Original Research Article

PREVALENCE OF HEPATITIS B AND D ANTIBODIES AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC AT EKITI STATE UNIVERSITY TEACHING HOSPITAL, ADO-EKITI, EKITI STATE, NIGERIA

Abstract

This study was conducted to determine the prevalence of Hepatitis B and D antibodies among pregnant women attending antenatal clinic at Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti. Structured questionnaires were administered to obtain their socio- demographic data. A total of 350 pregnant women between the ages 18 and 44 years were examined for the presence of hepatitis B using HbsAb rapid kit and presence of both HBV and HDV were confirmed using enzyme linked immunosorbent assay (ELISA). Liver enzymes of ELISA positive subjects were assayed. This study found the prevalence of HBV and HDV among pregnant women to be 7.7% and 0% respectively. There was no significant difference between the prevalence of HBV antibodies as related to their ages when screened with ELISA. Although more positive results were recorded between ages 18 – 34 when compared to other age groups, there was significant association in their age difference, (P =

0.007). Of the subjects that tested positive to HBV antibody, 2 (10%) were recorded to have raised liver enzymes. However, three 3 (15%) out of the ELISA-confirmed positive samples were at the upper limit of normal. The kit's specificity, sensitivity, positive prevalence and negative prevalence values were: 100%, 74%, 100% and 72% respectively. This study shows screening with ELISA for hepatitis B is much more viable than that of rapid kit and has a significant effect on the liver enzymes when tested positive. The study therefore suggests that health workers should always confirm all negative results using ELISA method

Keywords: Hepatitis, liver, HAV, HBV, HCV, HDV, pregnant women

Introduction

Hepatitis is an infection of the liver caused by several viruses; it is characterized by inflammation of the liver. It has a wide range of presentations that vary from a complete lack of symptoms to severe liver failure (Dienstag, 2015). The acute form of hepatitis is generally characterized by constitutional symptoms that are usually self-limiting, chronic hepatitis presents with similar characteristics, but can exhibit

signs and symptoms specific to liver dysfunction with long-term inflammation and damage to the organ (Khalili and Burman, 2013). There are five main types of viral hepatitis: type A, B, C, D, and E; the commonest of which are hepatitis A, B and C. Hepatitis B virus (HBV), C (HCV) and D virus (HDV), are spread mainly through contaminated blood and blood products, sexual contact and contaminated needles while Hepatitis A (HAV) are majorly through fecal – oral route (Rikabi *et al.*, 2009).

Hepatitis B virus (HBV) belongs to a family of DNA viruses known as hepadnaviridae and is the causative organism of Hepatitis B infection (Pungpapong *et al.,* 2007). HBV is also known as serum hepatitis and it is an important form of both acute and chronic viral hepatitis (James *et al.,* 2011), there is no age specificity in the infection and development of the disease (Ayolabi *et al.,* 2006). Hepatitis B is 50-100 times more infectious than human immunodeficiency virus (HIV) and 10 times more infectious than hepatitis C virus and because it replicates profusely and produces high titre in the blood any parenteral or mucosal exposure to infected blood poses a high risk of HBV infection (Pennap *et al.,* 2010). Hepatitis D virus (HDV) is mostly transmitted through exposure to infective blood. This may happen through transfusions of HDV- contaminated blood and blood

products, contaminated injections during medical procedures, and through sharing of sharp objects among drug addicts. Sexual transmission is also possible, but is much less common. There is no vaccine for HDV. The special features associated with both hepatitis B and D is jaundice, liver cancer, and liver damage (Ayolabi *et al.,* 2006).

Although much is known about the epidemiology of HBV in Ekiti state, Nigeria, limited investigations have been carried out on the co-infection of HBV and HDV among pregnant women in hospitals in Ekiti state, Nigeria. Studies carried out by various authors have shown that HBV and HDV infections are highly prevalent among Nigerians (Imarenezor *et al.,* 2016). Even though publications on Hepatitis B and D virus infection in Nigeria are increasing, still, there is little information among women of reproductive age, especially the pregnant ones who form the majority of the group required for regular routine diagnosis (Pennap *et al*., 2011). Screening pregnant women for HBV and HDV is essential to get reliable prevalence of the disease in that sub-population, since they fall within the sexually active group and are prone to the risk factors (Isa *et al.,* 2015).

