KNOWLEDGE, ATTITUDE, PRACTICE AND DETECTION OF HEPATITIS B VIRUS INFECTION AMONG FRESH UNDERGRADUATE STUDENTS OF OLABISI ONABANJO UNIVERSITY, AGO-IWOYE, NIGERIA

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ABSTRACT

Hepatitis is an important liver disease caused by Hepatitis B virus (HBV). Majority of people infected with HBV are unaware, presenting symptoms only at an advanced stage of the disease. Therefore this study determines the knowledge, attitude, practice and sero-prevalence of Hepatitis B virus infection among fresh undergraduate students of Olabisi Onabanjo University, so as to detect the infection early and institute treatment.

Sera from fresh undergraduate students on medical checkup were screened for Hepatitis B surface antigen (HBsAg) using Rapid Diagnostic Test strip, Enzyme Linked Immunosorbent Assay (ELISA) and molecular method using Random Amplified Polymorphic DNA analysis (RAPD). Structured questionnaire was administered to obtain socio-demographic information, risk factors and knowledge of the students about Hepatitis.

The awareness of HBV infection was 182 (45.5%). Major sources of information include electronic media 71 (39%), internet 64 (35%) and health workers 60 (33%). Majority of the students 352 (88%) had poor knowledge and 260 (65%) had negative attitude towards HBV infection. The prevalence of HBsAg is 12 (3%) for both the Rapid Diagnostic Test strip and ELISA methods. The presence of HBV DNA in the HBsAg positive samples also confirmed the presence of Hepatitis B virus and RAPD resolved the DNA into different polymorphic bands.

The carriage of HBsAg by these students called for concern as they could be potential source of spread for the infection. There is genetic variation in the HBV DNA. Education on risk factors of Hepatitis B virus in order to prevent transmission of the virus is required.

KEYWORDS: HBsAg, Knowledge Attitude and Practice, Risk factors

Accepted: 20th Dec. 2019

INTRODUCTION

Background

Hepatitis B is an important human viral hepatitis infection that is caused by Hepatitis B Virus (HBV), which is an enveloped in the family hepadnavirus. Other name for HBV infections includes serum hepatitis, homologous serum jaundice and type B hepatitis (WHO, 2002). The virus has affinity for the cells of the liver, multiply in the hepatocytes thus interfering with its functions (WHO, 2002). The virus is the major cause of acute and chronic liver disease which includes cirrhosis and hepatocellular carcinoma (Carvalho *et al*., 2012).

The World Health Organisation reported that over 350 million people infected with hepatitis B virus developed lifelong chronic infection (WHO, 2011). Chronic carrier rates of Hepatitis B Virus have been shown to range from 9 to 20% and 9 to 39% for sub-Saharan Africa (Ballah *et al*., 2012) and Nigeria (Emechebe *et al*., 2009) respectively.

Although, people living with chronic HBV infection do not present any active hepatic disease, this category of people are referred to as inactive carriers. Prolonged carriage of infection may result

in cirrhosis, hepatic failure, or hepatocellular carcinoma in future (Carvalho *et al*. 2012). The established mode of infection of the virus includes vertical (mother to child at birth), horizontal (contact with an infected person), sexual contact as well as parenteral (exposure to infected blood or body fluids) (Abilgaard and Peterslund, 1991; Luby *et al*., 1997; Akbar *et al*., 1997; Previsani & Lavanchy, 2002).

Studies have shown that the burden of HBV infections varies worldwide with age, studied population, impact of vaccination programmes, control measures (Liang, 2009; Ott, 2012; MacLachlan & Cowie, 2015; Abdela *et al.,* 2016). Implementation of vaccination campaign and immunization against hepatitis B has changed the distribution pattern of the disease (Carvalho, 2012). Furthermore, the prevalence varies substantively all over the world; reports from Nigeria are greater than 8% (Lesi *et al.,* 2004; Olokoba *et al.,* 2011; Oje *et al.,* 2012; Musa *et al.,* 2015) which is high as categorized by WHO (2002). Chronic HBV Infections in majority of people are asymptomatic and go unnoticed (WHO, 2002). Considering the high prevalence of HBsAg in general (Lesi *et al.,* 2004; Olokoba *et al.,* 2011; Oje *et al.,* 2012; Musa *et al.,* 2015), most reports centered on the general populace, health professional, medical students, children and those perceived at risk of infections (Liang, 2009; Carvalho, 2012; Abdela, 2016; Ikobah, 2016).

