Prevalence of Hepatitis D Virus among hepatitis B positive Blood Donors in Port Harcourt, Nigeria.

ABSTRACT

INTRODUCTION: Hepatitis D (Hepatitis Delta) in a disease caused by the hepatitis D virus (HDV). It is considered to be a subviral satellite because it can propagate only in the presence of the hepatitis B virus. The prevalence of HDV in Port Harcourt has not been reported hence this study, to bridge this knowledge gap.

METHODS: A total of 300 blood donors were recruited, 222(74.00%) males and 78 (26.00%) females, all within 20-59years. Of this number, 86(28.70%) were positive for HBV while 214(71.30%) Hepatitis B negative served as control. Samples collected were analysed at Braithwaite Memorial Specialist Hospital Port Harcourt. Hepatitis B surface antigen (HBsAg), and anti-hepatitis D antibodies (anti-HDV) for the presence of HBV and HDV infections were detected by one step Hepatitis B surface antigen (HBsAg) in serum, and enzyme linked immunosorbent assay for the detection of HDV.

RESULTS:

Of the 300 subjects, 86(28.6%) were positive for Hepatitis B surface antigen (HBsAg). Of these HBV positive subject, 9(10.4%) were positive for Hepatitis D virus (HDV). Age and gender of the study participants were not found to be risk factors for its prevalence ($p > 0.05$). There was no statistically significant difference in the PCV of those infected when compared with the non infected group. Using Pearson correlation analysis, HDV was not found to associate significantly with PCV ($r = 0.2849$, $p > 0.05$). This study recorded a prevalence rate of 10.4% among the HBsAg positive blood donors.

CONCLUSION AND IMPLICATIONS FOR TRANSLATION: There is a 10.4% prevalence of HDV among the HBsAg positive blood donors. To increase the safety level of blood products, the screening process should therefore be extended to the HDV.

KEY WORDS: Hepatitis B surface antigen (HBsAg), Hepatitis D virus (HDV)

1. INTRODUCTION

1.1 Background of the Study

Hepatitis D or delta hepatitis is a liver disease which is transmitted through percutaneous or mucosal contact with infectious blood (1). It occurs in both acute and chronic forms. Although there are also other viruses such as, (i) cytomegalovirus (ii) Epstein-Barr virus (iii) Adenovirus (iv) Herpes simplex virus, which though do not infect the liver, yet cause hepatitis (2), Hepatitis D is caused by hepatitis delta virus (HDV), a defective RNA virus that requires the help of other

viruses like hepatitis B virus (HBV) for its own replication (3). It is therefore tagged Delta antigen having been taken to be a new protein enclosed by HBV (4). Subsequently, experiment with chimpanzees showed Hepatitis Delta antigen to be a structural part of a pathogen required for the replication of HBV infection (4). It was then placed in to the genus – Deltavirus. Three of the genome, (GI, GII and GIII) genotypes have so far been identified, although research has shown that there exist at least 8 genotypes of the Hepatitis D – Viral pathogen. In its spartial distribution, Genotype I has been identified in Europe, North America, Africa and Asia. It varies from fulminant Hepatitis to asymptomatic chronic disease (5).

The HD virion is composed of an outer lipoprotein envelope made of the surface antigen of the HBV (HBsAg) and an inner ribonucleoprotein structure in which the HDV genome resides. The HDV genome consists of a single stranded RNA which is folded as a rod-like structure through internal base-pairing. It is complexed with the only HDV-encoded. The HDAg can elicit a specific immune response in the infected host, consisting of antibodies of the IgM and IgG class (anti-HDV). In HDV infected individuals, the timing of appearance and level of HDV RNA, HDAg, and anti-HDV in serum allow the three HDV-related clinical entities to be discriminated:

Although variable, the clinical course of HDV is typically more severe than that of the other hepatitis viruses. After an incubation period of 3-7 weeks, nonspecific clinical symptoms, including fatigue, lethargy, nausea, and anorexia, begin and last for about 3-7 days. Viral replication is usually diminished during this phase. Jaundice occurs in the next phase of symptoms. Fatigue and nausea usually continue, and the serum bilirubin level becomes abnormal. At the same time, the infected person may have clay-colored stool and dark urine. This is evidence of the liver's diminished ability to excrete bilirubin (6).

