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T-Cells Expression in HBV Infected Subjects in Port Harcourt, Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Hepatitis B virus infection is a potential life-threatening liver infection caused by hepatitis B virus capable of causing chronic infection and puts people at high risk of death from cirrhosis and liver cancer. This study 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, Military Hospital, and University of Port Harcourt Teaching Hospital. The aim of this study was to evaluate T-Cells expression in HBV Infected Subjects in Port Harcourt, Rivers state, Nigeria. HBV 5-parameter (panel) Rapid Test kit was used to assess HBV serological markers; BD Fascount automated machine was used in determining CD4, CD8, CD3, and CD4/CD8 ratio. SOP, GLP, External/Internal Quality Control were used accordingly and Quality Assurance ensued. All statistical tests conducted were 2-tailed, and probability value of < 0.05 was used as the threshold for declaring statistical significance. Data management and statistical analyses were conducted using Statistical Analyses System SAS 9.4 (SAS Institute, Cary, North Carolina, USA). 84.2% participants were males, 15.8% females aged between 19 and 65 years, Mean ±SD age 30.57±9.70. Participants from 20 states, South-South, South-East, and other Geo-political Zones of

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Nigeria, resident in the cosmopolitan city of Port Harcourt were recruited for the study. 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. The serological markers were grouped into four (4) categories based on HBsAg positivity: (i) HBV positive 1 – 'Occult HBV prior to treatment' (naïve previously unknown HBV: HBsAg -ve, other HBV markers +ve) 7.8% positive, [n=11]; (ii) HBV positive 2 (HBsAg +ve, other HBV markers +ve) 73.76% positive, [n=104]; (iii) HBV positive 3 – 'chronic or post treatment occult HBV' (known HBV case now occult': HBsAg -ve, other markers +ve) 14.18% positive, [n=20]; (iv) HBV positive 4 (HBsAg +ve, other markers -ve) 4.26% positive, [n=6]. CD3 and CD8 were significantly decreased in HBV infected subjects compared to healthy controls. CD4/CD8 ratio was significantly increased in HBV infected subjects compared to control group. CD4 count was decreased in HBV infected subjects than in healthy control though it was not statistically significant. CD3 and CD8 were significantly decreased ($p<0.0207$ and $P<0.0041$ respectively), in HBV positive subjects who were HBsAg negative but positive for other HBV serological markers, (HBV positive 3), when test subjects were compared by HBV panel assay. CD3 and CD4 showed very strong positive correlation ($p<0.0001$) among test subjects. CD8 and CD4, DC8 and CD3 also showed strong positive correlations ($p=0.0070$ and $p<0.0001$ respectively); CD4/CD8 ratio showed strong positive correlation with CD4, ($p=0.0002$). CD4, CD3, CD8, and CD4/CD8 ratio showed no statistically significant difference when compared by demographic indices including sex partner(s), marital status, and age group. CD4, CD3, CD8, and CD4/CD8 ratio may serve as prognostic markers in HBV infected subjects. Regular evaluation of these markers in HBV patients is advocated as it could be helpful for improved patient care/management. Periodic screening of some target population for HBV infection is recommended for our environment to check spread. Cost of diagnostic assays and treatment should be subsidized by government and capable cooperate organizations to help patients access regular and comprehensive health care.

Keywords: T-cell; CD4; CD3; CD8; CD4/CD8 ratio.

1. INTRODUCTION

In spite of the constant 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].

"Clinical outcomes of HBV infection largely depend on the quality and strength of the host's immune response. Studies have revealed that T cellular immune responses are essential for disease pathogenesis" [4,5,6] and have identified CD8+ T lymphocytes as the main cellular subset responsible for viral control [7,8]. "Compared with acute self-limiting infection, lack of vigorous and multi-specific T cell response in chronic HBV infection has been observed, which leads to the failure of viral clearance and the progression of disease" [4]. "The composition of peripheral T cell subpopulations, on the other hand, serves as a valuable index for evaluating T immune status in chronic HBV infection" [6]. "Impaired balance of peripheral T subpopulations has been reported at various stages of chronic HBV infections, associated with HBV replication levels, and can be partially restored after antiviral therapy" [9,6].

