Madonna University Journal of Medicine and Health Sciences Volume 2 issue 1 (2022), Pp. 159-184

http://madonnauniversity.edu.ng/journals/index.php/medicine

Comparative Studies on Hepatitis B Virus Infection in Ebonyi State Nigeria Using HBsAg as indices

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 and HBeAg as indices were carried out between the years 2018 to 2021. 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 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

Chima, C.O. D.C. Nwosu, D.C., Opara, A.U. Dike-Ndudim, J.N. Ahiara, C.O. and Obeagu, E.I. (2022). Comparative Studies on Hepatitis B Virus Infection in Ebonyi State Nigeria Using HBsAg as indices. Madonna University Journal of Medicine and Health Sciences. 2(1): 159-184

significant (Ø<0.05). In phase 2 of this work, six hundred and fifty six (656) positive samples to HBsAg were subjected to molecular analysis using HBeAg ELISA kit. The result showed 640 positives giving an overall prevalence of 6.7% in the study population which is still statistically significant. According to vaccination, out of the 4 positive samples from vaccinated participants only 1 sample remained positive using HBeAg Biomarker giving a vaccination - dependent prevalence of 0.3% in the study population. Out of the 652 non vaccinated persons that were positive in phase 1, 639 samples remained positive giving a non-vaccinated – dependent prevalence of 7.1% in the study population. According to age cohort, there was still variation in prevalence with age group, 3.6% for those aged 16-20 years, those aged 21-25 years had the same prevalence of 4.4%, 6.1% for those aged 26-30 years, 8.9% for those aged 31-35 years, 11.5% for those aged 36-40 years, 8.4% prevalence for those aged 41-45 and 4.2% for those aged 46-50 years. Prevalence of HBV infection according to sex in the study population still remained statistically insignificant. The result of the molecular analysis of this study according to location showed difference in prevalence as follows; Ebonyi Central 6.8%, Ebonyi North 8.6% and Ebonyi South 5.2% with Ebonyi North still maintaining the lead and it is 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*

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(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).

By the early 1980s the genome of the Hepatitis B virus had been sequenced and also the first vaccine tested (Petersen and Buti, 2012).

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).

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. 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).

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).

Comparative Study was done on Hepatitis B Virus infection in Ebonyi State, Nigeria using HBsAg and HBeAg as indices

MATERIALS AND METHOD

Study Area

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

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 Rulers 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

This work was done in two phases as follows;

1) 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.

2) Wet laboratory work (molecular assay) was carried out to determine the epidemiological status of the state through the detection of the HBeAg Biomarker.

Sample Collection

Phase 1

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. The positive samples were stored at -20°C for subsequent molecular analysis in phase 2 of this work to determine the Biomarkers of Hepatitis B virus.

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.

Phase 2

HBeAg Biomarker

All the positive samples were subjected to; molecular analysis for Hepatitis B Virus e Antigen (HBeAg) Biomarker using qualitative wet enzyme-linked immunosorbent assay (ELISA) at Divine Health Diagnostic Medical Laboratories Ltd. All reagents (ELISA Kits) were commercially purchased and the manufacturer's standard operational procedure (S.O.P) was followed strictly during the wet laboratory work.

Determination of HBeAg

Enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of human hepatitis B virus e antigen (HBeAg) concentrations in serum as modified by Biotech, Life Science Manufacturing Company San Diego USA (MyBioSource) with **Catalog No. MBS701371** was used.

Statistical Analysis

The data generated from the work (phases 1 and 2) 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

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

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

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

RESULTS

Table 1: 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 2: 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 3: 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 4: 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.

Table5: 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 6: 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 7: 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.

Table 8: Prevalence of HBeAg Biomarker among the positive population in phase 1

Biomarker.	No. Examined	No. Positive	No. Negative	Prevalence (%)
HBeAg.	656	640	16	97.6

Out of the 656 HBsAg positive samples examined for HBeAg biomarker, 640 was positive giving a prevalence of 97.6%.

Table 9: Prevalence of HBeAg Biomarker among the positive population in phase 1 according to vaccination

Vaccination.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Vaccinated.	4	1	3	25.0
Non-vaccinated.	652	639	13	98.0
Total	**656**	**640**	**16**	**97.6**

Out of 4 vaccinated people that were tested for HBeAg, 1 was positive giving a prevalence of 25.0% while 652 that were not vaccinated 639 were positive giving a prevalence of 98.0%.

