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Hepatitis C and delta viruses among HBV positive cohort in Abuja Nigeria

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Abstract

Introduction: Hepatitis B virus (HBV) is a transfusion transmissible viral pathogen known to cause chronic liver diseases associated with cirrhosis and hepatocellular carcinoma. The disease becomes more aggressive and severe in Hepatitis B, C and D co or tri-infected population which further complicates treatment options. Therefore, this study aimed to evaluate the burden of HCV and HDV infections among prospective donors tested positive for Hepatitis B surface Antigen (HBsAg) in selected health facilities in Abuja, Nigeria.

Methodology: This cross-sectional study was carried out among 193 (M=99; F=94) consenting HBV infected prospective blood donors, between age range of 18 to 60 years with mean age 31.6 (SD=12.4) years, initially intended to donate blood but were disqualified due to their HBV status in four health facilities in Abuja Nigeria. The demographic and other relevant information were captured using a structured questionnaire anti-HCV, anti-HDV and HBsAg were detected by commercial qualitative ELISA kits according to the manufacturer's instructions.

Results: Overall rates of 5.2% and 5.7% were detected for anti-HCV and anti-HDV among HBV infected cohort respectively. The rates were similar in male (7.1%) for HCV and HDV but higher in male (7.1%) than in female (3.4% and 4.3%) counterparts respectively. Furthermore, the males have 1.5 times higher risk of HCV/HDV with significant association (p=0.0065) than in females (OR=1.47, 95%CI 1.03-2.21) for both infections. The rate (7.7%) for HCV peaked at age group ≤20 years while anti-HDV rate (10.3%) was highest within the age groups 41-50 years. However, HCV/HDV/HBV tri-infection rate (3.6%) was only found in male age ranged 21-30 years. Among other predisposing risk factors for HCV/HDV/HBV co and tri-infections multiple sexual partnership was significantly associated (p<0.0423; OR=1.19, 95%CI 0.93-1.60) However, no significant association (p=0.059; OR=1.06, 95%CI 1.23-2.11) was found between study participants with HCV/HDV/HBV tri-infection and age/sex.

Conclusion: The study identified that the rate of HCV and HDV co-infection was high while tri-infection was rare among the study population. Therefore, blood screening for HCV and HDV is recommended among individuals with chronic HBV infection.

Keywords: *HCV, HDV/HBV co-infection, Tri-infection, Blood donors, ELISA, Abuja, Nigeria*

Résumé

Introduction : Le virus de l'hépatite B (VHB) est un agent pathogène viral transmissible par transfusion connu pour provoquer des maladies hépatiques chroniques associées à la cirrhose et au carcinome hépatocellulaire. La maladie devient plus agressive et plus grave chez les co-patients atteints d'hépatite B, C et D ou les trois infections, ce qui complique encore les options de traitement. Par conséquent, cette étude visait à évaluer le fardeau des infections à VHC et à VHD chez les donneurs potentiels testés positifs à l'antigène de surface de l'hépatite B (AgHBs) dans des établissements de santé sélectionnés à Abuja, au Nigéria.

Méthodologie : Cette étude transversale a été menée auprès de 193 (M = 99; F = 94) donneurs éventuels de sang infectés par le VHB, âgés de 18 à 60 ans et âgés en moyenne de 31,6 ans (SD = 12,4 ans), initialement destinés à ont donné du sang mais ont été disqualifiés en raison de leur statut au VHB dans quatre centres de santé à Abuja au Nigéria. Les informations démographiques et autres informations pertinentes ont été saisies à l'aide d'un questionnaire structuré anti-VHC, anti-HDV et AgHBs ont été détectées par des kits qualitatifs commercial ELISA, conformément aux instructions du fabricant.

Résultats : Des taux globaux de 5,2% et 5,7% ont été détectés pour les anticorps anti-VHC et anti-VHD dans la cohorte infectée par le VHB, respectivement. Les taux étaient similaires chez les hommes (7,1%) pour le VHC et le VHD, mais plus élevés chez les hommes (7,1%) que chez les femmes (3,4% et 4,3%) respectivement. De plus, les hommes