"In addition to other key indicators, e.g. liver function parameters, HBV DNA, etc., chronic hepatitis B is further characterized by marked changes in lymphocyte subpopulations and their activation status. Discordant T cell profiles in chronic hepatitis B patients, with decreased counts of CD8+ T cells and robust CD8+ activation, determined by an increase in the proportions of CD8+CD38+ T cells" [6]. "CD8+ and CD4+ T cells are two major components of the cellular immune system. CD8+ T cells play an important role in clearance of the virus and progression of the disease" [4]. Both CD8+ and CD4+ T cell levels in chronic hepatitis B patients and HBV carriers are often reduced, which might reflect the T cell disturbance and suppression [6]. "Upon administration of adefovir dipivoxil monotherapy, a marked elevation of CD8+ T cell levels occurred, which demonstrated a partial

restoration of T cell subsets and T cell immunity after the treatment. Other studies have suggested that antiviral therapy can also overcome CD8+ T cell hypo-responsiveness in chronic HBV infection" [10,11].

"Although the CD4+ and CD8+ T-cell responses to the hepatitis B virus (HBV) are observed to be crucial for the control of HBV infection, CD8+ cells are the main effector cells responsible for viral clearance and disease pathogenesis during acute HBV infection, and viral clearance is mediated by both noncytolytic and cytolytic effector functions of the CD8+-T-cell response" [4].

Aside from HBV DNA level and liver function parameters, chronic hepatitis B is characterized by marked changes in lymphocyte subpopulations and their activation status [6]; identifiable discordant T cell profiles in chronic hepatitis B patients, with decreased counts of CD8+ T cells and robust CD8+ T cell activation, determined by an increase in the proportions of CD8+CD38+ T cells. "CD8+ cells are required for the control of HBV since CD8 depletion in an animal study greatly prolonged the infection and delayed the onset of viral clearance and liver disease until CD8+ T cells reappeared in the circulation and virus-specific CD8+ T cells entered the liver" [4]. "In contrast, the duration of infection was unaffected by CD4 depletion. Interestingly, all of these events coincided with the appearance of HBV-specific T cells and the induction of both CD3 and IFN-γ mRNA in the liver" [4]. Thus, Thimme et al. [4] conclude that "CD8+ cells contribute importantly to the noncytolytic control of HBV replication in the liver of infected animals and also to the cytolytic process that regularly accompanies viral clearance".

"CD8+ and CD4+ T cells are two major components of the cellular immune system. Studies have revealed that CD8+ T cells play an important role in clearance of the virus and progression of the disease. Reductions of both CD8+ and CD4+ T cell levels in chronic hepatitis B patients and HBV carriers has been reported, which might reflect the T cell disturbance and suppression" [6]. "Furthermore, in conjunction with the adefovir dipivoxil monotherapy, a marked elevation of CD8+ T cell levels took place, which demonstrated a partial restoration of T cell subsets and T cell immunity after the treatment. Other studies have suggested that antiviral therapy can also overcome CD8+ T cell hypo-responsiveness in chronic HBV infection" [6].

2. MATERIALS 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.

2.2 Study 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.

2.3 Sample Size

The sample size was calculated using the formula. Prevalence of Hepatitis B virus in Nigeria is 8.12%. $N = Z2 \times P (1-P) / d2$ Where N = minimum sample Size, D = desired level of significance (0.05), Z = Confidence Interval (1.96), P = prevalence rate (9.9%). From the formula, 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.

2.4 Inclusion Criteria

1. Known hepatitis B patients without any other chronic disease condition e.g. diabetes, HIV/AIDS, etc.

2. Asymptomatic hepatitis B patients.
3. Blood donors positive for HBV, or Occult HBV.
4. Blood donors negative for HBV, and occult HBV were recruited as control.
5. Males and females from age 18 years old to 65 years.

2.5 Exclusion Criteria

1. Pregnant women.
2. Hepatitis B patients with any other chronic disease condition e.g. diabetes, HIV/AIDS, etc.
3. Subjects who could not voluntarily give informed consent.
4. Subjects less than 18 years of age were considered minors hence excluded.

2.6 Study 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.

2.7 Sample 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.

2.8 Sampling 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.

2.9 Study Location

The samples were analysed for HBV serological markers/occult HBV markers, ESR, in Rivers State University teaching Hospital, University of Port Harcourt Teaching Hospital, and Military Hospital, Port Harcourt. LFT, Biochemical iron assay, haematological indices, PT, and INR were carried out in UPTH. Immunological indices were carried out at RSUTH.

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

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. Negative: Only one purple bar (control line) in the control C zone. Positive: Both C and T bands are developed (two purple bars in the control C and test T zone). Invalid: There is no purple bar in the control C zone. HBeAb, HBcAb (Competitive method) Negative: Detecting T zone there are two purple bars in the control zone. Positive: Only one purple bar (control-line) in the control C zone. (Weakly positive sample may appear a very thin response line at the test line). Invalid: Detecting T zone there is no purple bar in the control C zone.