Co-infection occurs when both HDV and HBV are contracted simultaneously and causes chronic HDV infections in less than 5% of co-infected patients. Although clinical symptoms disappear, fatigue and lethargy may persist for weeks or months. **S**uperinfection occurs when chronic HBV carriers are infected with HDV. This leads to severe acute hepatitis and chronic Hepatitis D infection in 80% of the cases. Superinfection is associated with the fulminant form of viral hepatitis which is the most severe form of acute disease, characterized by hepatic encephalopathy that is manifested by changes in personality, disturbances in sleep, confusion, difficulty concentrating, and sometimes abnormal behavior and coma (7).

Clinically, it is the smallest virus affecting humans, being the major cause of liver cirrhosis and fulminant hepatitis (2). When compared with other hepatitis infections, HDV appears to have the greatest fatality rate, with a very high tendency to degenerate or progress into hepatocellular carcinoma and liver cirrhosis, (9). Super-infection with HDV likely causes more clinical impediment than a single infection with only HBV. HDV entry into the hepatic cells follows the same pattern as HBV, by accessing this cell and recognizing its receptor through the N-terminal domain of the large HBsAg, having gained entrance into the hepatic cells through the NTCP bile transporter (9,10).This receptor binding site is of the amino acid residue 9-15 as shown by mutagenic mapping. The virus on gaining full access into the liver cells and through a signal in HDAg, shed off its coat and transfer its nucleocapsid to the nucleus (11*)*. The virus then, through the aid of cellular RNA polymerases, replicates itself. This is because the nucleocapsid lacks an RNA polymerase used in viral genomic replication. Therefore, RNA pol II, 1 and III are utilized in the process of HDV replication (13). The cellular damage caused by HDV affects the liver mainly (14) and some direct cytopathologic effect on the hepatocytes as revealed by

analysis of HDV on chimpanzees (15). A cell culture experiment has also revealed that, in acute HDV infection, there occur a degenerative change in the effected liver cells. This is evident through traces of inflammatory cells in the parenchyma of the liver, which continues as long as the hepatocellular damage persists. This degenerative change is also proved by the evidence of a shrunken eosinophilic cytoplasm and pyknotic nuclei (15). In the pathogenesis of HDV infection two expressions are noted: (i) Expression of a trace of the delta antigen by the infected liver cell is thought to be the cause of the direct cytoplasmic effect of the HDV, while (ii) the expression of considerable delta antigen, which is also said to confer no cytotoxic effect, is thought to be responsible for the HDV chronicity and undermined hepatocellular immunity, thus making it vulnerable to immune mediated damages (16).

Amount of HBsAg in circulation basically serve as key to assess duration of therapy, such HDV markers as IgM and IgG are scarcely found soon after therapy. The stage of liver cirrhosis however, is achieved using liver biopsy, especially in patients already screened positive for HDV RNA. Diagnosis of HDV is best done alongside with anti HBV antibodies, especially of the acute HBV infection which is detectable following an incubation period of 6weeks but no specific symptom (17). Important markers such as HBsAg and anti-HBV core antibodies (anti-HBc-IgM) are useful, in that, (i) negative HBsAg rule out the presence of acute HBV infection (ii) positive HBsAg shows an indication of HBV infection and (iii) anti HBc are often used when HBsAg are negative, hence a marker mostly used to monitor early HBV convalescence. Positive anti-HBc is therefore an indication for either current or resolved HBV infection, while negative anti-HBc is an indication for total absence of current HBV infection (21). Therefore, anti-HBc-IgM which shows positive for HBV infection is not often necessary for the diagnosis of active HBV-infection, since chronic HBV-carrier shows positive anti-HBc- IgM for years

following convalescence (19). In the diagnosis to monitor ongoing HDV-infection, the use of reverse transcriptase-polymerase chain reaction (RT-PCR) is a better approach. This is because RT-PCR is capable of detecting and identifying up to 10 to 100 copies of the HDV genome in any HDV infected serum (20,21).

HDV infection has become a global menace affecting about 15 – 20 million HBV positive individuals. It is the major cause of hepatitis in older children and adult and common in adults with its maximum prevalence geographically distributed (22,23). In Africa, especially in Gabon, HDV prevalence is endemic about 9.2% positive cases is noted among 15.6% of HBV carriers (24). In Nigeria, there is record of 4.3% of HDV prevalence in patients with acute hepatitis and about 15% prevalence in those with chronic hepatitis, hepatocellular carcinoma and liver cirrhosis (25). The prevalence of HDV in Port Harcourt has not been reported amidst a prevailing percentage of HB infection in the state - a predisposing factor for HD infection, hence this study, to bridge this knowledge gap.