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présentent un risque 1,5 fois plus élevé de VHC / VHD avec association significative (p = 0,0065) que les femmes (OR = 1,47, IC 95% 1,03-2,21) pour les deux infections. Le taux (7,7%) pour le VHC a atteint un sommet au groupe d'âge ≤20 ans alors que le taux d'anti-VHD (10,3%) était le plus élevé au sein des groupes d'âge 41-50 ans. Cependant, le taux de tri-infection par le VHC / VHD / VHB (3,6%) n'a été observé que chez les hommes âgés de 21 à 30 ans. Parmi les autres facteurs de risque prédisposant de co et de tri-infections VHC / VHD / VHB, le partenariat sexuel multiple était associé de manière significative (p <0,0423 ; OR = 1,19 ; IC à 95% 0,93-1,60). Cependant, aucune association significative (p = 0,059; OR = 1,06 ; IC 95% 1,23-2,11) a été trouvé entre les participants à l'étude atteints de tri-infection VHC / VHD / VHB et leur âge / sexe.

Conclusion : L'étude a révélé que le taux de co-infection par le VHC et le VHD était élevé, alors que l'infection par trois était rare dans la population étudiée. Par conséquent, le dépistage sanguin du VHC et du VHD est recommandé chez les personnes présentant une infection chronique au VHB.

Mots-clés : VHC, co-infection VHD / VHB, tri-infection, donneurs de sang, ELISA, Abuja, Nigeria

Introduction

Hepatitis B virus (HBV), Hepatitis Delta virus (HDV) and Hepatitis C virus (HCV) share some significant similarities such as modes of transmission, considerable global spread, ability to infect the liver and the capacity to induce a chronic infection which may result to liver cirrhosis and hepatocellular carcinoma (HCC) [1]. In 2015, the number of individuals living with chronic HBV and HCV infection globally was estimated to be about 257 and 71 million respectively, of which African region was most affected [2]. Additionally, HBV, HCV and HDV co-existence is common especially in regions with high endemicity for the viruses [3]. Specifically about 5-20% of people with chronic HBV infection are also co-infected with HCV [4,5,6] and an estimated 5% rate of HBV/HDV co and /or super-infection have been reported [7].

According to a WHO report about 1.34 million deaths was caused by viral hepatitis in 2015 while this was similar to the number of deaths from tuberculosis, it was higher than the number of deaths from HIV [2]. Of this number, while HCV was responsible for 30%, HBV and /or HDV accounted for 66% while the remaining 4% was due to other viral hepatitis agents [2]. Reports have suggested an increase in mortality caused by liver diseases in the last two decades making liver cancer the second foremost cause of cancer deaths globally, after lung cancer [2,8]. Specifically, increased and rapid progression rate to complications associated with viral hepatitis has been reported in tri and/ or co-infection than in mono-infection [9].

Although unsafe blood may no longer be a health concern in the developed countries, the situation may be different in low and middle income countries, where the prevalence of transfusion transmissible infections is high [10,11]. Consequently due to the rising health risks associated viral hepatitis with co and/or tri-infections [3], we therefore aimed to assess the rates of HDV and HCV co and/or tri infections in HBsAg positive prospective blood donors in Abuja, Nigeria.

Materials and methods

Study location

A cross sectional sampling method was carried out among consenting prospective unremunerated blood donors who tested positive for HBsAg in a cohort from blood banks of four selected healthcare facilities in Abuja, the Federal Capital Territory of Nigeria. The four locations within Abuja community in which the sampling was done included the following: General Hospital Wuse, General Hospital Nyanya, General Hospital Kuje, and General Hospital Asokoro. The consenting participants were asymptomatic dwellers located in densely populated part of the city who had come to donate blood in the blood bank of the selected health facilities. They claimed not to be intravenous drug users.

Enrolment of the participants

The enrolment of the consenting participants was done between April and October 2016. During this period, a total of 193 HBV-positive cohort including 99 male and 94 female participants, age ranged 18 to 60 years, were enrolled (mean age = 31.6; SD = 12.4). They were unremunerated potential blood donors who were disqualified from donation solely due to their HBV status. A well-structured questionnaire was used to capture demographic and other relevant information from each participant while ethical approval for the study was obtained from the Federal Capital Territory Health and Research Ethics Committee (FHREC/2016/01/24/ 06–04-16).

Sample collection

Blood volume of about 5ml collected by venipuncture from each participant after obtaining their consent and dispensed into an appropriately

labeled EDTA sterile container. These samples were subsequently conveyed to the laboratory in an ice-filled Jablow box. They were centrifuged at a low speed of 500 g for 5 minutes, the plasma was then separated and two aliquots were made into a well-labeled cryovials for each sample using a sterile disposable pipette. Each aliquot was stored at "80°C

performed according to manufacturer's instructions. The optical density was read using the Emax endpoint ELISA micro-plate reader (Molecular Devices, Sunnyvale, CA, USA), and the results were interpreted according to the manufacturer's instructions.