Methodology

Study Area

This study was carried out among pregnant women attending antenatal clinic in EKSUTH, Ado-Ekiti is a city, in Southwest Nigeria,, Ado Ekiti, lies between latitude $7^{o}35`$ and $7^{o}38`$ North of the equator and Longitude $5^{o}10`$ and $5^{o}15`$ East of the Greenwich Meridian. It has a population of 308, 626. (Oriye, 2008; Adebayo and Jegede, 2010).

Study Population

The study made use of 350 consenting pregnant women accessing care at EKSUTH, who were consecutively sampled and enrolled into the study.

Ethical clearance

Ethical approval for the study was obtained from the ethical and research committee of Ekiti State teaching hospital, Ado Ekiti.

Sample collection

A semi-structured questionnaire was used to obtain data on subjects' sexual behaviours, previous health challenges and general medical

history. The blood was collected aseptically from by venipuncture into Ethylene diamine tetracetic acid, (EDTA) bottles, during their regular antenatal hospital visits.

Sample analysis

The blood was allowed to clot and the serum was separated by centrifugation at room temperature for 3000 revolution per minute (rpm) for 10 minutes into screw- capped plain bottles stored at -20^{o}C, and were analysed in less than 24 hours from collection time. (Tammen *et al*., 2005).

The serum samples were analyzed using both One – stage rapid test kit (RTK) (Diaspot diagnostics) and Enzymes linked immunosorbent assay (ELISA) respectively for identification and confirmation of the presence of antibodies to Hepatitis B and D viruses. Positive HBV samples were further screened to assay the level of Alanine aminotransferase and Aspartate aminotransferase.

RESULTS

Assessment of sero – prevalence of Hepatitis B and D was carried out among 350 pregnant women attending antenatal clinics of Ekiti State Teaching Hospital, Ado Ekiti. The socio – demographic of the subjects

are presented in Table 1. The subjects were within the age of 18 – 45 years with 18.6% having awareness of hepatitis infections and 20.3% vaccination against Hepatitis B. Twenty (5.7%) out of 350 samples analyzed were positive for Hepatitis B, while none were positive to Hepatitis D using the rapid test kit. Indicating the infectivity ratio of Hepatitis B and D were 5.7% and 0% respectively. There was significant association of HBV prevalence with advancement in age (P = 0.007). Hepatitis B virus infectionwas equally found to be associated with occupation (P = 0.007), stage of trimester (P = 0.001), HBV vaccination (P = 0.002), HBV awareness (P = 0.002) and HIV status (P = 0.004) (Table 1). Both the positive samples and negative samples by kit were subjected to ELISA screening and out of total (20) negative samples screened by kit, 7 (15.6%) were positive by ELISA while the whole 20 (44.4%) said to be positive by kit were also positive when screened with ELISA but a total of 27 subjects were confirmed positive by ELISA as against that of kit (Table 2). The sero prevalence of HBV among the pregnant women screened using ELISA is 7.7%, and this shows the prevalence is significantly moderate, according to WHO classification of assessing severity HBV. Two (10%) of the ELISA positive samples recorded had raised liver enzymes. However, 3 (15%) of the ELISA positive samples were at the upper limit of normal. The results of the

liver enzymes assay are shown in Table 3. Table 4 shows the comparison of the mean and standard deviation of the liver enzymes of the hepatitis B virus infected subjects and the control samples. There is no significant difference in the ALT activities of the HBV positive subjects and the negative control (P = 0.098), but there is significant increase in the AST activities of HBV positive subjects and AST negative control (P = 0.002). The sensitivity and specificity of Hepatitis B rapid kit are 74% and 100% respectively, which means 74% of subjects who actually have Hepatitis B were correctly identified by the kit, this indicates that 26% of the subjects who are positive with Hepatitis will be wrongly diagnosed by the kit, while 100% specificity indicates that all the positive subject having the infection was diagnosed by the kit. The positive predictive value is 100%, which also indicates that there is 0% chance of misdiagnoses using rapid kit, it confirms that all positive subjects by kit were actually positive for HBV. There was 72% chances that someone who tested negative using kit does not have Hepatitis B infection when screened with ELISA, this means there was 28% chances that someone with a negative result will actually have the disease.