It is noteworthy that Olabisi Onabanjo University mandate HBV test as one of the medical fitness check for her new entrants who are at risk of peer pressure and its vices which includes high risk sexual behavior, intravenous drug use, sharing sharp objects and tattooing amongst other, which are established sources of transmission. Over the years, observations from these medical check call for concern because more cases of newly admitted students with hepatitis B virus are being detected. It was therefore necessary to know the awareness level of these new entrants, so as to create more awareness on the risk factors associated with HBV infection, to forestall imminent danger.

This study is to determine the knowledge, attitude, practice and sero-prevalence of hepatitis B virus among newly admitted Olabisi Onabanjo University undergraduate students.

Materials and Methods

Ethical consideration

This study was conducted in accordance with the Declaration of Helsinki ethical principles for medical research involving human subjects, taking into account the confidentiality, freedom, anonymity, benefits, and safety of the study participants. An approval was sought and obtained from the Directorate of Health Services of Olabisi Onabanjo University, Ago-Iwoye. Informed consent was sought from the participants after the purpose of the study have been explained to them, only those that consented and signed the consent form were recruited.

Study population and size

The study population was newly admitted students undergoing Medical Fitness Test, which included Hepatitis B surface antigen test (HBsAg). The sample size was calculated using a prevalence value of 11.4% of HBsAg infection (Isa *et al*., 2015) to obtain a sample size of 400 including 10 attritions. Simple random sampling (ballot technique) was used to select 20 out of every 80 students that registered per day at the Laboratory Department of Olabisi Onabanjo University Health Centre, Ago-Iwoye.

Specimen collection

Structured questionnaire was administered to the participant to obtain social-demographic information such as age, sex, educational level, previous history of blood or blood products transfusion, knowledge of Hepatitis B virus infection and vaccination etc. Two milliliters of venous blood was collected aseptically from participants by venipuncture into well labeled plain sample bottles using sterile needle and syringe. Specimens were allowed to clot, retract, sera were separated and stored at -20^{o}C for further analysis.

Processing of specimens

Rapid Test Kit: Hepatitis B surface antigen was detected qualitatively using Micropoint Hepatitis B Rapid Test Kit. The test strip and serum were allowed to attain room temperature, the strip was dipped vertically into the serum sample with the arrows on the strip pointing downward, ensuring that the maximum line (MAX) on the test strip was not exceeded. Result was read within 5 - 20 minutes and interpreted according to the manufacturer's instruction.

Enzyme Linked Immunosorbent Assay (ELISA) method: HBsAg was confirmed using Fortress Diagnostics ELISA Kit (Fortress Diagnostics Limited Antrim Technological Park, Antrim (United Kingdom)) following the manufacturer's manual. The assay was carried in the Medical Microbiology and Parasitology Department, Olabisi Onabanjo University, Sagamu. The reagents and samples were allowed to attain room temperature for at least 15-30 minutes, 50µL of positive controls, negative controls and specimen were added into labelled wells using micropipette with fresh disposable tips for each specimen. The HRP-Conjugate (50µL) was added to all the wells except the blank well, mixed by gentle tapping, the plate was covered and incubated for 60 minutes at 37^{o}C. Each well was washed 5 times with Stock Wash Buffer (diluted 1 in 20 with distilled water), the plate was turned upside down onto blotting paper and tapped to remove excess buffer after the washing. Chromogen A and Chromogen B solution (50µL) were added into each well including the blank, mixed by gentle tapping and incubated at 37^{o}C for 15 minutes. A change in colour of the positive control and HBsAg positive sample wells to blue were observed. Then 50µL Stop Solution was added into wells and gently mixed. Change of the initial blue colour to intensive yellow colour indicates positive result. The plate reader was calibrated using the blank well and the absorbance reads at 450nm. The optical density and cut off valve were noted for each sample.