Table 1: Overall seroprevalence of anti-HCV, anti-HDV among HBV positive cohort in Abuja

Age Range (years)	No Tested	No (%) HCV Positive	No (%) HDV-Ab Positive
≤ 20	26	2(7.7)	1(3.8)
21-30	68	2(2.9)	5(7.4)
31-40	54	4(7.4)	2(3.7)
41-50	29	2(6.9)	3(10.3)
≥ 51	16	0(0.0)	0(0.0)
Total	193	10(5.2)	11(5.7)

at the Defense Research Laboratory repository. At the end of the whole sample collection, the stored cryovials were moved using an ice-filled chest container to the Institute for Advance Medical Research and Training, College of Medicine, University of Ibadan, where they were stored at

Statistical analysis

Statistical analyses were performed using SPSS software, version 21. Chi square ($\chi 2$) test was used to determine the association of HCV, HDV, and HBV markers with age and gender. Results were considered statistically significant at $p < 0.05$.

Table 2: Gender distribution of HCV among HBV positive population in Abuja (n=193)

Age range (years)	Male		Female		Total	Total
	No Tested	No (%) Positive	No Tested	No (%) Positive	No Tested	No (%) Positive
≤ 20	10	2(20.0)	16	0(0.0)	26	2(7.7)
21-30	28	2(7.1)	38	0(0.0)	64	2(3.1)
31-40	29	2(6.9)	27	2(7.4)	58	4(6.9)
41-50	20	1(5.0)	9	1(11.1)	29	2(6.9)
≥ 51	12	0(0.0)	4	0(0.0)	16	0(0.0)
Total	99	7(7.1)	94	3(3.4)	193	10(5.2)

p=0.735

(OR=1.49, 95%CI 1.03-2.21)

"80°C until tested using one of the vials while the other set was used for further studies.

ELISA screening for HBsAg, anti-HCV and anti-HDV

All the 193 blood samples were retested for HBsAg to ascertain their true status and subsequently tested for HCV and HDV antibodies using enzyme-linked immunosorbent assay (ELISA) kits (Diagnostic Automation/Cortez Diagnostic, Woodland Hills, California, USA). Both sensitivity and specificity of these test kits are 100%. The assays were

Results

This study reported overall rates of 5.2% and 5.7% for anti-HCV and anti-HDV among HBV infected population respectively (Table 1). The same rate (7.1%) was found in male for HCV and HDV while in female counterparts, the rate detected for HCV (3.4%) was lower than 4.3% found for HDV (Tables 2 and 3). Also by gender, the males have 1.5 times higher risk of HCV/HDV with significant association (p=0.0065) than in females (OR=1.47, 95%CI 1.03-2.21) for both infections respectively (Tables 3). The infection rate (7.7%) for HCV peaked within age

Table 3: Gender distribution rate of anti-HDV among HBV positive population

Age range (years)	Male		Female		Total	Total
	No Tested	No (%) Positive	No Tested	No (%) Positive	No Tested	No (%) Positive
≤20	10	0(0.0)	16	1(0.0)	26	1(3.8)
21-30	28	2(7.1)	38	3(5.3)	64	5(7.8)
31-40	29	2(6.9)	27	0(0.0)	58	2(3.4)
41-50	20	3(15.0)	9	0(0.0)	29	3(10.3)
≥51	12	0(0.0)	4	0(0.0)	16	0(0.0)
Total	99	7(7.1)	94	4(4.3)	193	11(5.7)

p=0.0065
(OR=0.99, 95%CI 0.63-1.57)

group ≤20 years while that for anti-HDV (10.3%) detected among participants at age groups 41-50 years (Tables 2 and 3). However, HBV/HCV/HDV tri-infection rate (1.6%) was only found in male participants among age group 21-30 years. There were no established significant associations (p=0.059; OR=1.06, 95%CI 1.23-2.11) between study participants with HCV/HDV/HBV tri-infection and age/sex (table 4).

had only primary education, 26(13.5%) did not have any formal education while the rest 66(34.2% and 18(9.2%) had secondary and tertiary education respectively. The subjects also consisted of 74(38.3%) individuals with multiple sexual partners and 119(61.7%) people with one or no sexual partner while 63(32.6%) had history of blood/ and blood products transfusion against 130(67.4%) who did not. Of these predisposing risk factors, multiple