Table 1: Distribution of Hepatitis B infection along socio-demographic indices

Indices		HbsAb kit Negative	Positive	Significance	Total
Age (years)	18-24	32(94.1)	2(5.9)	0.007*	34
	25-34	48(81.4)	11(18.6)		59
	35-44	23(76.7)	7(23.3)		30
Occupation	Civil Servants	22(75.9)	7(24)	0.007*	29
	Private	22(84.6)	4(15.4)		26
	Self employed	19(76)	6(24)		25
	Non-employed	41(93.1)	3(6.8)		44
First Pregnancy	No	51(86.4)	8(13.6)	0.312	59
	Yes	53(81.5)	12(18.5)		65
Stage of Trimester	1st	39(72.2)	15(27.8)	0.001*	54
	2nd	39(97.5	1(2.5)		40

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	3rd	26(86.7)	4(13.3)		30
Vaccinatio n	No	79(79.8)	20(20. 2)	0.002*	99
	Yes	25(100)	0(0)		25
Hbs Awareness	No	34(91.9)	3(8.1)	0.002*	37
	Yes	69(80.2)	17(24. 6)		86
HIV status	Negativ e	88(88)	12(12)	0.040*	100
	Positive	16(69.6)	7(30.4)		23

P- value = < 0.05 (Significant)*. (%) in Percentage

Table 2: Comparison of sero-prevalence of HBV using ELISA and Kit

		ELISA		
		POSITIVE	NEGATIVE	TOTAL
KIT	POSTIVE	20	0	20 P= 0.000*

NEGATIVE	7	18	25
TOTAL	27	18	45

P - value = < 0.05(Significant)*

Table 3: Alanine aminotransferace (ALT) and aspartate aminotranseferace (AST) concentrations of HBV positive samples.

Serial Number	Alanine Aminotransferase (ALT) (U/I)	Aspartate Aminotransferase (AST) (U/I)
1	1.0	2.0
2	3.0	2.0
3	1.0	2.0
4	2.0	5.0
5	11.0	9.0
6	3.0	13.0
7	3.0	8.0
8	1.0	10.0
9	7.0	8.0
10	1.0	2.0
11	3.0	3.0
12	1.0	2.0
13	4.0	4.0
14	1.0	5.0

15	2.0	7.0
16	1.0	2.0
17	4.0	7.0
18	13.0	10.0
19	1.0	2.0
20	2.0	2.0

Table 4: Statistical analysis of Liver enzymes assay

LIVER ENZYMES	**ALT**	**AST**
Hb Positive	3.25 ± 1.55	4.86± 2.68
Hb Negative	2.59 ± 1.42	3.20± 2.55
P value	0.098	0.002*

P value = <0.05 (Significant)*; Values are Mean ± SD

DISCUSSION AND CONCLUSION

Hepatitis B and D and related liver diseases are emerging as significant health problems even in apparently healthy individuals (WHO, 1998). Screening asymptomatic people is an important instrument in disease detection for prompt diagnosis and intervention especially in silent killers like HBV and HDV infections (Pennap *et al.,* 2010). An overall prevalence

rate of 7.7% was recorded for HBV and 0% was recorded for HDV in this research. According to WHO classification of assessing severity of HBV infections in HBV endemic countries, this prevalence is regarded as moderate and low respectively, WHO defines low prevalence to be <2%, moderate to be 2-8% and high prevalence to be >8% HBV positivity (WHO, 2010). The pooled prevalence of HBV in Nigeria which was reported as 13.6% and 14.1% for pregnant women attending antenatal clinic in University of Calabar teaching hospital, Calabar, (UCTH), Nigeria. It was recorded that 83.5% of pregnant women already diagnosed with chronic liver disease were positive to HbsAg, while 60.5% of these HbsAg positive women had co-infection with HDV, 39.47% were negative to HDV. In this said research, most subjects screened were already suffering from chronic liver disease and mostly male with chronic liver disease had a higher HBV and HDV infection 27 (58.7%), as compared to female 9 (41.3%) (Okpokam *et al.*, 2013). Sero prevalence of HBV in this research work is relatively lower when compared to the research work in the above stated teaching hospital and this is because the subjects are not apparently healthy pregnant women or individuals like the subjects used in this study. Also, different methodology was employed in the previous research study to arrive at the prevalence rate, in the previous study; 12.3% by using ELISA,

