 95%CI
	Positive *N* (%)	Negative *N* (%)			
Surgery					
Yes	2 (16.7)	10(83.3)	**1**	**0.00**	**1.0000**
No	14 (17.3)	67 (82.7)			
Blood Transfusion					
Yes	3 (11.1)	5 (89.9)	**1**	**0.00**	**1.0000**
No	13 (15.3)	72(84.7)			
Sharing sharp objects					
Yes	3 (17.6)	14 (82.4)	**1**	**0.00**	**1.0000**
No	13(17.1)	63 (82.9)			
Knowledge					
Yes	1 (11.1)	8 (88.9)	**1**	**0.00**	**1.0000**
No	15(17.9)	69 (82.1)			
Is there vaccine					
Yes	0 (0)	2 (100)	**1**	**0.00**	**1.0000**
No	16(17.5)	75 (82.5)			

DISCUSSION

The overall prevalence of HBV positivity, indicated by the presence of HBsAg was 17.2% (16/93).

The 17.2% prevalence of HBsAg among pregnant women in Sokoto is similar to a report of Ndako, *et.al.* (2012) with a prevalence of 17.2% among pregnant women in Bayara Bauchi, 15.8% in Maiduguri (Baba, et.al., 1999), and with a prevalence range from 2-15% (Eke, *et.al*., 2011). However, it is higher than 12.6% in Plateau State (Ndako*, et al.,* 2016), 12.3% in Minna (Ndams*, et.al.,* 2008), 9.3% in Awka, (Ezegbudo, *et.al,*. 2004), 8.3% in Nnewi, (Eke, et.al., 2011) and 4.6% in Enugu (Obi, et.al., 2006). However, it is lower than the 23.3% report of Alvarado-Mora, et al. (2011) in Kano. This goes to show that despite the endemicity of HBV in Nigeria, there is a higher prevalence in Northern Nigeria, compared to the South. Prevalence of HBV in this study shows endemicity of the infection according to WHO criteria (WHO/EPI, 1990).

The HBV prevalence of 17.2% seen in this study is higher when compared to the 6.51% reported in an earlier study by Saidu, et.al. (2015) among pregnant women in Sokoto. This varying reports from Sokoto are similar to the reports from Benin City where Ugbebor, et.al.(2011) documented a high prevalence of 12.5% and Oladeinde, et.al. (2013) reported a low prevalence of 2.2% among pregnant women in the same Benin City. This implies that prevalence may vary in similar geographic locality nonetheless, the explanations for such may be due to the sample size.

On the basis of age, the age group 18-25 has the highest rate of infection of 20.3%, similar to studies conducted by Saidu, et.al. (2015). Other variables such as occupation, level of education, trimester of pregnancy and knowledge of HBV were evaluated with highest percentages among non-formally educated women, housewives and those without knowledge of hepatitis. The gestational stage of the pregnancy showed a higher prevalence, though not statistically significant in the second trimester with 24.1% and this is similar to the record of the highest prevalence in the second trimester of pregnancy of 13.4% by Ndams, et.al. (2008) and 11.3% by Ndako, et.al. (2016) in Plateau State.

Changes in Liver enzymes in the study conducted by Tsai et.al. (2008), showed that Liver function tests are used to determine if the liver has been damaged or its function impaired. Elevations of certain liver tests in relation to others aids in that determination. The aminotransferases (which include ALT and AST) are notably elevated in liver damage caused by liver cell disease (hepatocellular disease). Alanine aminotransferase (ALT), Pregnant women screened in this study recorded 26.3% Abnormality in ALT levels which have been correlated positively with liver inflammation, while patients with persistently normal ALT levels had significantly lower liver damage compared with patients with either intermittent of persistently elevated ALT Levels (Zhang et.al., 2011).Therefore measurement of aminotransferase levels by serial observations and analysis remain the most common and convenient way to identify liver inflammation in patients particularly with chronic HBV infection.

It was also observed that samples that tested positive had significantly high mean ALT level of 14.34. Independent sample t-test showed statistical significance (P-value = 0.015, CI=95%) as shown in table 2, indicating an association between ALT and Hepatitis. This corresponds to Tsai, et.al. (1997) were raised serum ALT activity was found in HBV infections in Tawian, and research by Chang, et.al. (2000), who also found significantly raised ALT levels in HBV in Kaohsiung, Taiwan. Chi square analysis showed no statistical significance between ALT levels in HBV Infection among Pregnant Women in Sokoto, North-Western Nigeria, at 95% CI (P- value>0.05: $\chi 2$ = 0.00, P-value = 1.0000).

HBV induces TNF-α expression in human liver and human hepatoma cell lines (Gonzalez-Amaro, et.al., 1994) and patients with chronic hepatitis have elevated plasma TNF-α levels, and their peripheral blood mononuclear cells show enhanced TNF-α production in vitro (Bradham,et.al., 1998).

In this study, it was observed that samples that tested positive had significantly high mean TNF-alpha level of 30.21. independent sample t-test showed statistical significance (P-value < 0.029, CI=95%) indicating an association between TNF-alpha and viral hepatitis. This is similar to Sabry, et.al. (2015) where statistical analysis revealed highly significant increase in the mean value of serum TNF-α in patients with viral liver diseases. It is also similar to findings by Tilg et.al. (2002), that serum level of TNF-α increases significantly in liver diseases, and this agrees with research by Bilgic, et.al. (2015) that TNF- α levels were increased both in serums and liver tissues of patients with Hepatitis B. Nagaki, et.al. (2000), reported increased serum concentrations of soluble TNF- alpha receptors in persons with viral hepatitis and liver disease, and this agrees with Bradham, et.al. (1998) that serum levels of soluble TNF- alpha receptors are significantly elevated in chronic hepatitis infection.

Sabry, et.al. (2015) proposed that high serum level of TNF-α is a key mediator in the pathogenesis of liver necrosis and serum level of TNF-α reflects progression of inflammation, therefore, increased TNF-α in HBV contribute to role of innate immunity in stimulating the adaptive immune responses, thus signifying the role of TNF-α in antibody production.Chi square analysis showed no statistical significance between TNF-alpha levels in HBV (HBSAg) infection among Pregnant Women in Sokoto, North-Western Nigeria, at 95% CI (P-value > 0.05: $\chi 2 = 0.01$, P-value = 0.9350).

Though TNF-alpha and ALT values were significantly elevated in seropositive patients, Spearman Rho analysis showed no association between TNF-alpha and ALT level in HBV infection.

CONCLUSION AND RECOMMENDATIONS

The outcome of this study provides evidence that pregnant women are exposed to HBV infection. Findings also showed that TNF-alpha and ALT values were significantly elevated in seropositive patients, however, Spearman Rho analysis showed no association between TNF-alpha and ALT level in HBV infection.

Sensitization and Public enlightenment should be advocated to lower the burden of the disease among the study population and hence reducing the incidence of the disease.

Screening for and vaccination against HBV should be part of routine antenatal care for pregnant women.

ALT and TNF-alpha estimation should be introduced during pregnancy to reduce the risk of obstetric complications.

Our result underscores the need for molecular identification and characterization of the virus and to determine the relationship between the viral load and the immune response (TNF-alpha levels), level of liver damage (ALT levels) with viremia and to determine the pattern of distribution and the rate of transmission to infants at birth.

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