Original Research Article

Serological Pattern of Hepatitis B Virus Infection and Risk factors among Infected Subjects in Port Harcourt, Rivers state, Nigeria

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ABSTRACT

Hepatitis B virus infection is a serious global public health challenge that affects more than two billion people worldwide. This study aimed to evaluate the serological pattern of HBV infection in HBV infected patients in Port Harcourt, Nigeria.The main aim of this study was to evaluate the serological pattern of hepatitis B infection in Port Harcourt, Nigeria. This was a comparative cross sectional study carried out on 260 hepatitis B patients and blood donors attending hepatitis B clinics, and blood banks in Rivers State University Teaching Hospital, Port Harcourt, Military Hospital, Port Harcourt, and University of Port Harcourt Teaching Hospital, Choba. The study involved the use of hepatitis B panel assay, measurement of prevalence of hepatitis B virus infection in Port Harcourt, assessment of hepatitis B serological markers in all subjects, determination of the presence and prevalence of occult HBV among participants. HBV 5-parameter (panel) Rapid Test kit was used to assess HBV serological markers. SOP, GLP, External/Internal Quality Control were used accordingly and Quality Assurance ensued. 84.2% participants were males, 15.8% females aged between 19 and 65 years, Mean ±SD age 30.57±9.70 years, Participants from 20 states, South-South, South-East, and other Geo-political Zones of Nigeria, resident in the cosmopolitan city of Port Harcourt were enrolled. Result obtained showed serological markers among test subjects as 77.3% HBsAg, 43.97% HBsAb, 48.94% HBcAg, 36.17% HBcAb, and 46.81% HBeAg. Grouping of HBV panel assay result was HBV positive 1 (Occult HBV) 7.8% (n=11), HBV positive 2 73.76% (n=104), HBV positive 3 – (occult HBV post treatment) 14.18% (n=20), HBV positive 4 4.26% (n=6). All five serological markers of HBV in infected patients in PHC are evident in significant proportions indicating real infections at different stages of disease manifestation. Mass screening for HBV infection is recommended for our populace to check spread.

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Keywords: Occult hepatitis, serological markers, hepatitis panel assay

1. INTRODUCTION

In spite of continuing research, vaccination, and antiviral treatments, hepatitis B infection remains a serious global public health challenge that affects more than two billion people worldwide [1]. Hepatitis B is potentially a life-threatening liver infection caused by hepatitis B virus (HBV); a major global health problem capable of causing chronic infection and puts people at high risk of death from cirrhosis and liver cancer [2]. It involves inflammation of the liver, a condition that can be self-limiting or progress to fibrosis (scarring), cirrhosis or liver cancer. The virus belongs to the Hepadnaviridae family and is the most common cause of chronic liver disease; hepatocellular carcinoma and necrotizing vasculitis [3].

HBV serologic markers say a lot about the prognosis of hepatitis B [4]. In the study by Mohammed et al. [5] 1.1% of the participants had chronic HBV infection with high viral replication, 2.6% had acute infection with high viral replication, 4.6% were carriers with low viral replication, 1.4% were recently vaccinated, 16.0% were immune due to vaccination, 22.3% were immune due to previous natural exposure to the virus and the remaining 52.0% have never had any exposure to the virus. In contrast, a study in Benue State, Nigeria,

reported higher prevalence of 3.8% and 8.7% for chronic and acute infections respectively [6]. The Benue study recruited pregnant women, who often have low immunity.

Mohammed et al. [5] observed a significant association between gender and prevalence of HBsAg and HBeAb in their study ($p < 0.05$). Although differences in the prevalence of HBsAb, HBcAb and HBeAg were not statistically significant ($p > 0.05$), the prevalence of HBsAg, HBsAb, HBcAb, HBeAg and HBeAb were higher in participants who were male than female. These findings are similar to observations reported by Isa et al. [7] in North-Western Nigeria and Pennap et al. [8] in Keffi, Nigeria. Mustapha et al. [9] observed differently. Participants in Mohammed et al. [5] study were freshmen who had just left their various homes, the common culture that ensures young women spend most of their times at home on domestic activities with little chances of exposure to risk factors outside of home, while young men have more freedom of movement and association, might account for the higher prevalence of HBsAg in the male than female participants.

Mohammed et al. [5], also recorded significant association between marital status and prevalence of HBsAg among the participants ($p < 0.05$). The prevalence of HBsAg was higher among single participants than their married counterparts. This finding was collaborated by Ejele et al. [10] among HIV positive patients in Niger Delta, Nigeria; and Isa et al. [7], in a tertiary institution in North Western Nigeria; the differences in study populations notwithstanding. Moreover, history of blood transfusion was significantly associated with the prevalence of HBsAg and HBeAg ($p < 0.05$). Higher prevalence of HBsAg was observed among those who had received blood transfusion at some point in their lives. Until recently in Nigeria, testing of blood donors for hepatitis B virus infection was not routinely practiced in many clinical settings. This finding is in consonance with a previous report by Abah and Aminu [11] in Nigeria.

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Prevalence of HBsAg was significantly higher among participants who had multiple sex partners than those without ($p < 0.05$), [5]. This finding is supported by other studies including reports by Pennap et al. [8], among students of a Nigerian tertiary institution; Mboto and Edet [12], among students in University of Uyo, Nigeria. Statistically significant difference was observed between the prevalence of HBsAg and HBeAb in relation to scarification ($p < 0.05$), [5]. Participants with scarification marks were more likely to have HBV infection (HBsAg) than those without. This finding agrees with previous reports [8, 13] and participants in this category were likely from local homes where knowledge of transmission of the virus through the use of sharp unsterilized objects in making body-piercing marks is inadequate or lacking. Consumption of alcohol was not significantly associated with HBV infection in the study by Mohammed et al. [5]. This is not in agreement with previous reports that indicated alcohol consumption as a transmission risk [14]. It is possible that participants in Mohammed et al. [5] study were not sincere with their alcohol consumption habits, making our data on this not to be a true reflection of the reality.

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Moreover, higher prevalence of HBsAg was recorded among those who shared sharp objects than those who did not. This result is in consonance with other studies done in Nigeria [12, 15]; These findings further confirm that practices such as sharing of sharp unsterilized objects is a risk for transmission of the virus. No statistically significant association between the prevalence of HBV serologic markers in relation to sharing of clothes and bed spaces among the participants ($p > 0.05$)[5]. Ndako et al. [15], in North Central Nigeria and Isa et al. [7] in North Western Nigeria, made similar observations. However, this should not preclude the fact that HBV can be transmitted through those means since the virus can be found in saliva, tears, urine, breast milk and any other body fluid, (Isa et al., 2015).