Table 10: Prevalence of HBeAg Biomarker among the positive population in phase 1 according to sex

Sex	No. Examined	No. Positive	No. Negative	Prevalence (%)
Male	258	246	12	95.3
Female	398	394	4	99.0
Total	**656**	**640**	**16**	**97.6**

Out of 640 people infected, males had 246(95.3%) and females 394(99.0%).

Table 11: Prevalence of HBeAg Biomarker among the positive population in phase 1 according to age

Age	No. Examined	No. Positive	No. Negative	Prevalence (%)
16-20	29	28	1	96.6
21-25	41	41	0	100.0
26-30	70	68	2	97.1
31-35	100	99	1	99.0
36-40	165	161	4	97.6
41-45	131	128	3	97.7
46-50	120	115	5	95.8
Total	**656**	**640**	**16**	**97.6**

Out of the 640 people that were positive, 28(96.6%) were in the age bracket of 16-20 years. 21-25 years had 41(100%), 26-30years 68(97.1%), 31-35 years 99(99.0%), 36-40 years 161(97.6%), 41-45 years 128(97.7%) and 46-50 years 115(95.8%).

Table12: Prevalence of HBeAg Biomarker among the positive population in phase 1 according to occupation

Occupation.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Applicants	32	32	0	100
Artisans	105	102	3	97.1
Civil Servants	46	46	0	100
Clergy	6	6	0	100
Drivers	55	55	0	100
Farmers	244	240	4	98.4
Health workers	22	21	1	95.5
Students	22	22	0	100
Teachers	17	16	1	94.1
Traders	107	100	7	93.5
Total	656	640	16	97.6

Out of the 640 people that were positive to HBeAg in Ebonyi State, Applicants had 32(100%), Artisans 102(97.1%), Civil Servants 46(100%), Clergy 6(100%), Drivers 55(100%), Farmers 240(98.4%), Health workers 21(95.5%), Students 22(100%), Teachers 16(94.1%) and Traders 100(93.5%).

Table 13: Prevalence of HBeAg Biomarker among the positive population in phase 1 according to L.G.As

LGAs.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Abakaliki	58	56	2	96.6
Afikpo North	45	45	0	100.0
Afikpo South	52	51	1	98.0
Ebonyi	50	47	3	94.0
Ezza North	68	64	4	94.1
Ezza South	63	61	2	96.8
Ikwo	47	47	0	100.0
Ishielu	60	59	1	98.3
Ivo	25	25	0	100.0
Izzi	64	63	1	96.9
Ohaozara	38	38	0	100.0
Ohaukwu	55	53	2	96.4
Onicha	31	31	0	100.0
Total	**656**	**640**	**16**	**97.6**

Out of 640 people that were positive for HBeAg Biomarker in Ebonyi State, Abakaliki LGA had 56(96.6%), Afikpo North 45(100%), Afikpo South 51(98.0%), Ebonyi 47(94%), Ezza North 64(94.1%), Ezza South 61(96.8%), Ikwo 47(100%), Ishelu 59(98.3%), Ivo 25(100%), Izzi 63(96.9%), Ohaozara 38(100%), Ohaukwu 53(96.4%) and Onicha LGA 31(100%).

Table 14: Prevalence of HBeAg Biomarker among the positive population in phase 1 according to geopolitical zones

Zones.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Ebonyi Central	236	228	8	96.6
Ebonyi North	229	222	7	96.9
Ebonyi South	191	190	1	99.5
Total	**656**	**640**	**16**	**97.6**

Out of the 640 people that were positive for HBeAg Biomarker, Ebonyi central had 228(96.6%), Ebonyi North had 222(96.9%) and Ebonyi South had 190(99.5%).

Table15: The overall prevalence of HBV infection using HBeAg Biomarker in the study population

Study Population.	No. Examined	No. Positive	No. Negative	Prevalence (%)
16-50	9570	640	8930	6.7

Out of the 9570 people examined, 640 were positive using HBeAg Biomarker giving an overall seroprevalence of 6.7%. This is in contrast to 6.9% prevalence using HBsAg rapid screening technique.