2.11 CD4, CD8, CD3 Lymphocyte Count

Tabs of the reagent tubes were labelled with patient's laboratory number, the tube was then

vortexed upside down and upright for 5-seconds each. The reagent tube was opened with the coring station, patients' whole blood was mixed by inversion. 50 µL of patient's whole blood sample was pipetted into the reagent tube, the tube was subsequently capped and vortexed upright for 5 seconds and then incubated at room temperature in a dark chamber for 60-120 minutes. After incubating the tube, it was then uncapped and 50 µL of fixative solution was pipetted into it, it was then recapped, vortexed for 5 seconds and then run using the BDFascount machine. On the machine, the "enter" button was touched on the screen, the reagent lot cock and bid counts were verified. The "enter" button was touched again and the patient's laboratory number was inputted. The CD4 tube was uncapped, placed on the sample holder and "run" button was touched again. The Sample was aspirated and after about a minute the result were shown.

2.12 Data Analyses

The results were presented as Mean ± SD and in percentages where necessary. Student t-Test and analyses of variance were used to detect statistical significance were necessary. Results were considered significant at $p<0.05$. 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 hapatitis 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 (Mean ± SD 37.27±9.22 for test subjects, and 23.82±4.59 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%).

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

Fig. 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.

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

Fig. 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.

3.4 HBV Risk Factors Associated with the Study Population

Table 2 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.

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

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

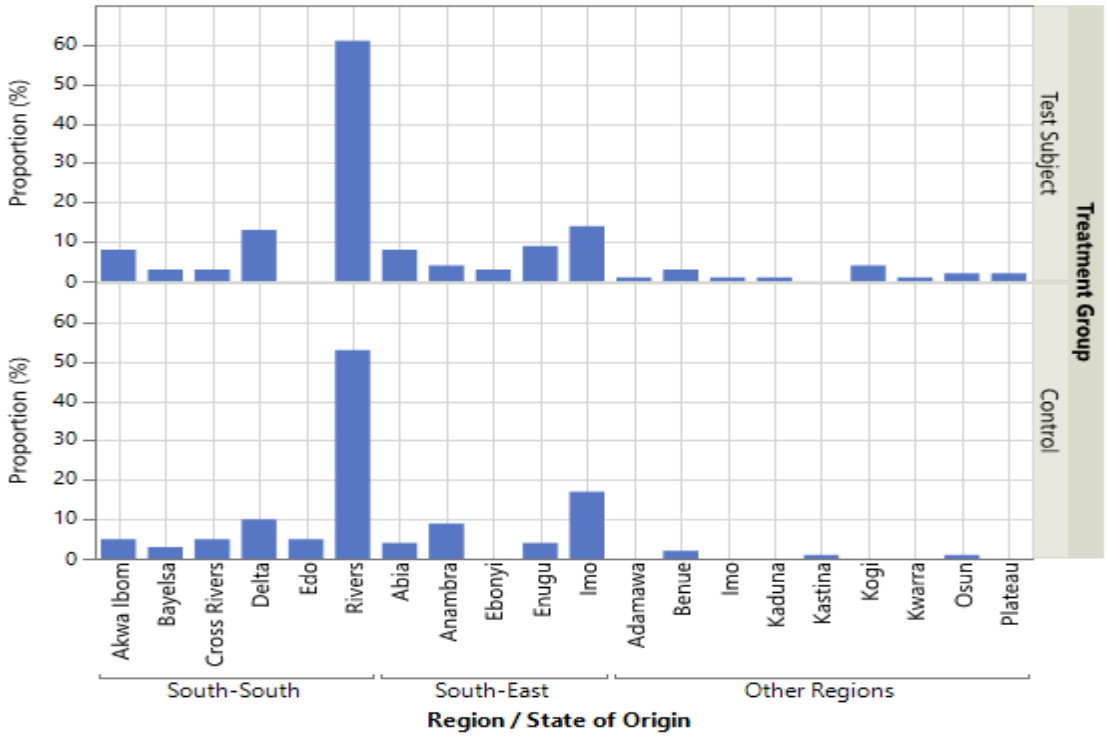

Fig. 1. Distribution of test subjects and control group by state of origin and geographical region