Molecular identification of HBV

Viral DNA was isolated and purified from tenfold dilutions of HBV-positive serum using the QIAamp DNA Blood Mini Kit (QIAamp, Qiagen) in accordance with the manufacturer's instruction. The purified HBV DNA was used to screen and optimized a total of 16 RAPD primers for polymorphisms and annealing temperature (Tm). Optimal PCR amplification across the Viral DNA was achieved at Tm between 34 and 40^{o}C. The primer that shows good and highest polymorphism with the PCR products was therefore used for the study. The primers were OPX 07 (GAGCGAGGCT), OPR 16 (CTCTGCGCGT) and OPA 01(CAGGCCCTTC). Each 25 µL PCR reaction contained 12.5 µL master mix (2×) (0.05 45 units/µL *Taq* DNA polymerase in reaction buffer; 4 mM $MgCl_2$, 0.4 mM dATP, 0.4 mM dCTP, 0.4 mM dGTP and 0.4 mM dTTP), 40 pmol oligonucleotide primer and 1 µg of template DNA. The DNA was first denatured for 2 minutes at 95^{o}C followed by 40 cycles of 15 sec denaturation at 95^{o}C, the annealing temperature was progressively decreased by 0.5^{o}C every cycle from 40^{o}C to 34^{o}C for 1 min and 2 min elongation at 72^{o}C with a final elongation for 2 min. The amplified products were separated on 1.5% TBE agarose gels stained with ethidium bromide and viewed under a UV Transilluminator (Bardakci, 2001). The analyses of the amplification products were done manually with consideration of the number of fragments and repeatability of the reaction following the procedures described by Behroozian *et al.* (2002). Each lane of amplified product was checked manually and scored for presence (+) or absence (-) of fragments.

The data generated from this study were presented using descriptive statistics. The data was subjected to statistical analysis using SPSS computer software version 19 for Windows to determine any significant relationship between infection rate, age and gender. Knowledge, attitude and practices were defined and scored as described by Abdela *et al*. (2016).

RESULTS

A total of 400 fresh students, males 208 (52%) and females 192 (48%) participated in this study. Majority 227 (56.8%) were between ages of 16 and 20 years, and 367 (91.8%) were 100 level students. Most of the participants were single 387 (96.8%) and 204 (51%) live with their parents/family (Table 1). Their awareness of the hepatitis B virus infection is less than average 182 (45.5%) while sources of awareness include electronic media 71 (39.0%), internet 64 (35.2%), health workers 60 (33.0%), schools 58 (31.9%), print media 23 (12.6%) and other sources 35 (8.8%) (Table 2). The knowledge of the students concerning the mode of transmission of the HBV was poor, very few believed the virus could be transmitted through blood transfusion 143 (35.8%), sexual intercourse 116 (29.0%), sharing needles 65 (16.3%), nose/ear piercing 25 (6.3%), tattooing 31 (7.8%), mother to child during childbirth 44 (11.0%), sharing of toothbrush 35 (8.8%). High percentage 386 (96.0%) of the students knew the virus cannot be contracted through inhalation, food /water and contact such as

holding /shaking hands with infected person (Table 3). Only 226 (56.5%) of the students have positive attitude on vaccination of healthy people against Hepatitis B virus, while 210 (52.5%) were not aware of vaccination against hepatitis B virus infection, only 188 (47%) agreed to be vaccinated and just 156 (39%) were willing to pay for the cost of vaccination if necessary (Table 4). Based on the knowledge of the students, only 48 (12%) have good knowledge of and attitude 140 (35%) to HBV infection

HBsAg was detected in blood samples of 12 (3%) of the students using both immuno-chromatographic method for Hepatitis B surface antigen and ELISA method which detects immunoglobulin G (IgG) class of antibodies against viral structural protein.

The Random Amplified Polymorphic DNA (RAPD) analysis of the Hepatitis B virus is shown in figure 1. The DNA from the positive samples were resolved into different haplotypes (subtypes) based on the amplification Random DNA of different molecular sizes. The amplified DNA from sample 5993 was discriminated into four distinct bands as against 5, 2 and 3, in samples 5916, 6004 and 5976 respectively. The analysis of the population structure of the Hepatitis B virus revealed varying banding patterns while the Random Amplified Polymorphic DNA band frequencies ranged from 0.25 for OPX 01-150, OPX 01-500 and OPX 01-750 through 0.50 for OPX 01-250 and OPX 01-520 to 0.75 for OPX 01-350 (Figure 1 and Table 5). The most predominant RAPD band was OPX 01-350 with a RAPD frequency of 75%. The least seen bands were OPX 01-150, OPX 01-500 and OPX 01-750kbp having RAPD frequency of 25%.

