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STUDIES ON HEPATITIS B VIRUS INFECTION IN EBONYI STATE NIGERIA USING HBSAG AS MARKERS: RAPID ASSESSMENT SURVEY

C.O. Chima[1], D.C. Nwosu[1], A.U. Opara[1], J.N. Dike-Ndudim[1], C.O. Ahiara[1] and *Emmanuel Ifeanyi Obeagu[1,2] 0000-0002-4538-0161

[1]Department of Medical Laboratory Science, Imo State University, Owerri, Imo State, Nigeria.

[2]Department of Medical Laboratory Science, Madonna University, Elele Campus, Elele, Rivers State, Nigeria.

emmanuelobeagu@yahoo.com

ABSTRACT

Studies on Hepatitis B Virus infection in Ebonyi State Nigeria using HBsAg for Rapid Assessment Survey were carried out between the years 2018 to 2020. Ethical approval for the work was obtained from the ministry of Health Ebonyi State. Thereafter, appropriate advocacy visits were made to the authorities of the LGAs and communities in the state. Five communities in each of the thirteen (13) LGAs of the state were randomly selected for the survey. Informed Consent was also obtained from the participants aged 16-50 years of both sexes. Nine thousand five hundred and seventy (9570) participants (3543 males and 6027 females) were recruited for the study. Blood samples were appropriately collected from the subjects and screened for Hepatitis B Virus infection using antibody (Ab) rapid test kit. Serum from the positive samples

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were separated and stored at -20°C for subsequent molecular analysis in phase 2 of this work. The screening result showed 656 positives cases giving a significant prevalence of 6.9% in the study population. According to vaccination, 1.0% prevalence was of those vaccinated and 7.1% for non- vaccinated. This increase was found to be statistically significant. There was variation in prevalence with relation to age group: 3.8% was recorded for those aged 16-20 years, 4.4% for those aged 21-25 years, 6.3% for those aged 26-30 years, 9.0% for those aged 31-35 years, 11.8% for those aged 36-40 years, and a decline of 8.6% for those aged 41-45 and 4.4% for those aged 46-50 years. This variation was found to be statistically significant (Ø<0.05). There was no significant difference in prevalence according to sex, 7.3% for males and 6.6% for females. The result of this study with regard to location also showed significant difference in prevalence, (Ebonyi Central7.1%, Ebonyi North 8.9% and Ebonyi South 5.2%) which was statistically significant (Ø<0.05). The above result is alarming and looking at the age bracket involved, the work force of Ebonyi State is in danger and it calls for serious concern to the government and health authorities at all levels to save the state from a total collapse in the area of manpower.

Keywords*: hepatitis B virus, infection HBsAG as markers, rapid assessment survey*

INTRODUCTION

Hepatitis B virus is a member of the family Hepadnaviridae and genus Orthohepadnavirus. Hepatitis B is a double stranded DNA virus with a lipid-containing outer envelope. It has three important antigens; surface antigen (HBsAg), Core antigen (HBcAg) and e-antigen (HBeAg) (Zuckerman, 1996). Electron microscopy of hepatitis B positive serum reveals three morphological forms, namely; the spherical particles measuring 20nm in diameter, the filamentous forms of about 20 × 200nm long and the Dane particles measuring 42nm, which is the infectious form of hepatitis B virus (Obeagu *et al.*, 2017; Franscica *et al.*, 2017; Obeagu, 2018).

.The viral genome consists of partially double – stranded DNA of about 3200kbp (kilo-base pairs) in length (Carman *et al*., 1989).

The HBV genotypes differ by at least 8% of the sequence and have distinct geographical distributions. There are eight known genotypes labeled A to H (Kramvis *et al.*, 2005). Two additional genotypes have also been found, though not yet universally accepted, making the current listing to run from A to J with several other subtypes also identified (Hernandez *et al.*, 2014).

These virus-specific markers are useful in epidemiological investigations because the secondary cases have the same subtype as the index case (Araujo, 2015).

The stability of HBsAg does not always coincide with that of the infectious agents, however, both are stable at -20°C for a period more than 20 years and also stable to repeated freezing and thawing. The virus is also stable at 25°C for at least 1 week. Hepatitis B virus is sensitive to higher temperatures, up to 100°C for 1 minute and to longer incubation period of 10 hours at 60°C unlike HBsAg. HBsAg is stable at a pH of 2.4 for up to 6 hours, but hepatitis B virus infectivity is lost. A solution of 0.5% Sodium hypochlorite (1:10 bleach), destroys the Hepatitis B virus antigenicity within 3 minutes. HBsAg and its viral infectivity are not destroyed by ultraviolent irradiation of plasma or other blood product (Deuffic - Burban *et al*., 2011).