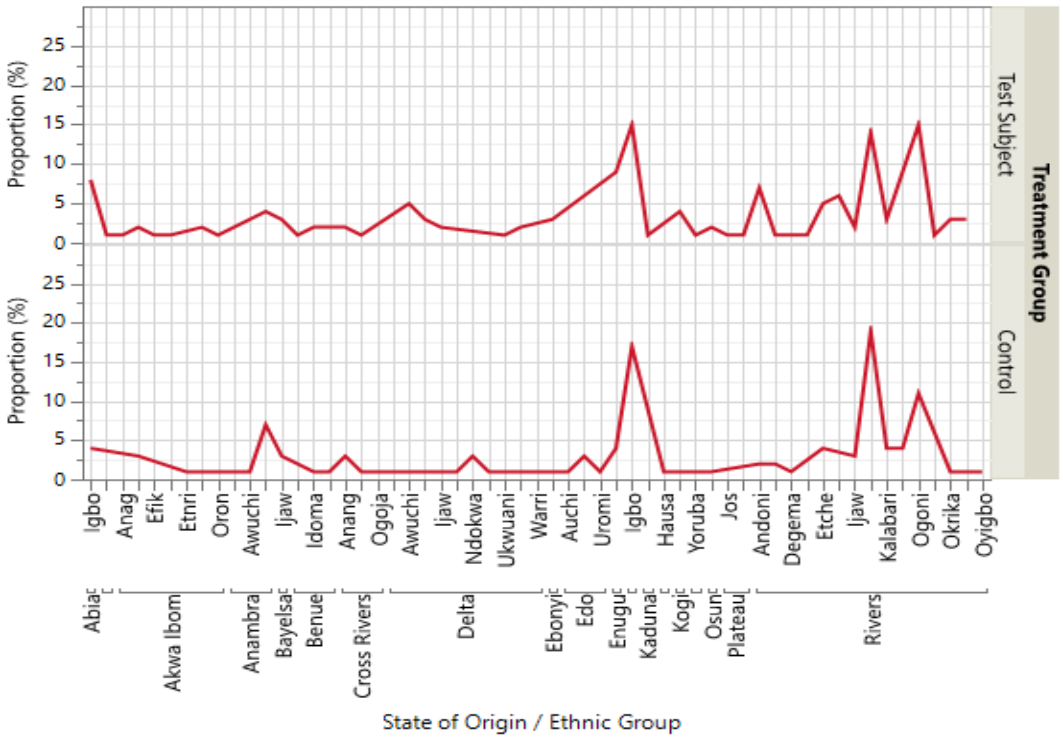

Fig. 2. Distribution of test subjects and control group by ethnic group and state of origin

Table 2. 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	239 (91.9)	132	50.8	107	41.2	1.191	0.2752[ns]
No	21 (8.1)	9	3.5	12	4.6		
Yes							
Current Smoking Status	237 (91.2)	131	50.4	106	40.8	1.175	0.2783[ns]
No	23 (8.9)	10	3.9	13	5.0		
Yes							
Prior Alcohol Status	229 (88.1)	127	48.9	102	39.2	1.166	0.2801[ns]
No	31 (11.9)	14	5.4	17	6.5		
Yes							
Current Alcohol Status	217 (83.5)	122	46.9	95	36.5	2.094	0.1478[ns]
No	43 (16.5)	19	7.3	24	9.2		
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							

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)

3.5 Association between Hepatitis B Virus Serological Markers among Test Subjects

Table 3 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.

3.6 Grouping of HBV Panel Assay Result Based on HBsAg Positivity in Test Subjects

Table 4 shows grouping of HBV panel assay result based on HBsAg positivity in 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.

3.7 Immunological Markers of Test Subjects and Control Group

As shown in Table 5, Mean ± SEM CD3, CD8, and CD4/CD8 ratio all showed high significant difference when compared between test subjects and control group, except CD4 which showed no statistically significant difference.

3.8 Comparisons of Immunological Markers in Test Subjects β by HBV Panel Assay Results

Table 6 shows comparisons of immunological markers in test subjects β by hbv panel assay results. Mean ± SEM CD3, CD8< and CD4/CD8 ratio were significantly different at p<0.0207, p<0.0041, and p<0.0380 when compared by HBV panel assay. Mean ± SEM CD4 showed no significant difference.