This study aimed to evaluate the serological pattern of HBV infection in HBV infected patients in Port Harcourt, Nigeria. In order to achieve this, we performed hepatitis B panel assay on all participants, evaluated the prevalence of hepatitis B virus infection, measured the hepatitis B serological markers and determined the prevalence of occult HBV among participants.

2. MATERIAL AND METHODS

2.1 Study area

This study was carried out in Port Harcourt, which is the capital of Rivers state, southern Nigeria. It lies along the Bonny River, 41 miles (66 kilometer) upstream from the Gulf of Guinea, and is located in the Niger Delta with a metro area population of 3,325,000. Subjects were recruited from the Rivers State University Teaching Hospital (RSUTH), University of Port Harcourt Teaching Hospital, and Military Hospital, Port Harcourt.

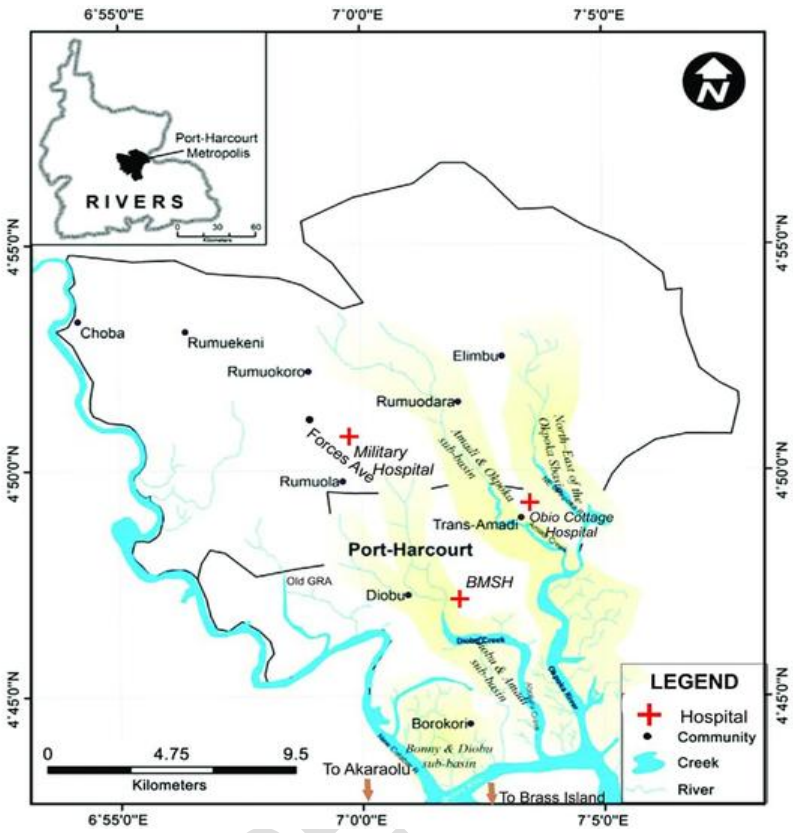

Image 1. Map of Port Harcourt showing study area and sampling locations.

2.2Study Population

A total of 260 subjects aged between nineteen (19) and sixty-five (65) years attending blood banks and hepatitis Clinics of the Rivers State University teaching Hospital, University of Port Harcourt Teaching Hospital, and Military Hospital, Port Harcourt were recruited for the study. 130 blood donors were recruited from the Rivers State University teaching Hospital, University of Port Harcourt Teaching Hospital, and Military Hospital blood banks, whereas known 130 hepatitis B positive patients were recruited from Rivers State University teaching Hospital, and Military Hospital hepatitis clinics. The 130 known hepatitis B positive patients served as the test subjects, while the 130 blood donors who tested negative for HbsAg were accepted by the blood banks as donors served as the control.

2.3Sample Size Calculation

The sample size was calculated using the formula method. Prevalence of Hepatitis B virus in Nigeria is 8.12%. Using this prevalence in the standard equation: $n = \frac{ZxZxP\ (1-p)}{\mathrm{dxd}}$, where n = sample size, z = 95% statistic for level of confidence (1.96), P = population proportion (8.12% or 0.0812), and d = margin of error (degree of accuracy desired (d = 0.05). From the calculation, the minimum sample size of 115 should be used, but for attrition purposes, a total of 130 samples from hepatitis B positive subjects were used in this study.

2.4Inclusion and Exclusion Criteria

The inclusion criteria for the study include: known hepatitis B patients without any other chronic disease condition e.g. diabetes, HIV/AIDS, etc.,asymptomatic hepatitis B patients, blood donors positive for HBV, or

occult HBV, Blood donors negative for HBV, and occult HBV were recruited as control, and males and females from age 18 years old to 65 years. The exclusion criteria were: Pregnant women, Hepatitis B patients with any other chronic disease condition e.g. diabetes, HIV/AIDS, etc., subjects who could not voluntarily give informed consent, and subjects less than 18 years of age were considered minors hence excluded.

2.5Study Design

This was a comparative cross sectional study carried out for hepatitis B patients attending hepatitis clinic in Rivers State University Teaching Hospital, Port Harcourt, Military Hospital Port Harcourt, and blood donors attending the blood banks of Rivers State University Teaching Hospital, Port Harcourt, University of Port Harcourt Teaching Hospital, Choba, and Military Hospital Port Harcourt. One hundred and thirty (130) blood donors who were pre-screened for HBsAg and accepted for blood donation were further screened for occult Hepatitis B infection using the five (5) parameter HBV panel assay. One hundred and nineteen (119) of them who were negative for occult HBV screening were used as control. Eleven (11) blood donors who were positive for occult HBV were added to one hundred and thirty Hepatitis B positive patients who met the inclusion criteria, making the test subjects a total of one hundred and forty-one (141). All 141 test subjects were evaluated for serological pattern of HBV infection.

Comment [D5]: This is repetation (study population)

2.6Sample Collection

Prior to sample collection, adequate protective equipment (PPE) were worn. The site of collection was cleaned using 70% Ethanol and 6ml of whole blood was obtained via venipuncture into appropriate sample container already labelled with patient's name, sex and age. Analysis was carried out within two hours of sample collection.

2.7Sampling Method

Samples for Hepatitis serological markers and biochemical iron parameters were collected into plain sample bottles, spun, and serum separated for analysis, and frozen where necessary. Samples for haematological parameters were collected into EDTA bottles and analysed immediately, and not later two (2) hours where necessary. Samples for liver function tests were be collected into lithium heparin sample bottles, spun, and serum separated for the assay. Samples for prothrombin time and International Normalized ratio were collected into sodium citrate sample bottles for the assay. Samples for CD4, CD8, and CD3 assay were collected into EDTA bottles and analysed immediately.