Table 16: Prevalence of HBV infection using HBeAg Biomarker in the study population according to vaccination

Vaccination.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Vaccinated	384	1	383	0.3
Non-vaccinated	9186	639	8547	7.0
Total	**9570**	**640**	**8930**	**6.7**

Out of 384 people that were vaccinated, 1 was positive using HBeAg Biomarker giving a prevalence of 0.3%.

Out of 9186 that were not vaccinated, 639 were positive using HBeAg Biomarker giving a prevalence of 7.0%.

Table 17: Prevalence of HBV infection using HBeAg Biomarker in the study population according to age

Age	No. Examined	No. Positive	No. Negative	Prevalence (%)
16-20	770	28	742	3.6
21-25	935	41	894	4.4
26-30	1112	68	1044	6.1
31-35	1110	99	1011	8.9
36-40	1400	161	1239	11.5
41-45	1522	128	1394	8.4
46-50	2721	115	2606	4.2
Total	**9570**	**640**	**8930**	**6.7**

Out of the 640 people that were positive using HBeAg Biomarker, 28(3.6%) were in the age bracket of 16-20 years. 21-25 years had 41(4.4%), 26-30years 68(6.1%), 31-35 years 99(8.9%), 36-40 years 161(11.5%), 41-45 years 128(8.4%) and 46-50 years 115(4.2%). There is still significant association in the prevalence according to age using HBeAg Biomarker.

Table18: Prevalence of HBV infection using HBeAg Biomarker in the study population according to sex

Sex	No. Examined	No. Positive	No. Negative	Prevalence (%)
Male	3543	246	3297	6.9
Female	6027	394	5633	6.5
Total	**9570**	**640**	**8930**	**6.7**

Out of 3543 male examined, 246(6.9%) were positive using HBeAg Biomarker and out of 6027 females examined, 394 were positive giving a prevalence of (6.5%).

There is still no significant association in prevalence with respect to sex.

Table19: Prevalence of HBV infection using HBeAg Biomarker in the study population according to occupation

Occupation.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Applicants	1203	32	1171	2.7
Artisans	986	102	884	10.3
Civil Servants	1027	46	981	4.5
Clergy	134	6	128	4.5
Drivers	523	55	468	10.5
Farmers	3050	240	2810	7.9

Health workers	202	21	181	10.4
Students	1204	22	1182	1.8
Total	9570	640	8930	6.7

Out of the 640 people that were positive to HBeAg Biomarker, Applicants had 32(2.7%), Artisans 102(10.3%), Civil Servants 46(4.5%), Clergy 6(4.5%), Drivers 55(10.5%), Farmers 240(7.9%), Health workers 21(10.4%), Students 22(1.8%), Teachers 16(6.8%) and Traders 100(9.9%).

There is still significant association in prevalence according to occupation in Ebonyi State using HBeAg Biomarker as indices with the highest prevalence of 10.5% among the Drivers and the least 1.8% among the Students.

Table 20: Prevalence of HBV infection using HBeAg Biomarker in the study population according to L.G.As

LGAs.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Abakaliki	835	56	779	6.7
Afikpo North	850	45	805	5.3
Afikpo South	920	51	869	5.5
Ebonyi	586	47	539	8.0
Ezza North	802	64	738	8.0
Ezza South	788	61	727	7.7
Ikwo	912	47	865	5.2
Ishielu	698	59	639	8.5
Ivo	520	25	495	4.8
Izzi	575	63	512	11.0

Ohaozara	740	38	702	5.1
Ohaukwu	711	53	658	7.5
Onicha	633	31	602	4.9
Total	**9570**	**640**	**8930**	**6.7**

Out of 640 people that were positive in Ebonyi State using HBeAg Biomarker, Abakaliki LGA had 56(6.7%), Afikpo North 45(5.3%), Afikpo South 51(5.5%), Ebonyi 47(8.0%), Ezza North 64(8.0%), Ezza South 61(7.7%), Ikwo 47(5.2%), Ishelu 59(8.5%), Ivo 25(4.8%), Izzi 63(11.0%), Ohaozara 38(5.1%), Ohaukwu 53(7.5%) and Onicha LGA 31(4.9%).

There is significant association in prevalence according to the Local Government Areas with the highest prevalence of 11.0% in Izzi and the least of 4.8% in Ivo LGA.