Table 3. Associations between Hepatitis B Virus serologic markers among test subjects

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

[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 4. Grouping of HBV panel assay result based on hbsag positivity in test subjects

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

*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 5. Immunological markers of test subjects and control group

Parameter	Treatment Group		Test Statistics	
	Test Subject [β][n=141]	Control [n=119]	t-Ratio	Prob >|t|
	Mean ± SEM	Mean ± SEM		
CD4 (Cells/µL), (500-1000)	839.11±16.051	876.01±17.472	1.555	0.1212[ns]
CD3 (Cells/µL), (600-2700)	1355.95±29.466	1558.76±32.075	4.656	<0.0001****
CD8 (Cells/µL), (500-1000)	517.53±19.904	700.40±21.665	6.216	<0.0001****
CD4/CD8 Ratio (1.0 - 4.0)	1.792±0.053	1.41±0.057	-4.914	<0.0001****

*SEM: Standard error of mean, CD4: Cluster of Differentiation 4 T-lymphocytes, CD3: Cluster of Differentiation 3 T-Lymphocytes, CD8: Cluster of Differentiation 8 T-Lymphocytes. [β] Persons infected with Hepatitis B Virus (HBV). Within each parameter, means ± SEM with different superscripts are significantly different at p<0.05. Significance Level: ****=p<0.0001; ns=Not Significant (p>0.05)*

Table 6. Comparisons of immunological markers in test subjects [β] by HBV panel assay results

Parameter	HBV Panel Assay Results				Test Statistics	
	HBV Positive 1 (Occult) [n=11]	HBV Positive 2 [n=104]	HBV Positive 3 (Occult) [n=20]	HBV Positive 4 (No DNA) [n=6]	F-value	P-Value
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM		
CD4 (Cells/µL) (500-1000)	832.00±45.54	851.70±14.81	765.85±33.77	878.17±61.66	1.95	0.1242[ns]
CD3 (Cells/µL) (600-2700)	1542.73±80.51[a]	1361.26±26.18 [ab]	1227.10±59.71[b]	1351.00±109.02 [ab]	3.36	0.0207*
CD8 (Cells/µL) (500-1000)	710.73±56.70 [a]	510.49±18.44 [b]	461.25±42.05 [b]	472.83±76.77 [b]	4.63	0.0041**
CD4/CD8 Ratio	1.32±0.17 [a]	1.82±0.06 [b]	1.83±0.13 [b]	2.01±0.24 [b]	2.89	0.0380*

*Within each parameter, means ± SEM with different superscripts are significantly different at p<0.05. Significance Level: *p<0.05; **p<0.01; ns=Not Significant (p>0.05)*

3.9 Comparisons of Immunological Markers in Test Subjects B by Selected Demographic Characteristics

Table 7 shows Comparisons of Immunological Markers in Test Subjects β by Selected Demographic Characteristics. Mean ± SEM CD4, CD3, CD8, and CD4/CD8 ratio showed no statistically significant difference when compared with demographic indices including sex, marital status, and age group.

3.10 Scatter Plot Showing the Correlations among Immunological Indices in Test Subjects and Control Group

Fig. 3 shows scatter plot showing the correlations among immunological indices in test subjects. CD3 and CD4 showed very strong positive correlation ($p<0.0001$), CD8 and CD4 showed strong positive correlation too, $p=0.0070$. DC8 and CD3 showed very strong positive correlation ($p<0.0001$) as well. CD4/CD8 ratio showed strong positive correlation with CD4, $p=0.0002$, whereas CD4/CD8 ratio showed very strong negative correlation with CD3 and CD8. As shown in Fig. 4, scatter plot of correlations among immunological indices in control group indicates very strong positive correlation between CD3 and CD4, CD8 and CD4, and also between DC8 and CD3 all at $p<0.0001$. There was positive correlation between CD4/CD8 ratio and CD4, $p=0.0052$, very strong inverse correlation between CD4/CD8 ratio and CD8, and a negative correlation between CD4/CD8 ratio and CD3 which was not statistically significant.

4. DISCUSSION

This study was carried out on hepatitis B patients and blood donors attending hepatitis clinics and blood bank in Rivers State University Teaching Hospital, Port Harcourt, Military Hospital Port Harcourt, and University of Port Harcourt Teaching Hospital, Choba. The main aim of this study was to evaluate T-Cells expression in HBV-infected subjects in Port Harcourt, Rivers State, Nigeria. Participants were from twenty (20) states, and more than fifteen (15) ethnic groups in Nigeria, (Figs. 1 and 2), of both sexes, between the age of 19 and 65 years old, (Table 1). Risk factors for HBV including prior and current smoking, prior and current alcohol consumption and multiple or single-sex partner, (Table 2) were not statistically significant upon analysis.