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2.8 Method of Assay

2.8.1Detection of HBV/Occult HBV Serologic Markers (HBV Panel Assay)

To detect HBV/occult HBV serologic markers (HBsAg, HBsAb, HBeAg, HBeAb and HBcAb), a HBV 5-parameter (panel) Rapid Test kit (Serum or plasma), (manufacturer/source) was used. Test and result interpretations were carried out according to the manufacturer's instructions.The samples and test board was brought to room temperature before use. The right side of the test board was kept horizontally from the original package, from left to right, respectively corresponding to HBsAg, HBsAb, HBeAg, HBeAb, HBcAb. With a Pasteur Pipette serum was taken and added into the wells of the test board by (70 per well of 2 drops). The result was recorded at exactly 15 minutes from when the assay started. The interpretation of the results were done as follows. Negative results only has one purple bar (control line) in the control C zonewhile positive had both C and T bands are developed (two purple bars in the control C and test T zone).The results were considered invalid if there is no purple bar in the control C zone.

2.9Data analyses

Data management and statistical analyses were conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA).

3. RESULTS

A total number of two hundred and sixty (260) participants were recruited for this study; 141 hepatitis B positive patients constituted the test subjects, whereas 119 hepatitis B negative subjects constituted the control group. Age of participants ranged from 19 to 65 years old. The results obtained in this study are presented in tables and figures below.

3.1 Demographic Characteristics of Study Population

Table 1 shows demographic characteristics of study participants. They were predominantly males (84.2%), while females constituted 15.8%. The age range of participants was between 19 and 65 years of age with Mean ±SD age 30.57±9.70 years (Mean ±SD 37.27±9.22 years for test subjects, and 23.82±4.59 years for control group). Majority (64.9%) of participants were singles, whereas 35.1% were married. Most of the participants (98.1%) were of the Christian religion; those of other religions were 1.9%. The South-South geo-political zone of Nigeria has the highest number (65%) of participants, followed by the South-East geo-political zone (27.7%), and followed by other regions (7.3%).

3.2Distribution of Test Subjects and Control Group by State of Origin and Geographical Region

Figure 1 shows distribution of test subjects and control group by state of origin and geographical region. Participants from 20 states in the country enrolled for the study. Majority of them were from the South-South geopolitical zone leading with Rivers State, followed by Delta State. The South-East Geopolitical Zone is next in participation leading with Imo State, followed by Anambra State. Then other zones leading with Benue and Kogi States.

3.3Distribution of Test Subjects and Control Group by Ethnic Group and State of Origin

Figure 2 shows distribution of test subjects and control by Ethnic group and state of origin. Subjects from many and diverse ethnic groups in Nigeria participated in the study. The Igbos from the eastern states were more in participation, followed by the Ijaws from the southern states, then the Ogonis, Anang, etc.

3.4Medical History of Study Participants

Table 2 shows the medical history of study participants. Number of participants who knew their HBV status to be positive prior to the study were 130 (50%), 43 (16.5) knew their status to be negative, while 87 (33.5%) did not know their HBV status prior to the study. 176 subjects (67.7%) had received no treatment for HBV prior to the study, whereas 84 (32.3%) had received some form of treatment for HBV. At the time of the study no participant was on any form of HBV treatment. 236 (90.8%) of the participants were having no other form of treatment or medication for any other condition. 16 (6.2%) were on antibiotics, 5 (1.9%) were on iron pills, 1 (0.4%) was on vasoprin, and 2 (0.8%) were on herbal drugs for other reasons aside from HBV. 235 (90.4%) have not been vaccinated for HBV, 8 (3.1%) were not sure, while 17 (6.5%) said to have received vaccination for HBV. At the time of this study 141 (54.23%) were HBV positive whereas 119 (45.77%) were HBV negative. At the time of this study 176 (67.7%) participants have not donated blood before, 48 (18.5%) had donated blood for 1-2 times, 17 (6.5%) had donated blood 3-4 times, and 19 (7.3%) had donated blood more than 5 times. All participants (n=260) said to have been on normal or regular meals. No special preferential dieting, no vegetarian.

Table 1: Demographic Characteristics of Study Population

Characteristic	N (%)	Treatment Group			
		Test Subject[β] (n=141)		Control (n=119)	
		n	%	n	%
Overall	260 (100)	141	54.23	119	45.77

Sex					
Female	41 (15.8)	41	15.8	----	0.0
Male	219 (84.2)	100	38.46	119	45.77
Age Group (Years)					
< 25	88 (33.9)	13	5.0	75	28.9
25 – 34	87 (33.5)	48	18.5	39	15.0
35 – 44	61 (23.5)	56	21.5	5	1.9
≥45	24 (9.2)	24	9.2	0	0.0
Age (Years) (Mean ±SD)	30.57±9.70	36.27±9.22		23.82±4.59	
Marital Status					
Single	168 (64.9)	56	21.6	112	
Married	91 (35.1)	84	32.4	7	
Religion					
Christianity	255 (98.1)	139	53.5	116	
Others	5 (1.9)	2	0.8	3	
Regions					
South-South	169 (65.0)	88	33.9	81	
South-East	72 (27.7)	38	14.6	34	
Other Regions	19 (7.3)	15	5.8	4	

[β] *Persons infected with Hepatitis B Virus (HBV). Note: within characteristics by treatment group, percentages may not add up to 100 due to rounding.*