Table 21: Prevalence of HBV infection using HBeAg Biomarker in the study population according to geopolitical zones

Zones.	No. Examined	No. Positive	No. Negative	Prevalence (%)
Ebonyi Central	3337	228	3109	6.8
Ebonyi North	2570	222	2348	8.6
Ebonyi South	3663	190	3473	5.2
Total	**9570**	**640**	**8930**	**6.7**

Out of the 640 people that were positive using HBeAg Biomarker, Ebonyi central had 228(6.8%), Ebonyi North had 222(8.6%) and Ebonyi South had 190(5.2%).

There is a significant association in the prevalence according to Senatorial Zones with Ebonyi North maintaining the lead.

DISCUSSION

The overall prevalence of Hepatitis B virus infection in the study population using HBeAg Biomarker was found to be 6.7% 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 have 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 1(0.3%) was positive and out of 9186 non vaccinated participants, 652 (7.0%) 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 3.6% in the study population of age (16-20) years, 4.4% for those within the age (21-25) years, 6.1% for those within the age (26-30) years, 8.9% for those within the age (31-35) years, 11.5% for those within the age (36-40) years, 8.4% for those within the

age (41-45) years and 4.2% 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 6.9% as against female, 6.5% (>0.05).

The prevalence of HBV infection according to location is statistically significant in this study (appendixes 35 and 36) with Ebonyi North having the highest prevalence of 8.6% followed by Ebonyi Central, 6.8% 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 6.7% 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.

REFERENCES

Agbede O.O, Iseniyi J.O, Kolewale M.O, Ojuawo A (2007). Risk factors and seroprevalence of hepatitis B antigenemia in mothers and their preschool children in Ilorin, Nigeria. *Therapy*. **4:**67–72

Akani CI, Ojule AC, Opurum HC, Ejilemele AA(2005). Seroprevalence of HBsAg in pregnant women in Port Harcourt. *Nigerian Postgraduate Medical Journal*. **12:**266–270**.**

Alpert E, Isselbacher K.J, Schur P.H(2007). The pathogenesis of arthritis associated with viral hepatitis. Complement-component studies. *New England Journal of Medicine* **285:**185–189**.**

*Araujo, N.M (2015). Hepatitis B virus intergenotypic recombinants worldwide: An overview. Infection, genetics and evolution. Journal of molecular epidemiology and evolutionary genetics in infectious diseases. **36:** 500–510.*

Aspinall , E. J, Hawkins G, Fraser A, Hutchison S.J, Golderag D(2011). Hepatitis B prevention, diagnosis, treatment and care. *Occupational medicine* **61**(8): 531-540.

Belo A.C (200). Prevalence of hepatitis B virus markers in surgeons in Lagos, Nigeria. *East African Medical Journal*.**77:**283–285.

Bhattarai S, Pradhan P.M, Lama S, Rijal S(2014). Hepatitis B vaccination status and needle-stick and sharps-related injuries among medical school students in Nepal: a cross-sectional study. BMC Res Notes. **7:**774.

*Carman W.F, Jacyna M.R, Hadziyannis S, Karayiannis P, McGarvey M.J, Makris A, Thomas HC (1989). Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. Lancet, **2** (8663): 588- 591.*

Chang M.H, You S.L, Chen C.J, Liu C.J, Lee C.M, Lin S.M, Chu H.C, Wu T.C, Yang S.S, Kuo H.S (2009). Decreased incidence of hepatocellular carcinoma in hepatitis B vaccinees: A 20-year follow-up study. *Journal of National Cancer Institute.* **101:** 1348–1355.

Davies J, Littlejohn M, Locarnini S.A, Whiting S, Hajkowicz K, Cowie B.C, Bowden D.S, Tong S.Y, Davis J.S (2013). The molecular epidemiology of hepatitis B in the indigenous people of Northern Australia. *Journal of Gastroenterology and Hepatology* **28**: 1234–1241.

Deuffic -Burban S , Delarocque -Astagneau E, Abiteboul D (2011). Blood- borne viruses in health care workers, Prevention and management. *Journal of clinical virology*. **52:**4.

Forbi JC, Onyemauwa N, Gyar S.D, Oyeleye A.O, Entonu P, Agwale S.M (2008). High prevalence of hepatitis B virus among female sex workers in Nigeria. *Institute of Tropical Medicine* Sao Paulo.**50:**219–221.

Ganem D and Prince A.M (2004). Hepatitis B virus infection--natural history and clinical consequences. England Journal of Medicine, **350:**1118–1129.