The virus was not discovered until 1966 when Baruch Blumberg found Australia antigen from blood of Australian people and the virus particle was later discovered by David Dane and his colleagues in 1970 using Electron microscopy (Greenberg *et al*., 1976).

Hepatitis B virus is the causative agent of a potentially life threatening liver infection which can be acute or chronic leading to hepatoma that puts people at high risk of death from cirrhosis and cancer (WHO, 2014).

More than 2 billion people worldwide are estimated to have had hepatitis B virus (HBV) infection, with 350–400 million being chronic carriers of the virus (Lok and McMahon, 2007).

Its prevalence varies throughout the world, but is highest in the tropical regions. It is estimated that 5–15% of adults in sub-Saharan Africa are chronically infected with HBV (Hou *et al*., 2005). In Nigeria, 11.6% prevalence has been reported from Maiduguri among blood donors and pregnant women (Harry *et al*.,1994), 4.3% from Port Harcourt among pregnant women (Akani *et al*.,2005), 5.7% from Ilorin in mothers and their preschool children (Agbede *et al*., 2007), 8.3% from Zaria among pregnant women (Luka *et al*., 2008), 17.1% from female sex workers in Nassarawa (Forbi *et al*., 2008), 14.9% from apparently healthy blood donors in Yola (Olokoba *et al*., 2009) and 25.7% among surgeons in Lagos (Belo, 2000).

Health-care workers have a 3- 5 fold higher prevalence of HBV than the general population, with surgeons and dentists having higher reported cases (Minuk *et al*., 2005). There is a 15–25% risk of dying prematurely in adulthood from HBV-related cirrhosis and hepatocellular carcinoma, while those with acute infections may also succumb to fulminant liver failure (Ganem and Prince, 2004).

In areas of high endemicity where at least 8% of the populations are chronic HBV carriers, HBV is mainly contracted at birth and early childhood (Hou *et al*., 2005). About 90% of those infected during the prenatal period, 30% of those infected in early childhood, and 6% of those infected after 5 years of age develop chronic infection (Lok and McMahon, 2007).

Transmission of HBV among adults occurs through contact with infected blood and body fluids such as semen, vaginal fluids, and saliva. Therefore transfusion of unscreened blood and its products, sexual activities, use of contaminated or inadequately sterilized instruments, sharing of sharp objects as usually occur during some cultural practices like local circumcision, are common means of spread. It could also occur by other means of iatrogenic or horizontal transmission such as long-term household contacts with no sexual involvements in regions of

high endemicity. HBV infection is also recognized as an occupational health hazard for health-care practitioners (Bhattarai *et al*., 2014).

In many regions of the world, such as Europe, the Americas, and Australia, there has been a downward trend in the prevalence of HBV infection due mainly to improved immunization against HBV and health-care practices. This includes screening of blood and blood products before transfusion, injection safety, and infection control policies (Ott *et al*., 2012).

The prevalence of this disease in Nigeria as a whole is not known although the country has long been considered to be among the highly endemic countries of sub-Saharan Africa. Data for chronic viral hepatitis are not routinely collected by the Integrated Disease Surveillance and Response system, which collects only acute viral hepatitis cases; therefore, chronic hepatitis infection remains largely underreported (WHO, 2014).

The study was done to assess Hepatitis B Virus infection in Ebonyi State, Nigeria using HBsAg for Rapid Assessment Survey.

MATERIALS AND METHOD

Study Area

The study was carried out in Ebonyi State, South Eastern Nigeria.

Study Population

Five (5) Development centers from each of the thirteen (13) local government areas of Ebonyi State were randomly selected for participation during the Rapid epidemiological survey. A maximum of two hundred (200) Participants, male and female apparently healthy, from each Development center between the ages of 16 and 50 years were recruited and screened for HBV infection.

Advocacy, Mobilization and Pre-survey Contacts

Advocacy visits were made to the local authorities in the study area with a letter of introduction from the head of Department of Medical Laboratory Science IMSU. The Hon. Commissioner of Health, Ebonyi State was the first approached for ethical approval. With the ethical approval letter from the Ministry of Health, I went to the Hon. Commissioner for Local Government and Chieftaincy Affairs, Chairmen of LGAs, LGA Health authorities, the Coordinators of the Development centers, His Royal Highness and Community leaders. Meetings were held with the Traditional Ruler in the selected Development Centers during which they were properly informed of the study and the benefits they stood to gain from it. Date, time and venue for the sample collection were agreed on. Field workers were recruited, evenly spread, from the communities in the Development centers and trained with respect to their job description. Announcements were made in worship centers, village meetings, markets and hamlets using town criers in all the communities visited. Fliers were also shared.