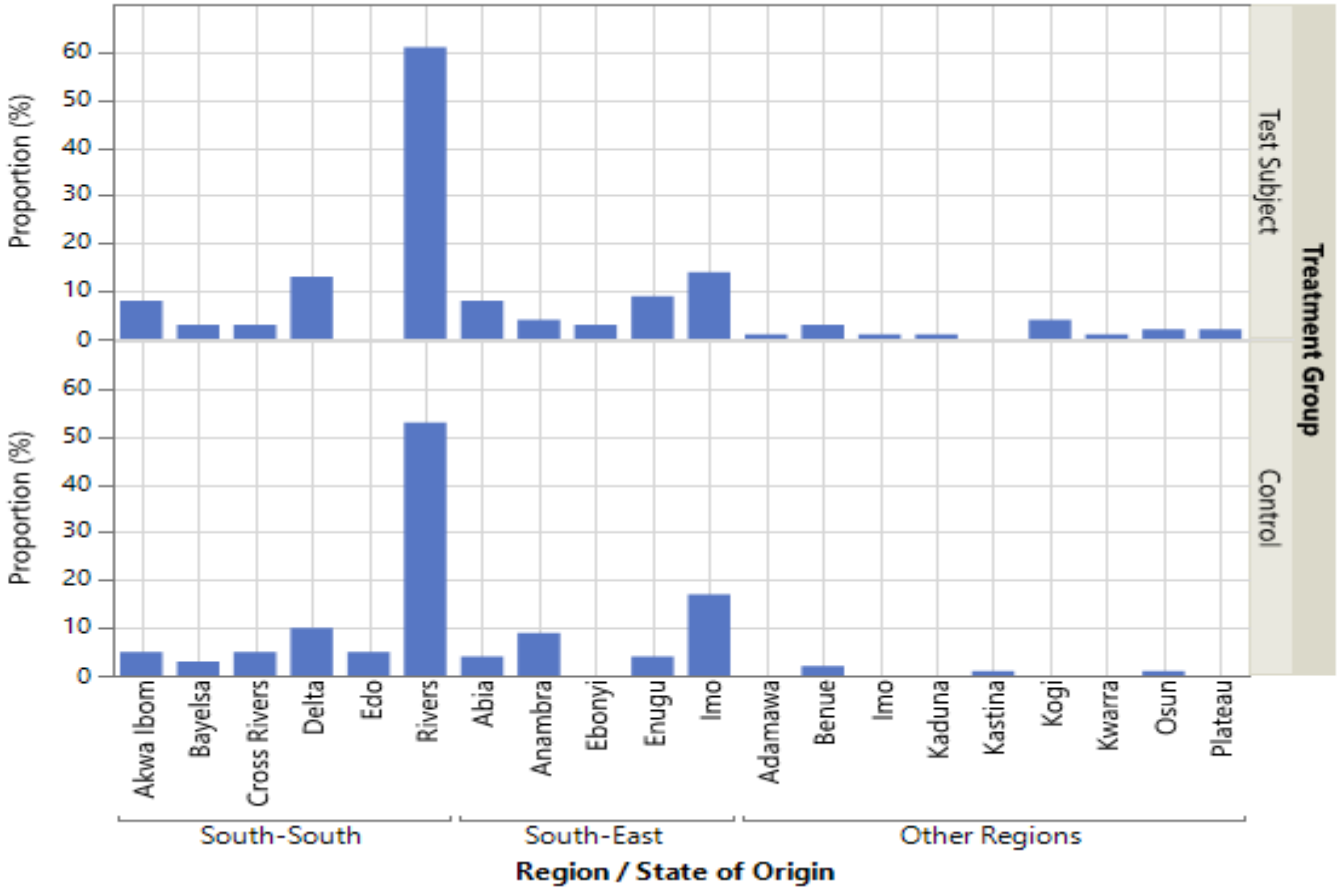

Fig.1. Distribution of Test Subjects and Control Group by State of Origin and Geographical Region

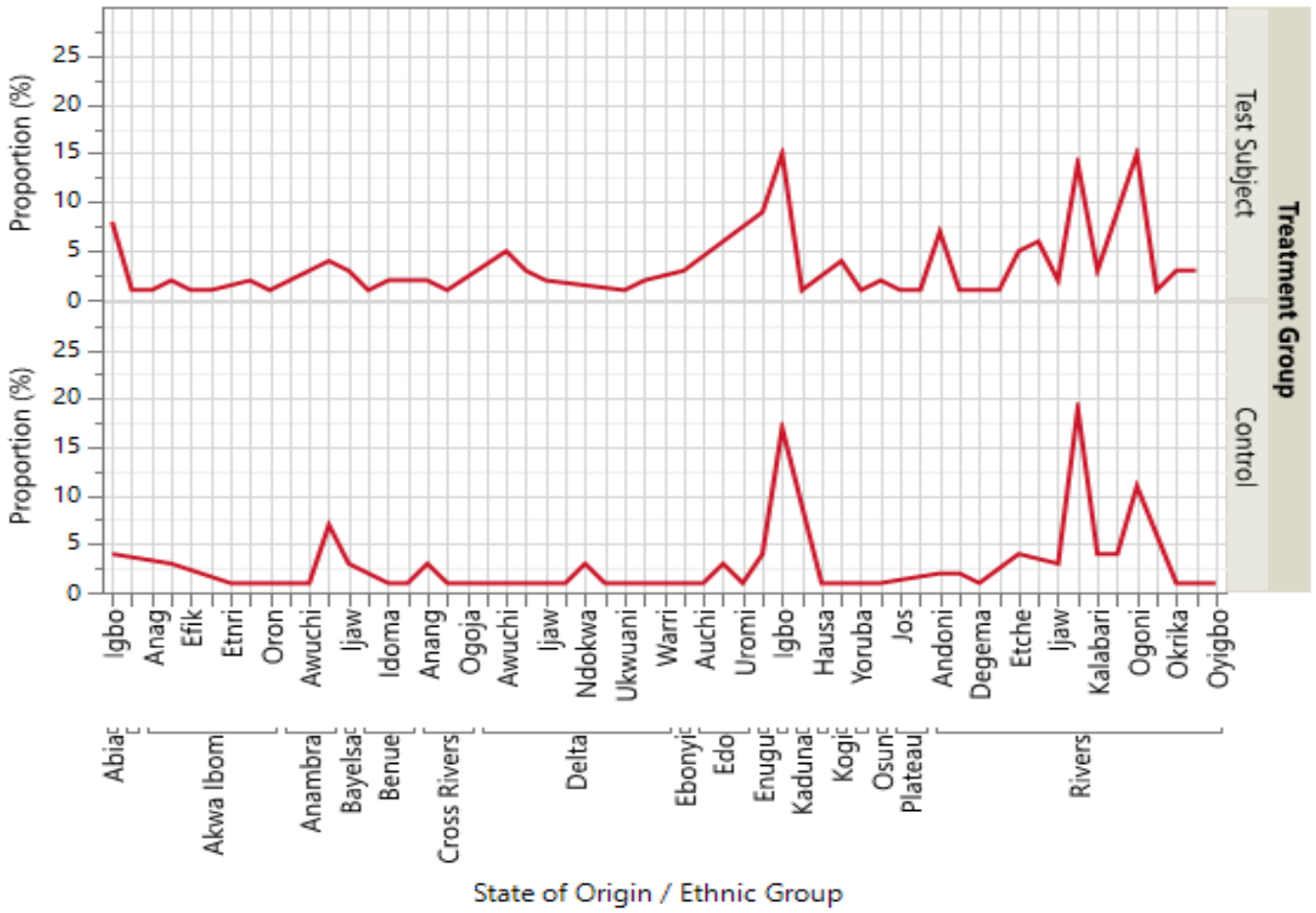

Fig.2. Distribution of Test Subjects and Control Group by Ethnic Group and State of Origin