1.2 Objectives of the Study

This research conducted a rapid test for Hepatitis B surface antigens for the presence of HBV, enzymatically determined the presence of acute (current) Hepatitis B virus infection and tested for the presence of anti- Hepatitis D Virus for co-infection of HBV and HDV among blood donors in Port Harcourt.

1.3 Specific Aims and Hypothesis

The aim of this study was to determine the prevalence of Hepatitis D viral infection among hepatitis B positive blood donors in Port Harcourt.

2. METHODS

2.1 Study Variables

A qualitative cross sectional design was employed to achieve general serological screening test on a 100 sampled males and females donors of 18 – 59 years age bracket. Using the non-anticoagulated vacuum system, 4mls each of venous blood of the subjects were collected and the serum separated and stored within 24 hours in the laboratory practical freezer at 15-30^{0}C then screened within 2 weeks following collection, for negative or positive for hepatitis B surface antigen (HBSAg), by the enzymatic method using ELISA.

2.2 Statistical Analysis

The percentage/frequency distribution, standard error of mean, Pearson correlation models were used at significance level of $P > 0.05$ using the SPSS statistical package.

2.3 Ethical Approval

Ethical approval for of this research was obtained from the Rivers State Research and Ethics Committee for the full analysis in the Braithwaite Memorial Specialist Hospital (BMSH) laboratory, Port Harcourt.

3. RESULTS

3.1 Percentage Frequency Distribution of the Demographic Details (Number of Subjects, Gender and Age) of Hepatitis B Positive and Control Subjects of the Study Population

Table 1. show that a total of 300 blood donors were recruited into this research out of which 86 (28.67%) cases were diagnosed Hepatitis B positive and 214 (71.33%) cases were free of Hepatitis B infection. Among the 86 Hepatitis B positive individuals 72(24%) were males and 14(4.7%) were females. From the 214 Control Subjects 150(50 %) were males while 64(21.33%) were females. The age range of the participants in this study was 20-59years; among the HBV positive subjects there were 19 (22.10%), 46 (53.50%), 19 (22.10%) and 2 (2.30%) of 20-29, 30-39, 40-49 and 50-59 years age ranges respectively. Then among the Control subjects 62 (28.97%), 94 (43.93%), 52 (24.30%) and 6 (2.80%) of 20-29, 30-39, 40-49 and 50-59 years age ranges respectively.

Table 1. Percentage Frequency Distribution of the Demographic Details (Numberof Subjects, Gender and Age) of Hepatitis B Positive and Control Subjects of the Study Population

Parameters	Hepatitis B Positive Subjects	Hepatitis B Negative Subjects
Number of Subjects	86 (28.67%)	214 (71.33%)
Gender		
Males	72 (24%)	150 (50%)
Females	14 (4.7%)	64 (21.33%)
Age (years)		
20-29	19 (22.10%)	62 (28.97%)
30-39	46 (53.50%)	94 (43.93%)
40-49	19 (22.10%)	52 (24.30%)
50-59	2 (2.33%)	6 (2.80%)

3.*2* *Percentage Frequency Distribution of Demographic Details ((Number of Subjects, Gender and Age) of Hepatitis D Positive Subjects among the Hepatitis B Positive Subjects of the Study Population*

Table 2. show that, among the 86 Hepatitis B positive subjects 77 (89.53%) were negative for Hepatitis D While 9(10.47%) were detected and confirmed Hepatitis D positive; from the later population 8(88.89%) were males while 1(11.11%) was a female. The percentage frequency distribution of age ranges (20-29, 30-39, 40-49 and 50-59 years) among the Hepatitis D positive subjects were 1 (11.11%), 6 (66.67%), 2 (22.22%) and 0 (0.00%) respectively

Table 2. Demographic Association (Number of Subjects, Gender and Age) of Hepatitis D Status of the Study Population

Parameters	**Hepatitis D Negative Subjects**	**Hepatitis D Positive Subjects**
Number of Subjects	77(89.53%)	9 (10.47%)
Gender		
Males	64 (74.42%)	8 (9.30 %)
Females	13 (15.12%)	1 (1.17%)
Age (years)		
20-29	18 (23.38%)	1 (11.11%)
30-39	40 (51.95%)	6 (66.67%)

40-49	17 (22.08%)	2 (22.22%)
50-59	2 (2.60%)	0 (0.00%)

3.3 Association of Sex and Age Group, and HBsAg Status

Table 3 shows the association of sex and age Group, and HBsAg status. With a P-Value of P = 0.940, there was no statistical significance of age and sex on the infection and spread of hepatitis B viral infection.