Table 1: Demographic Characteristics of Respondents

Variables	Frequency	Percentage
Age		
16-20	227	56.8
21-25	138	34.5
>25 years	35	8.8
Gender		
Male	208	52.0
Female	192	48.0
Level		
100	367	91.8
200	33	8.3
Religion		
Islam	119	29.8
Christianity	259	64.8
Others	22	5.5
Marital status		
Single	387	96.8
Married	13	3.3
Who do you live with		
Parent/family	204	51.0
Friends	52	13.0
By myself	136	34.0
Others	8	2.0

Table 2: Knowledge and Source of Information about Hepatitis B Virus Infection

	Frequency	Percentage
Knowledge of Hepatitis B		
Virus infection		
Yes	182	45.5
No	218	54.5
Source of information		
Electronic media		
Yes	71	39.0
No	111	61.0
Print media (newspaper)		
Yes	23	12.6
No	159	87.4
Internet		
Yes	64	35.2
No	118	64.8
Health workers		
Yes	60	33.0
No	122	67.0
School		
Yes	58	31.9
No	124	68.1
Family members		
Yes	22	12.1
No	160	87.9
Others		
Yes	35	8.8
No	147	91.3

Table 3: Knowledge of mode of Transmission of Hepatitis B Virus Infection

Variables	Frequency	Percentage
Mode of infection		
Sexual intercourse		
Yes	116	29.0
No	284	71.0
Sharing of sharp objects		
Yes	65	16.3
No	335	83.8
Blood transfusion		
Yes	143	35.8
No	257	64.3
Nose/ear piercing		
Yes	25	6.3
No	375	93.8
Tattooing		
Yes	31	7.8
No	369	92.3
Mother to child during childbirth		
Yes	44	11.0
No	356	89.0
Sharing toothbrush with an infected person		
Yes	35	8.8
No	365	91.3
By inhalation		
Yes	16	4.0
No	384	96.0
Through food and water		
Yes	16	4.0
No	384	96.0
Hand shaking/holding infected person		
Yes	16	4.0
No	384	96.0

Table 4: Attitude of Students towards Vaccination against Hepatitis B Virus

Variables	Frequency	Percentage
Healthy people should be vaccinated against HBV infection		
Yes	226	56.5
No	85	21.3
I don't know	89	22.3
Are you aware of vaccination against HBV infection		
Yes	114	28.5
No	210	52.5
I don't know	76	19.0
Would you like to receive HBV vaccine		
Yes	188	47.0
No	133	33.3
I don't know	79	19.8
Would you to pay for vaccination		
Yes	156	39.0
No	165	41.3
I don't know	79	19.8

Table 5: RAPD Band Frequencies of the Hepatitis B Virus

RAPD	1	2	3	4	RAPD band frequency
OPX 01- 150	-	-	+	-	0.25
OPX 01- 250	+	+	-	-	0.50
OPX 01- 350	+	+	-	+	0.75
OPX 01- 500	-	-	-	+	0.25
OPX 01- 520	+	+	-	-	0.50
OPX 01-750	-	+	-	-	0.25

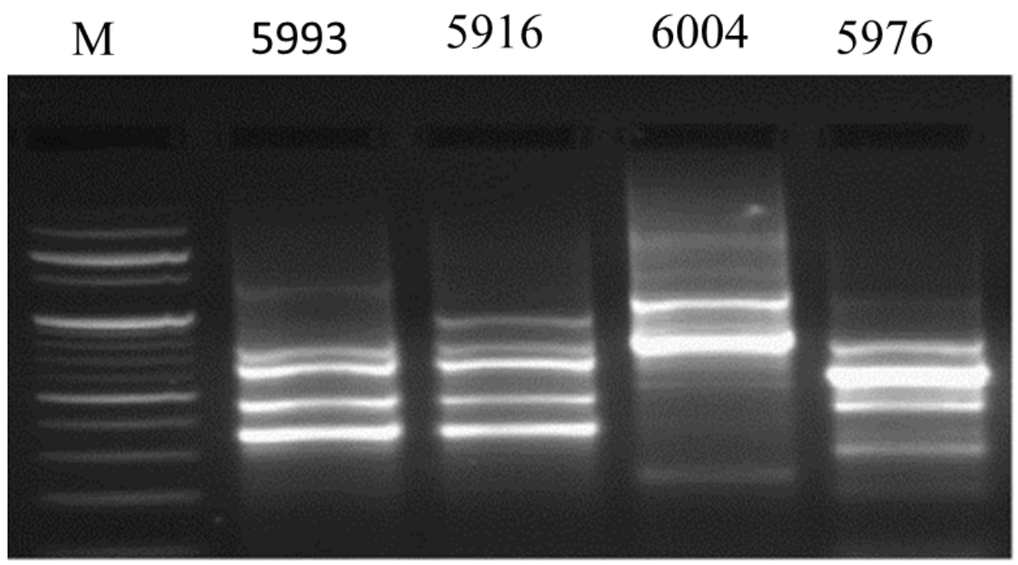

Figure 1: RAPD banding pattern amplified with primer OPX 01 (Operon Tech. Inc.) Lane M = DNA marker (1kbp-13kbp), Lanes 2 – 5 = Template DNA of Hepatitis B Virus (DNA from positive samples 5993, 5916, 6004, 5976