Table 2: Medical History of Study Participants

Characteristic	N (%)	Treatment Group			
		Test Subject[β] (n=141)		Control (n=119)	
		n	%	n	%
Prior HBV Status					
Negative	43 (16.5)	2	0.8	41	15.8
Positive	130 (50.0)	130	50.0	0	0.0
Unknown	87 (33.5)	9	3.5	78	30.0
Prior HBV Treatment					
No	176 (67.7)	59	22.7	119	45.8
Yes	84 (32.3)	82	31.5	0	0.00
Current HBV Treatment					
No	260 (100)	141	54.2	119	45.8
Yes	---	---	---	---	---
Other Medication Used					
None	236 (90.8)	117	45.0	119	45.8
Antibiotics	16 (6.2)	16	6.2	---	---
Iron	5 (1.9)	5	1.9	---	---
Vasoprin	1 (0.4)	1	0.4	---	---
Herbal Drug	2 (0.8)	2	0.8	---	---
Prior HBV Vaccination					
No	235 (90.4)	116	44.6	119	45.8
Not Sure	8 (3.1)	8	3.1	0	0.00
Yes	17 (6.5)	17	6.5	0	0.00
Current HBV Status					
Negative	119 (45.8)	0	0.0	119	45.8
Positive	141 (54.2)	141	54.2	0	0.00
Blood Donation Category					
None	176 (67.7)	133	51.2	43	16.5
1-2 times	48 (18.5)	6	2.3	42	16.2
3-4 times	17 (6.5)	1	0.4	16	6.2
5+ times	19 (7.3)	1	0.4	18	6.9
Vegetarian (*Nutritional Preference*)					
No	260 (100)	141	54.2	119	45.8
Yes	---	---	---	---	---

[β]Persons infected with Hepatitis B Virus (HBV). Note: within characteristics by treatment group, percentages may not add up to 100 due to rounding.

Table 3: Hepatitis B Virus (HBV) Risk Factors Associated with the Study Population

Characteristic	N (%)	Treatment Group				Test Statistics	
		Test Subject[β]		Control			
		n	%	n	%	X^2value	*p-value*
Prior Smoking Status							
No	239 (91.9)	132	50.8	107	41.2		
Yes	21 (8.1)	9	3.5	12	4.6	1.191	0.2752[ns]
Current Smoking Status	237 (91.2)	131	50.4	106	40.8		
No	23 (8.9)	10	3.9	13	5.0	1.175	0.2783[ns]
Yes							
Prior Alcohol Status	229 (88.1)	127	48.9	102	39.2		
No	31 (11.9)	14	5.4	17	6.5	1.166	0.2801[ns]
Yes							
Current Alcohol Status	217 (83.5)	122	46.9	95	36.5		
No	43 (16.5)	19	7.3	24	9.2	2.094	0.1478[ns]
Yes							
Prior Sex Partner(s)	260 (100)	141	54.2	119	45.8		
One	---	---	---	---	---	----€	----€
Multiple							
Current Sex Partner(s)	260 (100)	141	54.2	119	45.8		
One	---	---	---	---	---	----€	----€
Multiple							

[β] *Persons infected with Hepatitis B Virus (HBV).Note: within characteristics by treatment group, percentages may not add up to 100 due to rounding.€ Test statistics were inestimable because of constant distributions within characteristic across treatment groups. Significance level: ns=not significant (p>0.05).*

3.5HBV Risk Factors Associated with the Study Population

Table 3 shows HBV risk factors associated with the study population. 239 (91.9%) Participants responded 'NO' to prior smoking status before commencement the study, 21 (8.1%) responded YES. 237 (91.2%) participants responded 'NO' to current smoking status at the time of the study, while 23(8.9%) responded 'YES'. 229 (88.1%) participants responded 'NO' to prior alcohol status before commencement of the study, whereas 31 (11.9%) responded YES. 217 (83.5%) participants responded 'NO' to current alcohol consumption/status, while 43 (16.5%) responded YES. All participants (test subjects and controls) responded 'NO' to multiple sex partner, and 'YES' to single sex partner prior to recruitment for the study, and same response at the time of the study.

3.6Association between Hepatitis B Virus Serological Markers among Test Subjects

Table 4 shows the association between hepatitis B virus serological markers among test subjects. 32 (22.7%) of the test subjects tested negative for HBsAg while 109 (77.3%) tested positive which was significant at p<0.0001. 79 (56.03%) tested negative HBsAb, while 62 (43.97%) tested positive which was not significant (p=0.1522). 72 (51.06%) tested negative for HBcAg, while 69 (48.94%) tested positive and was not significant (p=0.8005). 90 (63.83%) tested negative to HBcAb, 51 (36.17%) tested positive which was significant at p>0.001. 75 (53.19%) tested negative to HBeAg whereas 66 (46.81%) tested positive and that was not significant at p=0.4485.

Comment [D10]: P<0.001

3.7Serological Pattern of Hepatitis B Infection among Test Subjects

Table 5 shows serological pattern of hepatitis B infection among test subjects. Serological pattern for HBV markers among test subjects were grouped into four (4) categories, HBV positive 1, HBV positive 2, HBV positive 3, and HBV positive 4, depending on whether HBsAg was negative (occult) or not. HBV positive 1 – 'Occult HBV pre-treatment' (HBsAg -ve, other markers +ve) had 130 (92.2%) participants who were negative and 11 (7.8%) who were positive, which was significant at p<0.0001. HBV positive 2 (HBsAg +ve, other markers +ve) had 37 (26.24%) participants who tested negative while 104 (73.76%) participants tested positive, and it was significant at p<0.0001. HBV positive 3 – 'occult HBV post treatment' (HBsAg -ve, other markers +ve) had 121 (85.82%) were negative whereas 20 (14.18%) participants were positive, significant at p<0.0001. HBV positive 4 (HBsAg +ve, other markers -ve) had 135 (95.74%) negative, while 6 (4.26%) participants were positive, and was significant at p<0.0001.

3.8Summary of Hepatitis B Virus Panel Assay Results for Test Subjects

Table 6 shows summary of HBV panel assay result for test subjects. Study subjects who tested positive to HBV panel assay and categorized as indicated in 4.4 above are summarized as follows: HBV positive 1 – 'Occult HBV pre-treatment' (HBsAg -ve, other markers +ve) 7.8% (n=11), 95% CI (4.41-13.43). HBV positive 2 (HBsAg +ve, other markers +ve) 73.76% (n=104), 95% CI (65.94-80.32). HBV positive 3 – 'occult HBV post treatment' (HBsAg -ve, other markers +ve) 14.18% (n=20), 95% CI (9.37-20.90). HBV positive 4 (HBsAg +ve, other markers -ve) 4.26% (n=6), 95% CI (1.96-8.97).

3.9Cell Plot of Hepatitis B Serologic Assay Results for Test Subjects by Sex and Age

Figure 4 show cell plot of hepatitis B serological test result by age and sex of study participants. The highest rate of positivity among males occurred within the age bracket of 35-44 years for all 5 HBV serological markers, while the lowest rate of positivity among males occurred at age bracket <25 years. The highest rate of positivity among females occurred within the age bracket of 25-34 years for all 5 HBV serological markers, whereas the lowest rate of positivity among females occurred at age bracket<25 years.