Sample Size

This was determined using Araoye (2004).

Formula

Sample size $\mathbf{N = z^2pq/d^2}$

N= Minimum Sample Size for Significant Survey

Z = Standard Normal Deviation Set Which is equivalent to 95% confidence interval and equal to 1.96

P = proportion in the target population in a previous study (Olokoba *et al*., 2009).

q = 1-p

d = margin of acceptance error= 5% (0.05)

Sample size N= 1.96^2 x 0.149 (1- 0.149)/0.05^2

= 3.8416 x 0.149(0.851)/ 0.0025

=0.572 x 0-851/0.0025

=0.4868/0.0025

Sample size N =194.7

N= approx. 195 (minimum size for significant survey)

Selection Criteria

A) Inclusion

a) Those within the age range of 16-50 years.

b) Those that gave their consent.

B) Exclusion

a) Those that refused consent.

b) Those outside the age bracket.

Study Design

Rapid assessment survey (RAS) or Rapid epidemiological mapping (REM) to locate the HBV infection belts in the State. This was done using rapid serological technique for qualitative detection of HBsAg in the population studied.

Sample Collection

About 5mls of venous blood was aseptically collected from each participant using sterile syringes and needles (by venipuncture technique) into dry plastic containers without anticoagulant. It was properly labeled and taken in cooler boxes to the laboratory (Divine Health Diagnostic Medical Laboratories Ltd Aba, Abia State) for separation and the serum was used for rapid serological technique for qualitative detection of HBsAg.

Laboratory Procedures

All reagents were commercially purchased and the manufacturer's standard operational procedure (S.O.P) was followed strictly during the screening test.

Determination of HBsAg

Screening Test: A rapid one step test strip for the qualitative detection of Hepatitis B Surface Antigen (HBsAg) in serum and plasma as modified by Zhejiang Orient Gene Biotech for Tell Company **catalog No: 20190420** was used.

Statistical Analysis

The data generated from the work were analyzed by Chi-square statistical tool. The values were expressed as percentage, mean and standard deviation, and results presented in tables and figures.

Calculation of Chi-square (y^2)

$$y^2 = \sum_{0-1}^{2} \sum_{E-1}^{2} \frac{(0_f - E_f)^2}{Ef}$$

Where Of = Observed frequency

Ef = Expected

and

$$Ef = \frac{\text{Row total} \quad X \quad \text{Column total}}{\text{Grand total}} = Ei.j$$

Where i = row position (1^{st}, 2^{nd})

j = Column position (1^{st}, 2^{nd})

RESULTS

Table 5: The overall seroprevalence of Hepatitis B virus infection using HBsAg in the study area in phase 1

Study Population.	No. Examined.	No. Positive.	No. Negative.	Prevalence (%)
16 – 50 years	9570	656	8914	6.9

Out of the 9570 people examined, 656 were positive giving an overall seroprevalence of 6.9%.

Table 6: Seroprevalence of HBV infection according to vaccination

Vaccination	No. Examined	No. Positive	No. Negative	Prevalence (%)
Vaccinated	384	4	380	1.0
Non-vaccinated	9186	652	8534	7.1

Total	9570	656	8914	6.9

Out of 384 people that were vaccinated, 4 were positive giving a prevalence of 1.0%, 9186 were not vaccinated and 652 were positive giving a prevalence of 7.1%.

Table 7: Seroprevalence of HBV infection according to age

Age(yrs)	No. Examined.	No. Positive.	No. Negative.	Prevalence (%)
16-20	770	29	741	3.8
21-25	935	41	894	4.4
26-30	1112	70	1042	6.3
31-35	1110	100	1010	9.0
36-40	1400	165	1235	11.8
41-45	1522	131	1391	8.6
46-50	2721	120	2601	4.4
Total	**9570**	**656**	**8914**	**6.9**

Out of the 656 people that were positive, 29(3.8%) were in the age bracket of 16-20 years. 21-25 years had 41(4.4%), 26-30years 70(6.3%), 31-35 years 100(9.0%), 36-40 years 165(11.8%), 41-45 years 131(8.6%) and 46-50 years 120(4.4%). There is a significant association in the prevalence according to age.