Table 7. Comparisons of immunological markers in test subjects [β] by selected demographic characteristics

Characteristic	n	CD4 (Cells/µL), (500-1000)	CD3 (Cells/µL), (600-2700)	CD8 (Cells/µL), (500-1000)	CD4:CD8 Ratio
		Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
Sex	41	822.63±23.85	1335.49±42.85	512.85±30.60	1.80±0.09
Female	100	845.87±15.27	1364.34±27.44	519.44±19.59	1.79±0.06
Male					
t-Ratio, Prob >|t|		0.82, 0.4134	0.57, 0.5716	0.18, 0.8564	-0.04, 0.9660
Marital Status	56	850.39±20.45	1374.57±36.77	525.88±26.25	1.84±0.08
Single	84	833.13±16.70	1344.51±30.03	511.41±21.44	1.77±0.06
Married					
t-Ratio, Prob >|t|		-0.65, 0.5143	-0.63, 0.5277	-0.43, 0.6701	-0.75, 0.4556
Age Group (Years) <	13	817.39±42.11	1455.77±76.09	638.39±53.53	1.51±0.16
25	48	806.73±21.92	1327.83±39.60	521.10±27.86	1.74±0.08
25 – 34	56	859.98±20.29	1349.29±36.66	491.04±25.79	1.87±0.08
35 – 44	24	866.96±31.00	1373.67±56.00	506.71±39.40	1.87±0.12
45^{+}					
F-Ratio, Prob > F		1.44, 0.2344	0.79, 0.5036	2.08, 0.1055	1.60, 0.1930

Abbreviations: SEM: Standard error of mean, CD4: CD4 T-lymphocytes, CD3: CD3 T-Lymphocytes, CD8: CD8 T-Lymphocytes

[β] Persons infected with Hepatitis B Virus (HBV). Within each parameter, means ± SEM are not significantly different (p>0.05)

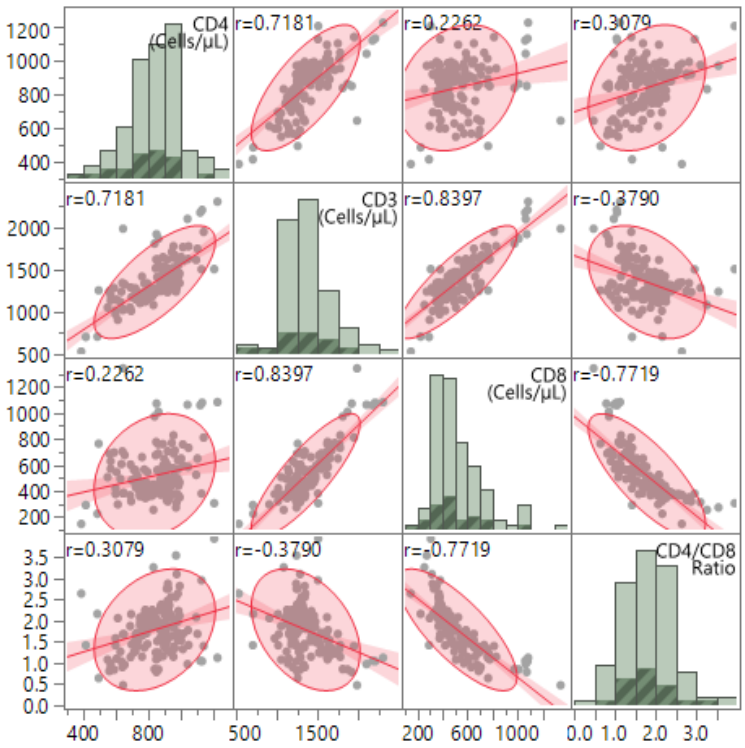

Fig. 3. Scatter plot showing the correlations among immunological indices in test subjects