Comment [D11]: group

Comment [D12]: group

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Comment [D14]: group

3.10Recursive Partitioning of Risk Factors Associated with HBV Panel Assay Results in Test Subjects

Figure 4 shows Recursive Partitioning of Risk Factors Associated with HBV Panel Assay Results in Test Subjects. Probability rate by recursive partitioning for prior smoking risk factor among test subjects by HBV panel assay was higher among those who reported 'NO', than in subjects who reported YES'. Probability rate for prior alcohol status was higher among those who reported 'YES', than in subjects who reported 'No'. Current alcohol consumption HBV risk factor was higher in those who reported 'YES' for HBV positive 1, than in those who reported 'NO', it was conversely higher for those who reported 'NO' to current alcohol consumption status for HBV positives 2, 3, and 4, than in those who reported 'NO' for same category of subjects. Overall, probability rate for contracting HBV for smoking status and alcohol consumption risk factors were 0.0780 for HBV positive 1; 0.7376 for HBV positive 2; 0.1418 for HBV positive 3; 0.0426 for HBV positive 4, not significant.

Table 4. Associations between Hepatitis B Viruses Serologic Markers among Test Subjects

Screening Test	Test Subject [β](n=141)			Test Statistics	
	n	%	95% CI	X^2 Value	P-value
HBsAg					
Negative	32	22.70	16.56-30.27		
Positive	109	77.30	69.72-83.44	42.05	<0.0001****
HBsAb					
Negative	79	56.03	47.78-63.95		
Positive	62	43.97	36.05-52.22	2.05	0.1522 [ns]
HBcAg					
Negative	72	51.06	42.89-59.18		
Positive	69	48.94	40.82-57.11	0.06	0.8005 [ns]
HBcAb					
Negative	90	63.83	55.63-71.30		
Positive	51	36.17	28.70-44.37	10.79	0.0010***
HBeAg					
Negative	75	53.19	44.98-61.23	0.57	0.4485[ns]
Positive	66	46.81	38.77-55.02		

[β] *Persons infected with Hepatitis B Virus (HBV).*
Note: within characteristics by treatment group, percentages may not add up to 100 due to rounding.
*Significance Level: ****=p<0.0001; ns=Not Significant (p>0.05).*

Table 5. Serological Pattern of Hepatitis B Infection among the Test Subjects

Parameter	Test Subject [β](n=141)			Test Statistics	
	n	%	95% CI	X^2 Value	P-value
HBV Positive 1 (occult HBV)					
Occult pre-treatment, HBsAg –ve, other markers+ve					
Negative	130	92.20	86.57-95.59		
Positive	11	7.80	4.41-13.43	100.43	<0.0001****
HBV Positive 2					
HBsAg +ve, other markers+ve					
Negative	37	26.24	19.68-34.06		
Positive	104	73.76	65.94-80.32	31.84	<0.0001****
HBV Positive 3					
Occult post treatment, HBsAg –ve, other markers+ve					
Negative	121	85.82	79.10-90.63		
Positive	20	14.18	9.37-20.90	72.35	<0.0001****
HBV positive 4					
HBsAg +ve, other markers -ve					
Negative	135	95.74	91.03-98.04		
Positive	6	4.26	1.96-8.97	118.02	<0.0001****

Abbreviations: 95% CI: 95% Confidence Interval.
[β] Persons infected with Hepatitis B Virus (HBV).
Note: within characteristics by treatment group, percentages may not add up to 100 due to rounding.
*Significance Level: ****=p<0.0001; ns=Not Significant (p>0.05).*

Table 6. Summary of Hepatitis B Virus Panel Assay Results

Panel Assay Result	Test Subject [β](n=141)			Test Statistics	
	n	%	95% CI	X^2 Value	P-value
HBV Positive 1: *Occult HBV pre-treatment: (HBsAg -ve, Other Markers +ve)*	11	7.80	4.41-13.43	181.64	<0.0001****
HBV Positive 2: (*HBsAg +ve, Other Markers +ve*)	104	73.76	65.94-80.32		
HBV Positive 3: *Occult HBV post-treatment (HBsAg -ve, Other Markers +ve)*	20	14.18	9.37-20.90		
HBV Positive 4: *(HBsAg +ve, Other Markers -ve)*	6	4.26	1.96-8.97		

*[β] Persons infected with Hepatitis B Virus (HBV). Note: within characteristics by treatment group, percentages may not add up to 100 due to rounding. Significance Level: ****=p<0.0001.*