Table 4: Co-infection of HCV/HDV by gender among HBV positive population (Tri-infection)

Age range (years)	Male		Female		Total	Total
	No Tested	No (%) Positive	No Tested	No (%) Positive	No Tested	No (%) Positive
≤20	10	0(0.0)	16	0(0.0)	26	0(0.0)
21-30	28	1(3.6)	38	0(0.0)	64	1(1.6)
31-40	29	0(0.0)	27	0(0.0)	58	0(0.0)
41-50	20	0(0.0)	9	0(0.0)	29	0(0.0)
≥51	12	0(0.0)	4	0(0.0)	16	0(0.0)
Total	99	1(1.0)	94	0(0.0)	193	1(0.5)

p=0.059
(OR=1.06, 95%CI 1.23-2.11)

Predisposing/risk factors for HDV in HBV and HCV infections

Association of sociodemgraphic profiles of the study participants with HDV in HBV and HCV infections showed that only 54 (28.0%) participants had prior knowledge while 139(72.0%) were ignorant. Fifty(25.6%) participants claimed to have been vaccinated against the virus against 143(74.4%) who were not vaccinated against HBV. Only 60(31.1) of them had no incisions on their bodies while 133 (68.9%) were incised at one point or the other in their lives. A total of 83(43.0%) of the participants sexual partnership was significantly associated (p<0.0423; OR=1.19, 95%CI 0.93-1.60) (Table 5).

Discussion

Dual and triple infections with hepatotropic viruses (HCV, HBV, HDV) are often associated with acute or chronic hepatitis with potential rapid progression to cirrhosis and Hepatocellular Carcinoma (HCC) [3,9]. This study has found overall rates of 5.2% and 5.7% for HCV and HDV antibody respectively among HBsAg positive cohort (Table 1). The rate for HCV/HBV co-infection detected in this study is

Table 5: Association of socio-demographic profiles with predisposing factors for HDV in HBV and HCV infections among the study population

Characteristic	No Tested (N=193)	No (%) Positive	p-value	OR (95% CI)
Age range (years)				
<20	26(13.5)	1(3.8)	0.609	1.22(159-1.91)
21-30	64(33.5)	5(7.8)		
31-40	58(30.1)	2(3.4)		
41-50	29(15.0)	3(10.3)		
≥ 51	16(8.9)	0(0.0)		
Marital status				
Single	62(32.1)	2(3.2)	0.087	1.13((0.78-1.90)
Married	94(48.7)	5(5.3)		
Separated	25(12.6)	3(12.0)		
Widowed	12(6.2)	1(0.0)		
Sex				
Male	99(51.3)	7(7.1)	0.0065*	0.99(0.63-1.57)
Female	94(48.7)	4(4.3)		
Level of Education				
Primary	83(43.0)	4(4.8)	0.215	0.8((0.58-1.88
Secondary	66(34.2)	2(5.0)		
Tertiary	18(9.3)	1(5.8)		
None	26(13.5)	4(16.4)		
Knowledge about hepatitis B/D/C viral infections				
Yes	54(28.0)	2(3.7)	0.370	
No	139(72.0)	9(5.7)		
Vaccination against HBV				
Yes	50(25.6)	3(6.0)	0.152	
No	143(74.4)	8(6.6)		
Having multiple sexual partners				
Yes	74(38.3)	6(8.2)	0.0423*	1.19(0.93-1.6)
No	119(61.7)	5(4.2)		
Have tattoo, incision/tribal in any part of the body				
Yes	133(68.9)	4(3.6)	0,216	
No	60(31.1)	7(11.7)		
History of blood/blood products transfusion?				
Yes	63(32.6)	4(6.3)	0,0721	
No	130(67.4)	7(5.4)		

**p<0.05 was considered statistically significant (using Chi square test)*

5.2% which connotes the fact that both virus share similar route of transmission. This rate while higher than 0.4% reported in a presumed low risk group in Jos [12] falls within the rate of 5.2% reported by Strickland, [13]. Our rate however is lower than the rates of 8.6%-14.5% found in other regions of the country [14,15,16]. Although variation in population may account for this difference, however, improved healthcare delivery which included but not limited to improved screening of blood units and safe needle practices in various health centers over the years may also account for the decline in rate of co-infection [10,11].