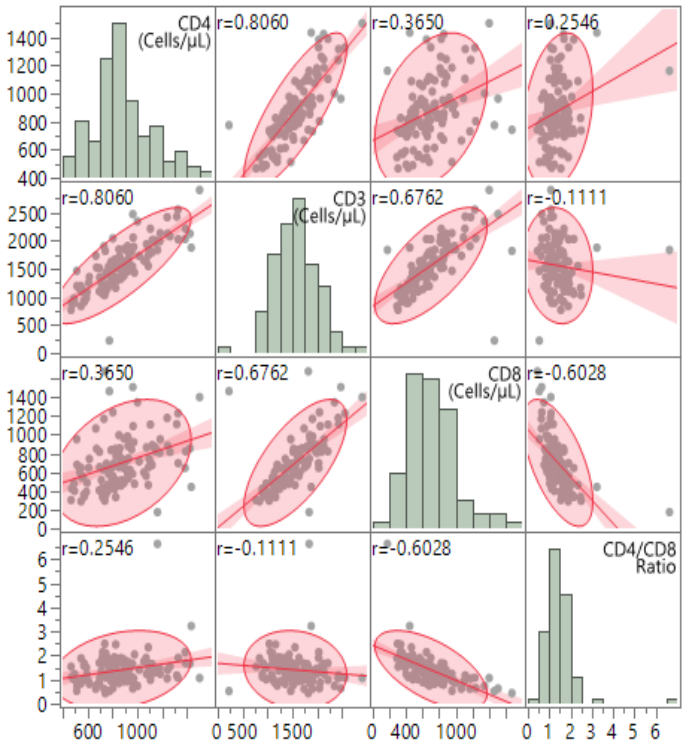

Fig. 4. Scatter plot showing the correlations among immunological indices in control group

Our study revealed that CD3 and CD8 were significantly decreased in HBV-infected subjects compared to healthy controls, (Table 5). Our result is in harmony with Thimme et al. [4] who reported identifiable discordant T cell profiles in chronic HBV patients, with decreased CD8+ T

cells and robust CD8+ T cell activation, determined by an increase in the proportions of CD8+CD38+ T cells. CD8+ cells are required for the control of HBV, accompanied by the appearance of HBV-specific T cells and the induction of both CD3 and IFN-γ mRNA in the liver [4]. Our result is also in agreement with Cao et al., (2011) who reported reductions of both CD8+ and CD4+ T cell levels in chronic hepatitis B patients and HBV carriers. This might reflect T cell disturbance and suppression. As observed by Cao et al. [6], adefovir dipivoxil monotherapy showed a marked elevation of CD8+ T cell levels, which demonstrated a partial restoration of T cell subsets and T cell immunity after the treatment. Other studies have suggested that antiviral therapy can also overcome CD8+ T cell hypo-responsiveness in chronic HBV infection [6].

CD4/CD8 ratio was significantly increased in HBV-infected subjects compared to control group. A reduced CD4+/CD8+ ratio is associated with reduced resistance to infection. A normal CD4/CD8 ratio is >1.0 with CD4 lymphocytes ranging from 500 to 1,200/mm^3 and CD8 lymphocytes ranging from 500 to 1,000/mm^3. CD4/CD8 ratio >1 could indicate a strong immune. Increased CD4/CD8 ratio in HBV infected subjects in our study could indicate an immune system that is stimulated and striving to contain the infection, even if the CD3, CD4, and CD8 counts were decreased in the HBV infected subjects than in the healthy control. Our test subjects appeared physically fit and were strong enough to attend clinics and attend to their concerns personally without any physical aid, reflecting a more stable immune control as observed in the CD4/CD8 ratio. Perhaps, some treatment a number of them may have received earlier could have contributed to enhancing their immune status against the infection.

CD4 count was decreased in HBV-infected subjects than in healthy control though the difference was not statistically significant. Our finding was corroborated by the result of Ahmad et al. [12] who observed no statistically significant difference in CD4 count between test subjects and control group. Our findings on CD4 are partly at variance with the report of Francisca et al. [13] which showed that CD4 count, absolute eosinophils count and monocytes count of HBsAg positive subjects were significantly lower than that of the HbsAg negative subjects ($p<0.05$). Perhaps, their patients were in a more severe disease state or had a more discordant T-cell expression.

CD3 and CD8 were significantly decreased ($p<0.0207$ and $p<0.0041$ respectively), in HBV positive subjects who were HBsAg negative but positive for other HBV serological markers, (HBV positive 3), when test subjects were compared by HBV panel assay (Table 6). The infection in this category is a more chronic condition and may have some impact on the result. CD4/CD8 ratio was significantly increased ($p<0.0380$) in HBV-positive subjects who were HBsAg positive but negative for other HBV serological markers when compared by HBV panel assay. This category of subjects is also a more chronic condition where the subject has lost HBV DNA markers, left with HBsAg. The immune struggle in favour of the patient may have resulted in an increased CD4/CD8 ratio. From our study, CD4, CD3, CD8, and CD4/CD8 ratios showed no statistically significant difference when compared with demographic indices including sex partner(s), marital status, and age group (Table 7).