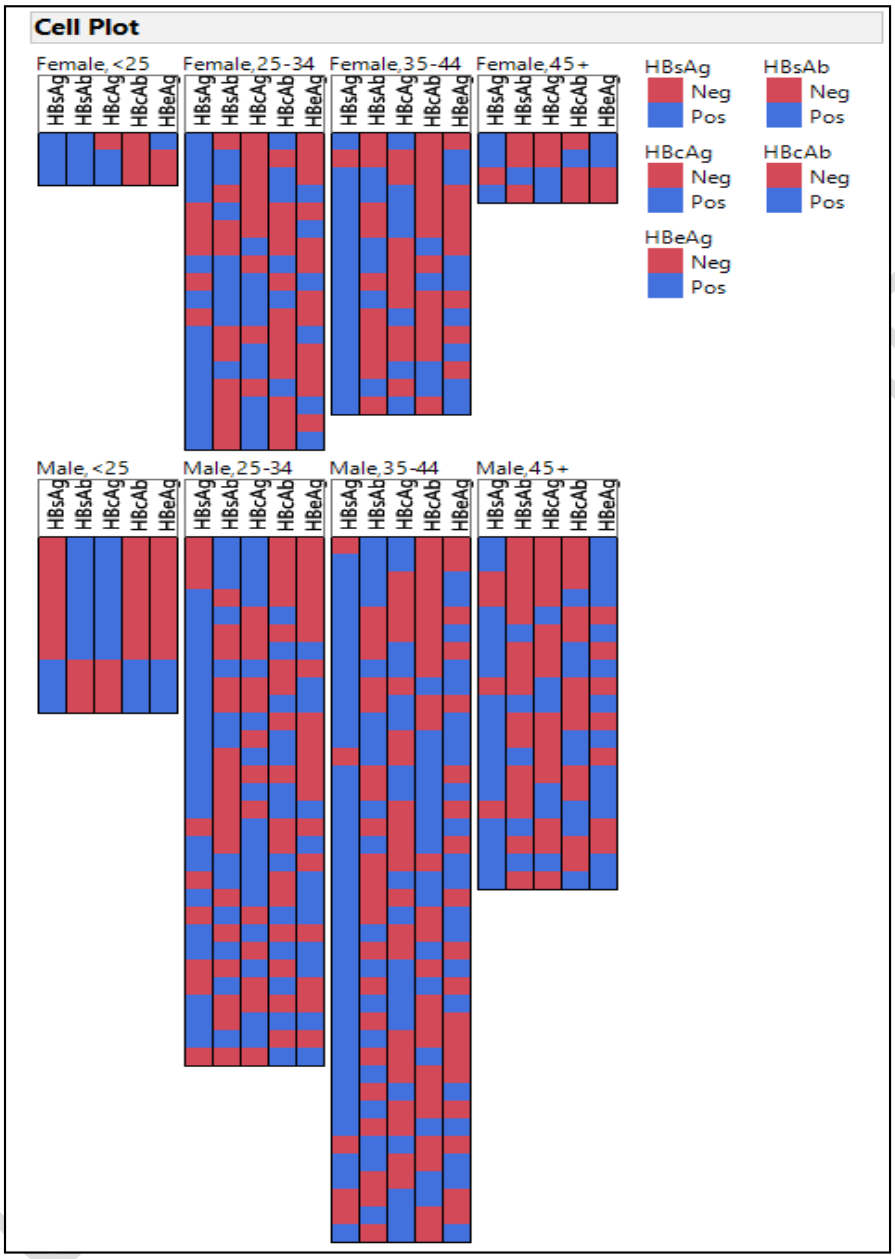

Fig. 3. Cell Plot of Hepatitis B Serologic Assay Results for Test Subjects by Sex and Age

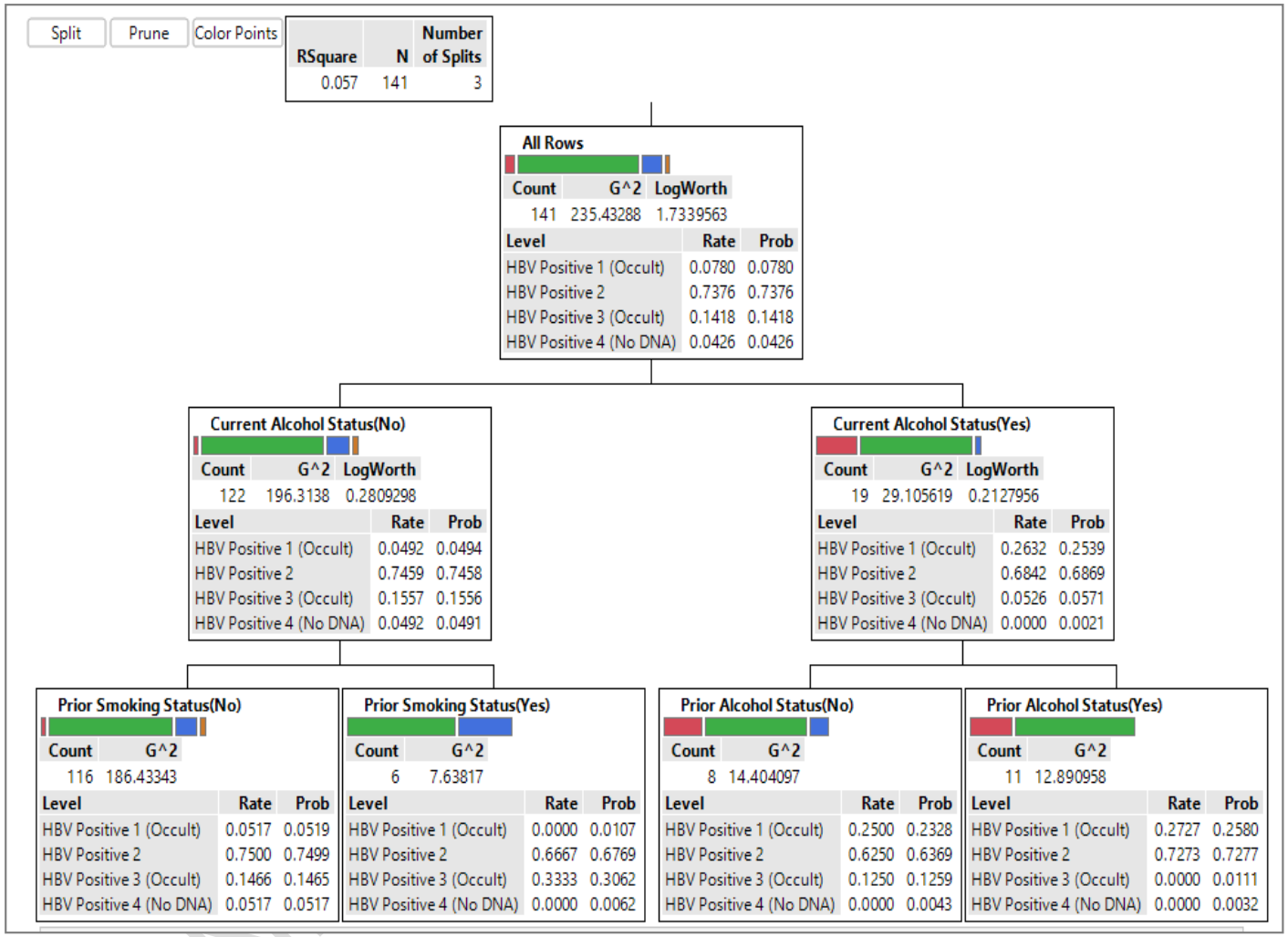

Fig. 4. Recursive Partitioning of Risk Factors Associated with HBV Panel Assay Results in Test Subjec

4. DISCUSSION

This study was carried out on hepatitis B patients attending hepatitis clinics in Rivers State University Teaching Hospital, Port Harcourt, Military Hospital Port Harcourt, and blood donors attending the blood banks of Rivers State University Teaching Hospital, Port Harcourt, University of Port Harcourt Teaching Hospital, Choba, and Military Hospital Port Harcourt. The main aim of this study was to evaluate serological pattern of HBV infection in PHC. Participants were from at least twenty (20) states, and more than fifteen (15) ethnic groups in Nigeria (Fig. 1 and 2) of both sexes, between the age of 19 and 65 years old, (Table 3). Risk factors for HBV including prior and current smoking, prior and current alcohol consumption, multiple or single sex partner, (Table 3) did not show any statistically significant difference.