17.5% by immunochromatography and 13.6% using HBV DNA polymerase chain reaction (Musa *et al.*, 2015). This research work only focused on the potency of HBsAb rapid kit compared to HbsAb ELISA screening techniques, so the prevalence from this study is low (8.26%) compared to that of ELISA (12.3%). A reviewed HBV infection reported at the University of Ibadan showed the peak in the age group of 20 to 30 years which is in accordance to the age group in table 1 of this study. The mean prevalence is `13.3% in contrast to the sero prevalence of 8.26% in this study. This report questions effectiveness of the Nigeria HBV vaccination program (Vandu and Aminu, 2014).

In contrast, the prevalence of 0% reported for HDV in this study is lower than the 12.5%, reported in a research work carried out in Tehran, Iran, (Sayeed *et al.*, 2004) as this study excluded other risk factors for HDV infection, which includes chronic hepatitis and liver cirrhosis. In patients with acute hepatitis and asymptomatic infection, the prevalence was 4.3% while in patients with chronic hepatitis, liver cirrhosis and primary liver cell carcinoma, the prevalence was 15%. These factors may be responsible for the prevalence of HDV recorded previously by other researchers as it emphasized HDV as more prevalent in HBV- related

liver disease in Nigeria in contrast to the findings in this study (Nwokediuko and Ijeoma, 2009).

Furthermore, the reasons for these variations may be related to the fact that infection usually varies from one locality to another and from one country to another depending on the level of associated risks. The study area in this research is a tertiary hospital in south western part of Nigeria that operates a closed system. In this system of operation, the movement and interaction of individuals with the outside world is limited and restricted to the financial capability of individuals, as a result, prevalence reported from other West Africa countries are in contrast to that obtained in this study. Previous studies in sub-Sahara Africa reported prevalence of HBV and HDV infection ranging from 0.0% - 44.4%. Other study conducted in Guinea Bissau found a high prevalence of HBV/HDV infection (25.0%) with no statistical difference according to HIV type, this agrees with the reports of this study in relation to subject's HIV status which shows low significance for HbsAb rapid kit and none for HbAb ELISA (Patric *et al*., 2017)**.** Statistics shows that the difference between the hepatitis B positive subjects' ALT results and ALT negative controls have no significant difference (P= 0.098), while values

of AST positive and AST negative controls are significantly difference (P = 0.002).

Sexual transmission of hepatitis D has been a subject of debate for a while now, since it does not exit alone, however, some literatures state that sexual practices that involve higher levels of trauma to the anal genital mucosa, such as anal penetrative sex could in some instances lead to the transmission of HDV (Taylor, 2006). Low prevalence of HDV in this study could be attributed to the fact that major risk factors such as liver cancer, liver cirrhosis and hepatitis liver disease in subjects screened were not put into consideration. Nigeria being an endemic area for hepatitis B and probably due to some failed vaccination practice (Ikobah *et al*., 2016), might also have contributed to the high prevalence rate of HBV infection (WHO, 2010).

The findings of this study show that HBV infection is highly endemic and HDV infection has a low endemicity among pregnant women at the obstetrics and gynecology unit of EKSUTH. Considering the liver enzymes activities as well, 70% of the pregnant women screened had relatively high ALT values (5 – 8 U/I) but not above the kit normal value while AST values has more raised activity and this could be due to presence of other form of co infection such as HIV and other bacterial infections. This difference is equally statistically significant.

This should call attention to the burden of HBV and HDV infection, AST, ALT infection among pregnant women in Ekiti State.

Recommendation

In confirming the HBV status of individuals, ELISA technique should be adopted solely as it assures accurate and precise diagnosis. It should also be brought to the attention of the target population that protection against HBV infection can be conferred by potent vaccine and use of immunoglobulin for positive individuals. Workshop at the grass root level, health centers and antenatal clinics should be organized more often to sensitize people about the viral infection, its route of infection, pathogenicity, diagnosis, treatment, prevention and control.

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