Large variations in anti-HDV prevalence across and within countries have been reported [17]. Our study detected, the prevalence of 5.6% for anti-HDV which falls within the range of 2.1% and 12.5% reported in different population groups from different parts of Nigeria [18,19,20]. Although there is low public awareness, medical interest, and research support for HDV co-infection probably due to the gains in HBV control [21]. There is need for renewed

interest in understanding the epidemiology as reports has shown sudden uprising in the prevalence of HDV infection even in regions previously known to be in HBV low endemicity region [22,23]. Specifically improved surveillance for HDV co/superinfection is of a major clinical importance since HDV in HBV causes the most aggressive form of viral hepatitis, with rapid progression to cirrhosis and hepatic decompensation in comparison to other viral hepatitis counterparts [23].

In this study, the rate of HCV and HDV co-infection by gender shows that the males have higher rate of infection (7.1%) than their female (3.4% and 4.3%) counterparts (p=0.0065) for both infections respectively and this is however not significant (Tables 2 and 3). This finding is in concordance with other reports which showed higher prevalence of hepatotropic virus co-infections in male than their female counterparts [5,19,20]. Furthermore differences in life styles of males associated with risky behaviours compared with their female counterparts who are known to eliminate the virus more frequently (24) may explain the reason for this.

Age-wise analysis in the present study found HCV to be higher (7.7%) among individuals belonging to the ≤20years (Tables 2 and 3). While this is in agreement with (16) which reported a lower co-infection rate among younger age group than with older age group for HCV/HBV, this however contrasted with the findings of Ifeorah *et al.* [20] which showed a higher infection rate for HDV in HBV among younger population who are more involved in high risky practices including multiple sexual partnerships (p<0.0423) than their older counterparts (Table 5). Nevertheless, a rate of 10.3% for anti-HDV in found among participants within the 41-50 years age groups agrees with the findings of Opaleye *et al.* [19], indicating that chronicity of HDV infection is proportional with advancement in age. A contrary finding was reported for heterotropic viruses investigated in another population group and this is not in consonance with our findings [1,16]. The reason for this difference seemed unclear but may be attributed to the variations in population involved in these studies among other reasons.

The present study also found HCV/HDV/HBV tri-infection rate of 0.5% (Table 4). This rate is lower but comparable with the findings of 0.7% and 0.8% respectively reported in similar other studies [20,25] among other population groups. The lower rates for triple infection could be associated with various mechanisms which bring about inhibition processes and repression of hepatotropic organisms in different scenario. Specifically while active HCV replication has been suggested to be involved in suppression of replication of other hepatotropic viruses and may dominate in triple infection [26], other reports provide evidence for suppression of HCV replication by HDV and HBV [27,28]. Now that the scenario in which the problems of tri-infection is becoming more obvious thereby making management of the victims more complex, it is therefore necessary for a renewed interest in areas of Hepatitis tri/co infection due to underlining health consequences.

Various confounders have been associated with predisposing/risk factors associated with hepatotropic viral infections including knowledge about HDV/HBV/HCV mode of transmission, vaccination history of HBV, level of education, multiple sexual partnerships, having tattoo, body incisions/tribal marks and history of blood/body fluid transfusions among others. As clearly demonstrated in this study, practice of multiple sexual partnerships was significantly and independently associated with HDV/HBV/HCV transmission (p<0.0423; OR=1.19, 95%CI 0.93-1.60) (Table 5). In line with this norm, Oliveira *et al.* (24) reported a significant association of practice of having multiple sexual partners predisposes such individuals to HDVHBV/HCV infections and in agreement with our study. However, lack of knowledge of the mode of transmission of hepatotropic viruses such as HDV in HBV and HCV helps to maintain the transmission (5,7) particularly among those with risky lifestyle such as injection drug users and others with tattoos, body incisions/tribal marks and blood transfusion. Hence, in places where appropriate laboratory investigations are lacking, many improperly screened blood/blood products may be certified pathogen free and this scenario of public health importance (19).

Conclusion

This study has reported high rates of HCV and HDV co infection in hepatitis B surface antigen-positive cohort and these findings are comparable with high risk population groups from previous studies. The tri-infection rate though low does not under rate the health implication especially with increased incidence of death due to liver cancer as reported by WHO in 2015. We therefore recommend further strengthening of the health care system with regards to viral hepatitis control especially among people already living with one form of viral hepatitis.

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