The study revealed association between hepatitis B virus serological markers among test subjects as 77.3% HBsAg, 43.97% HBsAb, 48.94% HBcAg, 36.17% HBcAb, and 46.81% HBeAg (Table 4), which agree with previous studies for serological pattern in HBV infected subjects which demonstrated 89% prevalence rate of HBsAg [16, 16 17]. Franscica et al. [19], also showed varying percentage of detection rates of HBV markers (HBsAg 88%, HBeAg 30.7%, HBcAb 13.3%, HBeAb 8.0%, and HBsAb 4.0%) indicated highest rate for HBsAg (88%) in subjects exposed to HBV infection.

Finding of 77.3% HBsAg by panel assay in our study is indicative of active HBV infection which is consistent with many other studies with high prevalence rate which buttress the fact that HBV is endemic in Nigeria[8, 20, 21, 7, 22]. Musa et al. [23] who used electronic databases to select systematic reviews and meta-analyses from 2000 to 2013, (Forty-six studies included, n = 34,376 persons) recorded that HBV infection is hyperendemic in Nigeria and may be the highest in Sub-Sahara Africa,

It was also revealed that 43.97% HBsAb which is similar to the findings of Mohammed et al. [5], who reported 38.3% of the participants had HBsAb. This could be either due to vaccination or previous natural exposure to HBV. These findings are consistent with the 22.7% prevalence of HBsAb reported among healthy individuals in Benue, Nigeria; 22.2% among surgeons in Lagos, Nigeria; and 28% among hospital personnel in Cairo, Egypt [24, 6].

Our study revealed 46.81% HBeAg. Some studies in other study populations have found lower HBeAg prevalence of 6.5% and 4.7% among pregnant Nigerian women and a set of individuals who were HBsAg positive [11, 25,]. These differences may have resulted from the peculiarities of the different study populations, since women of child bearing age are often given HBV vaccine as the attend antenatal clinics. Some of the HBV positive subjects in our study were naïve HBV patients who had not received treatment and new to the knowledge of their HBV positivity. This marker is indicative of active replication and transmission, there was a significant risk of transmission in this population with a potential impact on the incidence of the disease and a concomitant challenge to control initiatives. It has been established that HBsAg-positive individuals, who are as well HBeAg positive, have 70–90% chances of transmitting the virus to their contacts in addition to being at high risk of developing persistent liver disease leading to cirrhosis and primary liver cancer if not treated, [26, 27, 11].

Finding of 48.94% HBcAg is a marker of infectious viral replication. This also shows much acute infection because the antibody (HBcAb) is produced during and after an acute HBV infection. Some studies have reported higher prevalence of HBcAb in certain populations [28, 24, 6, 11]. Sadoh et. al. [29] found an 11.4% HBcAb prevalence in a population of infants in Benin, in contrast to a population young adults, or adults. The relatively higher prevalence in our study might be attributed to the age differences between the two populations.

We discovered that 36.17% HBcAb which is slightly higher than detection of HBcAb in 28.0% of the participants as reported by Francisca et al. [19]. Consequently, anti-HBc is considered to be a more specific marker for HBV infection during window period and it indicates incidence of post hepatitis B among subjects [18]. It implies earlier exposure to the virus by this proportion of the participants.

In this study, we discovered 7.8% occult HBV infection (HBV positive 1) by HBV panel assay among screened, approved, and accepted naïve blood donors by the existing donor screening protocol in our public health care set-up. The discovery of 7.8% occult HBV infection among blood donors in this study was a very key and significant finding because of its relevance to safe blood transfusion survive, the need for reviewed donor screening protocol, updated policy framework, and overall public health.

Our study revealed that the highest rate of positivity among males occurred within the age bracket of 35-44 years for all 5 HBV serological markers, while the lowest rate of positivity among males occurred at age bracket<25 years (Fig. 4). Rate of positivity in males was higher than rate of positivity in female which was highest in the 25-34 years age group and lowest in the <25 years age group for all 5 HBV serological markers. This is consistent with the observation of Isa et al. [7] in North Western Nigeria and Pennap et al. [8] in Keffi, Nigeria who reported that prevalence of HBsAg, HBsAb, HBcAb,

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HBeAg and HBeAb were higher in participants who were male than female. Higher rate of positivity in the 35-44 years than <25 years age group could be due the possibility of higher exposure of the 35-44 years age group to HBV via marriage, more frequent and, or longer sexual contacts, and exposure to other risk factors such as sharp objects, blood transfusion, etc. over time.

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From our study, HBV risk factors showed no statistical difference when compared with HBV serological markers. This is in agreement with findings by Mohammed et al. [5] who reported that prevalence of HBsAg was significantly higher among participants who had multiple sex partners than those without ($p < 0.05$) since our participants all reported 'NO' to multiple sex partners. Overall, probability rate for contracting HBV for smoking status and alcohol consumption risk factors were not significant in our study. This is not in agreement with previous reports that indicated alcohol consumption as a transmission risk [14]]. It is possible that participants in our study may not be completely sincere with their alcohol consumption, smoking, and sex habits, making our date on HBV risk factors not a complete reflection of the reality.

Serological pattern for HBV markers among test subjects were grouped into four (4) categories, HBV positive 1, HBV positive 2, HBV positive 3, and HBV positive 4, depending on whether HBsAg was negative (occult) or not, especially considering the prevalent HBV screening method in our health care system and the need to appreciate the trends and possible challenges in our environment. HBV positive 1 – 'Occult HBV pre-treatment' (HBsAg -ve, other markers +ve) had 7.8% (n=11) positive; HBV positive 2 (HBsAg +ve, other markers +ve) had 73.76% (n=104); HBV positive 3 – 'occult HBV post treatment' (HBsAg -ve, other markers +ve) had 14.18% (n=20) positive; HBV positive 4 (HBsAg +ve, other markers -ve) had 4.26% (n=6) positive, (Table 4).

5 CONCLUSION

The study revealed the association between hepatitis B virus serological markers among test subjects as 77.3% HBsAg, 43.97% HBsAb, 48.94% HBcAg, 36.17% HBcAb, and 46.81% HBeAg. Finding of 77.3% HBsAg by panel assay among our test subjects in our study is indicative of active HBV infection which further reemphasize the high prevalence and endemic nature of HBV in, Port Harcourt, and our country Nigeria. The discovery of 7.8% occult HBV infection among blood donors is a key and significant finding because of its relevance to safe blood transfusion survive, the need for reviewed policy and execution framework, and overall public health. The highest rate of positivity among males occurred within the age bracket of 35-44 years for all 5 HBV serological markers, while the lowest rate of positivity among males occurred at age bracket <25 years. Rate of positivity in males was higher than that of positivity in female which was highest in the 25-34 years age group and lowest <25 years age group for all 5 HBV serological markers.

ETHICAL APPROVAL

The study ethical approval was obtained from Ethics and Research Committee Rivers State Ministry of Health, Port Harcourt, and Rivers State. Written consent was obtained for all patients and personal information was handled with utmost confidentiality.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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