Table 3. Association of Sex and Age Group, and HBsAg Status

Characteristic	HBsAg		Test Statistics	
	Negative	Positive	X^2 (df)	P-Value
Sex				
Female	64 (29.91)	14 (16.28)		
Male	150 (70.09)	72 (83.72)	0.011 (1)	0.917^{ns}
Age Group (Years)				
20-29	62 (28.97)	19 (22.1)		
30-39	94 (43.93)	46 (53.50)		
40-49	52 (24.30)	19 (22.10)		
50+	6 (2.80)	2 (2.30)	0.415 (3)	0.940^{ns}

Note: Percentages may not add up to 100 due to rounding up; Frequency for each variable may vary due to nonresponses or missing values. ns=Not Significant at P >0.05.

3.4 Association of Sex and Age Group, and Anti-HD Status

Table 4 shows the a**ssociation of sex and age group, and anti-HD status.** A P-Value of P = 0.675, showed no statistical significance of age and sex on the infection and spread of hepatitis D viral infection.

Table 4. Association of Sex and Age Group, and Anti-HD Status

Characteristic	Anti-HD		Test Statistics	
	Negative	Positive	X^2 (df)	P-Value
Sex				
Female	13 (15.12)	1 (1.17)		
Male	64 (74.42)	8 (9.30)	0.259	0.611 ^{ns}
Age Group				
(Years)	18 (23.38)	1 (11.11)		
20-29	40 (51.95)	6 (66.67)		
30-39	17 (22.08)	2 (22.22)	1.530	0.675 ^{ns}
40-49	2 (2.60)	0 (0.00)		
50+				

Note: Percentages may not add up to 100 due to rounding up; Frequency for each variable may vary due to nonresponses or missing values. ns=Not Significant at P >0.05.

3.5. Comparison of Mean ± SEM of Weight and Packed Cells Volume(PCV)

Table 5 show a Comparison of Mean ± SEM of Weight and Packed Cells Volume (PCV) of the Study population. With a P-Value of P = 0.0001& P = 0.01, there is an observed specific statistical significance in the PCV status of the male subjects and the weight of subjects above the 50 years age group respectively, but there is no significance in the weight and PCV of both positive and negative subjects in the study population of both hepatitis B and D viral infection.

Table 5. Comparison of Mean ± SEM of Weight and Packed Cells Volume(PCV)

		Weight		PCV	
Characteristic	**N**	**Mean ± SEM**	**p-value**	**Mean ± SEM**	**p-value**
Sex					
Female	78	69.67±1.24		39.74±0.18	
Male	222	74.00±0.80	0.005	44.71±0.20	0.0001
Age Group (Years)					
20-29	79	70.44±1.22		42.92±0.39	
30-39	140	73.21±1.01		43.09±0.31	
40-49	73	73.49±1.34		44.47±0.34	
50+	8	85.25±5.42	0.006	44.75±0.73	0.011
HBsAg					
Negative	214	73.24±0.80		43.45±0.23	
Positive	86	71.97±1.03	0.399^{ns}	43.35±0.37	0.812 ^{ns}
Anti-HD					
Negative	291	72.99±0.70		43.45±0.20	
Positive	9	69.00±3.07	0.318 ^{ns}	42.67±1.35	0.502 ^{ns}

Note: Percentages may not add up to 100 due to rounding up; Frequency for each variable may vary due to nonresponses or missing values. ns=Not Significant at P >0.05.

3. 6 Correlation Analysis of Measured Parameters by HBsAg and anti-HDV Status

Table 6 shows a positive Pearson correlation of hepatitis B surface negative and blood donors with a non-significant correlation for weight, r = 0.070 & r = 0.489 and PCV, r = 0.1205 & r = 0.2408 respectively. Also, a positive Pearson correlation for hepatitis D virus negative and positive blood donors with a non-significant correlation for weight, r = 0.1613 & r = 0.4937 and PCV, r = 0.0228 & r = 0.1849 respectively.