Table 8: Seroprevalence of HBV infection according to sex

Sex	No. Examined.	No. Positive.	No. Negative.	Prevalence (%)
Male	3543	258	3285	7.3
Female	6027	398	5629	6.6
Total	**9570**	**656**	**8914**	**6.9**

Out of 656 people infected, males had 258(7.3%) and females 398(6.6%).

There is no significant association in prevalence with respect to sex

Table9: Seroprevalence of Hepatitis B virus infection according to occupation

Occupation.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Applicants	1203	32	1171	2.7
Artisans	986	105	881	10.6
Civil Servants	1027	46	981	4.5
Clergy	134	6	128	4.5
Drivers	523	55	468	10.5
Farmers	3050	244	2806	8.0

Health workers	202	22	180	10.9
Sex workers	0	0	0	0
Students	1204	22	1182	1.8
Teachers	235	17	218	7.2
Traders	1006	107	899	10.6
Total	9570	656	8914	6.9

Out of the 656 people that were positive to HBsAg, Applicants had 32(2.7%), Artisans 105(10.6%), Civil Servants 46(4.5%), Clergy 6(4.5%), Drivers 55(10.5%), Farmers 244(8.0%), Health workers 22(10.9%), Sex workers 0(0%), Students 22(1.8%), Teachers 17(7.2%) and Traders 107(10.6%).

There is significant association in prevalence according to occupation in Ebonyi State with the highest prevalence of 10.9% among the Health workers and the least 1.8% among the Students.

Table 10: Seroprevalence of HBV infection according to location (LGAs)

LGAs.	No. examined	No. Positive	No. Negative	Prevalence %
Abakaliki	835	58	777	6.9
Afikpo North	850	45	805	5.3
Afikpo South	920	52	868	5.7
Ebonyi	586	50	536	8.5
Ezza North	802	68	734	8.5

Ezza South	788	63	725	8.0
Ikwo	912	47	865	5.2
Ishielu	698	60	638	8.6
Ivo	520	25	495	4.8
Izzi	575	64	511	11.1
Ohaozara	740	38	702	5.1
Ohaukwu	711	55	656	7.7
Onicha	633	31	602	4.9
Total	**9570**	**656**	**8914**	**6.9**

Out of 656 people that were positive in Ebonyi State, Abakaliki LGA had 58(6.9%), Afikpo North 45(5.3%), Afikpo South 52(5.7%), Ebonyi 50(8.5%), Ezza North 68(8.5%), Ezza South 63(8.0%), Ikwo 47(5.2%), Ishelu 60(8.6%), Ivo 25(4.8%), Izzi 64(11.1%), Ohaozara 38(5.1%), Ohaukwu 55(7.7%) and Onicha LGA 31(4.9%).

There is significant association in prevalence according to the Local Government Areas with the highest prevalence of 11.1% in Izzi and the least of 4.8% in Ivo LGA.

Table 11: Seroprevalence of HBV infection according to geopolitical zones

Zones.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Ebonyi Central	3337	236	3101	7.1 Ebonyi North
2570	229	2341	8.9	

Ebonyi South	3663	191	3472	5.2
Total	**9570**	**656**	**8914**	**6.9**

Out of the 656 people that were positive, Ebonyi central had 236(7.1%), Ebonyi North had 229(8.9%) and Ebonyi South had 191(5.2%).

There is a significant association in the prevalence according to location

DISCUSSION

The overall prevalence of Hepatitis B virus infection in the study population using HBsAg Rapid Assessment Survey was found to be 6.9% which is far above 5.0% highest prevalence found in Sub Sahara Africa and 2% in America and Europe according to WHO (2017) and could be attributed to the poor or absence of vaccination program in Ebonyi State Nigeria. Other possible contributing factors may include; multiple sex partners, lack of awareness campaign, lack of screening facilities in the rural areas especially before blood transfusion, child delivery, traditional circumcision and even before marriage. The population of Ebonyi State is well above two million people and majority of them are rural dwellers. This study showed that over 90% of the population has no knowledge of Hepatitis B virus.

Therefore, ignorance of the disease has contributed to the high prevalence of HBV infection in the state. This is a serious threat to life and major challenge to manpower and economic advancement in Ebonyi State. The result of this work has shown that, the incidence reduction goal of the WHO which targeted less than 1% prevalence of HBV infection globally in 2020 (WHO, 2016) failed in Ebonyi State. Consequently, the 2030 target of reducing HBV infection and its associated complications to 0.1% (WHO, 2018) may not be feasible unless something drastic is done by the government of Ebonyi State.