The sero-prevalence of Hepatitis B surface antigen among the fresh undergraduate students of Olabisi Onabanjo University was 3% and this can be described as intermediate prevalence level. Previsani & Lavanchy (2002); MacLachlan & Cowie (2015) defined the epidemiology of hepatitis B in terms of the prevalence of hepatitis B surface antigen (HBsAg) in a population and broadly group the prevalence level as high (>8%), intermediate (2 - 7%) as well as low (2%). This prevalence rate was moderate and in line with 4.7% reported amongst University of Uyo students (Mboto and Edet, 2012). However, high prevalence of 11.5% and 12.5% were reported amongst Nasarawa State University and Ahmadu Bello University students respectively (Pennap *et al.*, 2011; Aminu *et al.*, 2013). The intermediate or moderate prevalence observed in this study population could be attributed to the impact of vaccination and other prevention programs which has been shown to decrease the seroprevalence of HBV (MacLachlan & Cowie, 2015). Most of the people still having HBV infection are those born before hepatitis B vaccine was widely available and included in childhood immunization (WHO, 2017).

Likewise the high seroprevalence reported by other studies (Pennap *et al.*, 2011; Aminu *et al.*, 2013), could be related to poor acceptance of immunization programmes in these areas. Abdulkarim *et al*. (2011), reported that Northern Nigeria has the lowest immunization rates in the world and barely 10 percent of children complete their routine vaccinations, probably because of weakened primary health care system in northern Nigeria over the years. The rumours on the safety of the polio vaccine also, disrupted routine immunization services which are either irregular or no longer available.

The students have poor knowledge about HBV infection; the source of information of the few that were aware of the viral infection includes the electronic media, internet and health care workers. Similarly, only 35% of them had a positive attitude towards the infection. Less than half of the students were aware of Hepatitis B, risk factors and the mode of transmission. This is in contrast to the studies carried out by Al-Jabri *et al*. (2004); Abdela *et al*. (2016) among undergraduate medical and non-medical students in Saudi Arabia and Northwest Ethiopia respectively. Furthermore, Kosisochi *et al.* (2017) reported very good knowledge and poor positive attitude to Hepatitis B

virus infection among undergraduate students of University of Nigeria. These differences can be attributed to the fact that unlike the other studies where majority of the participants were medical and health sciences undergraduate, the entire participants in this study were fresh undergraduate with little knowledge of health issues.

Both the immuno-chromatographic method and the ELISA method gave equal prevalence rate of HBsAg virus infection (3%) in this study. Adeyemi *et al.* (2013) reported 5.7% and 10.8% positive results for HBsAg using immune-chromatographic and ELISA technique respectively. This disparity might probably be due to sensitivity and specificity of the brand of detection kits, methods and the sample size used.

In this study, the DNA from the different participant was resolved into different RAPD polymorphic band which is an indication that there is variation in the hepatitis B DNA. The fact that each of the Hepatitis B DNA was discriminated into different banding types further stressed the genetic diversity of the isolate. This finding is not unexpected as the RAPD technique has long been affirmed as a reliable method for scoring diversity in different organisms (Deshwall, 2005; Kumar & Kumar, 2015).

The prevalence of HBsAg in the participant is moderate, the knowledge, attitude and practice of HBV infection is poor. There is genetic variation in the hepatitis B DNA from the participant.

It is highly recommended that sensitization and awareness campaign should be carried out amongst the seemingly unaware students given their level of ignorance about the transmission of the disease and certain risk factors, so as to prevent the transmission or spread of hepatitis B virus infection.

Competing interests: The authors declare that they have no competing interest

Acknowledgements: We appreciation the Management, the Director of Health Services Olabisi Onabanjo University Health Centre and all the fresh undergraduate students who participated in this study. We also appreciate all laboratory staff that assisted in the research.

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