From our study, CD3 and CD4 showed a very strong positive correlation ($p<0.0001$) among test subjects. CD8 and CD4, DC8 and CD3 also showed strong positive correlations ($p=0.0070$ and $p<0.0001$ respectively); CD4/CD8 ratio showed a strong positive correlation with CD4, ($p=0.0002$), (Fig. 3), all showing their agreement or similar progression by direction and proportion. Also, indicating that changes in one variable will relate to a similar type of change in the second variable. CD4/CD8 ratio showed a very strong negative correlation with CD3 and CD8 among test subjects indicating their inverse relationship; and strong tendency for the two variables to progress in opposite directions, or proportionate from one another.

Our result indicated a very strong positive correlation between CD3 and CD4, CD8 and CD4, and also between DC8 and CD3 all at $p<0.0001$ in the control group (Fig. 4). There was also a positive correlation between CD4/CD8 ratio and CD4, ($p=0.0052$), showing their agreeable direction and proportion. There was an inverse correlation between CD4/CD8 ratio and CD8, and a negative correlation between CD4/CD8 ratio and CD3 which were not statistically significant.

Our study also 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 3). Our result is in harmony with previous studies for serological pattern in HBV-infected subjects which demonstrated 89% prevalence rate of HBsAg, [14,15,16]. Francisca et al. [13] 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 high rate of HBsAg (88%) in subjects exposed to HBV infection.

Finding of 77.3% HBsAg by panel assay in our study could indicate active HBV infection which is consistent with many other studies with high HBV prevalence rate which buttress the fact that HBV is endemic in Nigeria [17,18,19,20,21]. Musa et al. [22] used electronic databases to select systematic reviews and meta-analyses from 2000 to 2013, (Forty-six studies included, n = 34,376 persons), reported that HBV infection is hyper-endemic in Nigeria and may be the highest in Sub-Sahara Africa. Decreased level of CD4 positive cells were noted in HBV patients in Rivers State University Teaching Hospital [23].

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 that HBsAg screening is the predominant HBV test method in our health care system and the need to assess the trend and possible challenges the serological screening approach may pose in our environment. Hence, the four groupings are as follows: (i) HBV positive 1 – 'Occult HBV prior to treatment' (naïve previously unknown HBV: HBsAg -ve, other HBV markers +ve) 7.8% positive, [n=11]; (ii) HBV positive 2 (HBsAg +ve, other HBV markers +ve) 73.76% positive, [n=104]; (iii) HBV positive 3 – 'chronic or post treatment occult HBV' (known HBV case now occult: HBsAg -ve, other markers +ve) 14.18% positive, [n=20]; (iv) HBV positive 4 (HBsAg +ve, other markers -ve) 4.26% positive, [n=6], (Table 4). As observed in this study, screening for HBsAg alone as serological marker for HBV, as obtainable in many low-income or under-resourced countries is grossly inadequate as a screening method for HBV infection. Going by the result of the study, an entire 21.99% of HBV positive subjects could have been missing or reported as false-negative except for the 5 parameter HBV panel assay, and these are occult HBV results.

5. CONCLUSION

A major finding from our study was the observation that CD3 and CD8 were significantly decreased in HBV infected subjects compared to healthy controls. CD4/CD8 ratio was significantly increased in HBV infected subjects compared to control group. CD4 count was decreased in HBV infected subjects than in healthy control though the difference was not statistically significant. CD3 and CD8 were significantly decreased, in HBV positive subjects who were HBsAg negative but positive for other HBV serological markers (HBV positive 3) when test subjects were compared by HBV panel assay. We also observed that CD3 and CD4, CD8 and CD4, DC8 and CD3 all showed strong positive correlation among test subjects. CD4/CD8 ratio showed strong positive correlation with CD4, while CD4/CD8 ratio showed very strong negative correlation with CD3 and CD8 among test subjects. Positive correlation also occurred between CD3 and CD4, CD8 and CD4, DC8 and CD3, and CD4/CD8 ratio and CD4 in control group. CD4, CD3, CD8, and CD4/CD8 ratio showed no statistically significant difference when compared by demographic indices including sex partner(s), marital status, and age group. Another major finding from our study is that it revealed the association between HBV 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 could indicate active HBV infection which further emphasize the high prevalence and endemic nature of HBV in, Port Harcourt, and our country Nigeria. Grouping of HBV serological pattern 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, considering that HBsAg screening is the predominant HBV test method in our health care system and the need to assess the trend and possible challenges the serological screening approach may pose was an important perspective.

CONSENT AND 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.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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