The major occupation of Ebonyians is farming which encourages polygamous marriage hence multiple sex partners which leads to wide spread of HBV infection according to Hughes (2000). However, the high prevalence of HBV infection in Ebonyi state is in agreement with Davies *et al*. (2013) which indicated that absence or ineffective primary health care facilities in the rural areas is a major factor in the spread of HBV infection. In Ebonyi State, women at the rural areas still deliver their babies in the homes of traditional birth attendants due to lack of health care facilities and qualified health professionals.

The seroprevalence of HBV infection according vaccination is statistically significant (<0.05). Out of 384 vaccinated participants, only 4(1. %) was positive and out of 9186 non vaccinated participants, 652(7.1%) were positive. This is in agreement with Chang *et al*. (2009) where Taiwan with 10% HBV infection prevalence, twenty years after introducing Hepatitis B virus vaccine, reduced to 1.2% prevalence. Wong *et al*. (2014) and Aspinall *et al*. (2011) findings also agreed with the work where vaccination enhanced reduction in the prevalence of HBV infection.

The age specific rate were 3.8% in the study population of age (16-20) years, 4.4% for those within the age (21-25) years, 6.3% for those within the age (26-30) years, 9.0% for those within the age (31-35) years, 11.8% for those within the age (36-40) years, 8.6% for those within the age (41-45) years and 4.4% for those within the age (46-50) years. There is a significant rise in the prevalence of HBV infection within the population as age increases from 16-40 years but declined from the age of 41-50 years. This could be attributed to the effect of the introduction of immunization program against HBV infection in Nigeria in 2004 and also in agreement with Aspinall *et al*. (2011) which indicated that the protective value of the vaccine decreases as age increases. The decline in the prevalence within the age of 41-50 years could be attributed to the health condition of those infected by HBV that they could not participate in the survey or majority of them have died.

From this study, there is no statistical significant increase in prevalence according to sex, male 7.3% as against female, 6.6% (>0.05).

The prevalence of HBV infection according to location is statistically significant in this study with Ebonyi North having the highest prevalence of 8.9% followed by Ebonyi Central, 7.1% and Ebonyi South 5.2%.

In Ebonyi North where we have the highest prevalence, the reasons are not farfetched. There is no secondary health care facility in the zone; the primary health care centers available are simply structural representation. There are virtually no preventive activities against HBV in Ebonyi North which is in agreement with Davies *et al*. (2013). The zone is also thickly populated with low income rural dwellers (peasant farmers, traders and artisans) that cannot afford to access improved health care services outside the zone. Ebonyi Central almost shared the same factors as Ebonyi North except the presence of Mile 4 Catholic Missionary Hospital Abakaliki founded in 1937 which provided secondary health care services to the zone. The creation of Ebonyi State in 1996 later attracted to the zone Tertiary Health Institution – Ebonyi State University Teaching Hospital (EBSUTH) and some standard private hospitals and Laboratories at the state capital which has contributed to the prevalence reduction. The lower prevalence in Ebonyi South could be attributed to the presence of two missionary hospitals strategically sited in the zone. The Presbyterian Joint Hospital Uburu established in 1912 and the Mater Misericordiae Hospital Afikpo founded in 1946 actually provided improved health care services that led to the lowest prevalence in Ebonyi State.

Conclusion

The result of this study seems very high with overall prevalence of 6.9% especially when compared to 1% WHO universal target for 2020 and 0.1% for 2030. The reason is not farfetched; Ebonyi state was created by joining the most neglected zones from former Abia and Enugu States. There was no secondary health institution owned by the government in the zones. The primary health care centers were poorly staffed with auxiliary health workers and no modern facilities especially in the area of medical laboratory services. Blood transfusion is apparently carried out without screening. The three missionary hospitals in Ebonyi South and Central were

there to serve the missionaries and the church members and could not replace government roles for the people. In Ebonyi North where none existed, the prevalence was the highest among the three zones. The immunization scheme against Hepatitis B virus may have existed only on paper or was not effectively carried out. There was no awareness campaign against HBV infection hence deaths recorded due to the infection were attributed to punishment from the gods of the land for offence committed by the victims or the hand work of the enemies. Considering the mortality, morbidity, social and economic effects of Hepatitis B virus infection on humanity, the prevalence rate in Ebonyi State is alarming and looking at the age bracket involved, the work force of Ebonyi State is in danger and it calls for serious concern to the Government and health authorities at all levels to save the state from a total collapse in the area of manpower. It is also deduced from the result of this work that Hepatitis B virus immunization scheme may be compromised in Ebonyi State. Therefore, there is urgent need to monitor the entire scheme for effective coverage and better result.

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