*Greenberg H.B, Pollard R.B, Lutwick L.I, Gregory P.B, Robinson W.S, Merigan T.C(1976). Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. England Journal of Medicine, **295**: 517-522.*

Harry T.O, Bajani M.D, Moses A.E (1994). Hepatitis B virus infection among blood donors and pregnant women in Maiduguri, Nigeria. *East African Medical Journal*, **70**:596–597.

Hernández S, Venegas M, Brahm J, Villanueva RA (2014) Full-genome sequence of a hepatitis B virus genotype. *Genome Announcement*, **2** (5): e01075–14

Hou J, Liu Z, Gu F (2005). Epidemiology and prevention of hepatitis B virus infection. *International Journal of Medical Science*, **2**:50–57.

Hughes R.A (200). Drug injectors and the cleaning of needles and syringes. *European Addiction Research,* **6**(1): 20-23.

Kramvis A, Kew M, François G (2005*).* Hepatitis B virus genotypes. *Vaccine*, **23** (19): 2409–2423

Kramvis A and Kew M.C(2005). Relationship of genotypes of hepatitis B virus to mutations, disease progression and response to antiviral therapy. *Journal of Viral Hepatology*, **12**: 456–464.

Lok A.S and McMahon B.J(2009). Chronic hepatitis B. *Hepatology*, **50**: 661–662.

Olokoba A.B, Salawu F.K, Danburam A, Desalu O.O, Olokoba L.B, Wahab K.W(2009). Viral hepatitis in voluntary blood donors in Yola, Nigeria. *European Journal of Scientific Research,* **31**:329–334.

Ott J.J, Stevens G. A, Groeger J, Wiersma S.T (2012). Global epidemiology of hepatitis B virus infection: new estimates of age-specific HBsAg seroprevalence and endemicity. *Vaccine,* **30**:2212–2219.

WHO (2018). Regional framework for the triple elimination of mother-to-child transmission of HIV, hepatitis B and syphilis in Asia and the Pacific 2018–2030. WPR/RC68/7, *Geneva.*

WHO (2017). Background – Epidemiology and natural history: Guidelines on Hepatitis B testing, *Geneva.*

WHO (2016).Global health sector strategy on viral hepatitis, 2016–2021,*Geneva.*

WHO (2014). Hepatitis B. http://www who.inf/inf-s/en/fact204.html

Wong F; Pai R; Van Schalkwyk J, Yoshida E.M (2014). Hepatitis B in pregnancy: a concise review of neonatal vertical transmission and antiviral prophylaxis. *Annals of Hepatology* , **13** (2): 187-195.

Zuckerman A.J (1996).Hepatitis Viruses. In: Baron's Medical Microbiology (4th Ed.). University of Texas Medical Branch. ISBN 0-9631172-1-1.

Okoroiwu I.J., Obeagu E.I., and Igbikwe C. Evaluation of Acute Phase Proteins (Alpha-Fetoprotein, Erythrocyte, Sedimentation Rate and Leucocytes Counts in Viral Hepatitis Patients in Aba. Madonna Journal of Medicine and Health Sciences. 2022 2(1) 98 – 107.

Obeagu, E.F., Onyenweaku, F.C., Nwobodo, H.A., Ochei, K.C., Ochiabuto,O.M.T.B. (2017) Impact of HIV and Hepatitis B Virus Coinfection on Selected Haematological Markers of the Patients in Umuahia, Abia State, Nigeria. Ann Clin Lab Res. 5:2. doi:10.21767/2386-5180.1000175

Francisca, O.U., Ihongbe, J.C., Ifeanyi, O.E. (2017). Evaluation of Some Immunological and Haematological Indices of Hepatitis B Infected Subjects in Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. J Biomedical Sci. 6:3. doi: 10.4172/2254-609X.100061

Obeagu, E I. (2018). A Review on Hepatitis B Virus and Hepatocellular Carcinoma. Canc

Chima, C.O. D.C. Nwosu, D.C., Opara, A.U. Dike-Ndudim, J.N. Ahiara, C.O. and Obeagu, E.I. (2022). Comparative Studies on Hepatitis B Virus Infection in Ebonyi State Nigeria Using HBsAg as indices. Madonna University Journal of Medicine and Health Sciences. 2(1): 159-184

Therapy & Oncol Int J.10(2): 555784. DOI: 10.19080/CTOIJ.2018.10.555784