Table 6. Correlation Analysis of Measured Parameters by HBsAg and anti-HDV Status

		Weight		PCV	
Characteristic	**N**	**R**	**p-value**	**r**	**p-value**
HBsAg					
Negative	214	0.070		0.1205	
Positive	86	0.489	0.399^{ns}	0.2408	0.812^{ns}
Anti-HD					
Negative	291	0.1613		0.0228	
Positive	9	0.4937	0.318^{ns}	0.1849	0.502^{ns}

4. DISCUSSION

4.1 Discussion

Blood is the body fluid considered to be most vital to the survival of human and the proper functioning of all the body cells, organs, tissues, and systems. Therefore, Clinical remedy for shortage of blood diagnosed by its low Hemoglobin (Hb), or packed cell volume (PCV) requires a transfusion of blood from healthy donors. Such donors are considered healthy if they are screened free of blood transmissible infections such as HIV, Hepatitis strains (Hepatitis A, B, C, D, & E), etc. However, for donors' safety, this study has observed a gap - the prevalence of HDV - in the screening of donors. Hence, this cross sectional study.

Intended donors less than 20 years were excluded being considered underage, while those above 59 years were considered older and weaker in strength as to withstand the stress accompanying blood donation, and moreover, were considered clinically not fit for donation owe to other health challenges at such advanced age as observed within this study population.

A statistically high prevalence (75.60%) of hepatitis B virus among adult of the 30-39 years and 40-49 years age bracket may be the period of its manifestation after such individuals got involved in active sexual activities or work exposures that placed them at risk; agreeing with Suerau and Aparna*et al.*, that, of the estimated 15 million people infected by HDV globally, high prevalence is common in adults than in children, with its maximum prevalence geographically distributed (23,24).

A comparison of the sex/genders of the healthy and infected subjects of both hepatitis B and hepatitis D were also considered, and with a P-Value of $P>0.05$, both sex and age were found to be of no significant determinant for the infection and spread of the disease among blood donors.

Although,74%, a far greater percentage of the male sex, were found to be infected by this blood disease than the females of just 26%; nevertheless, that proved no guaranteed of the male being prone to the disease than female. However, a holistic consideration showed that male show more readiness to blood donation than female. Again, most of the female are simply differed from blood donation either been found to be in the peak, just starting or ending their menstrual cycles, while others are either pregnant or in lactation. Therefore, with due consideration of their iron demand during the stages observed above, and the loss of iron experience during blood donation, they are simply differed, hence, not proceeded to be screened serologically for any Hepatitis strains. This simply account for the gender difference or group in the number of male and female found to be infected by this disease. HBV and HDV is hence not sex limited.

An observed statistically significant PCV specifically in the male subjects than the females is due to the genetically higher haemoglobin level in male than in female. Although, an observed significance in the weight of the male and female subjects of above 50years age group might be due to the sedentary lifestyle and inactivity associated with such age; nevertheless, a gross comparison thereof , with negative correlation, $r < 1$, shows no significant relationship in the subjects' weights and blood levels (PCV) due to hepatitis B and hepatitis D infections. Hence, neither weight nor hemoglobin level is a determining factor for the prevalence of the disease, so far as the condition has not progress to chronicity. This is because, negative progression of the disease could affect the liver and other related organs and thus affect the individual's general parameters, even weight. Also, the haemopoitic system, especially the bone morrow could be temporary distorted by hepatic viral infection (24, 25).

Conclusively, a cross sectional statistical analysis of this research shows a10.47% prevalence of Hepatitis D among 28.6% hepatitis B positive subjects in the study population, agreeing with

Makuwa *et al,* 2008; that of the 15.6-20% HBV positive cases in Africa, only about 9.2% cases are noted for HDV infection; compared to Nwokediuko *et al.,* 2009 record in Nigeria, that there occurred about 4.3% of HDV prevalence in patients with acute hepatitis and about 15% prevalence in those with chronic hepatitis, hepatocellular carcinoma and liver cirrhosis in Nigeria (25).

4.2 Limitations

This study was greatly challenged with finacial constraints.

4.3 Recommendation for further studies

Individual who are infected with Hepatitis B could also be co-infected with HDV therefore, all donors positive for HBV should be screened for HDV (ii) Since the disease seems to be asymptomatic from its onset, government and NGOs should embark on massive screening for Hepatitis D to advert its development, spread and chronicity. (iii) This study should be extended to other citizens outside the donors group.

5. CONCLUSION AND IMPLICATIONS FOR TRANSLATION

There is a low prevalence of HDV in Port Harcourt and the effect of the HDV disease appear to manifest in older people who probably are with lethargic liver disease.

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