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Are patients with Primary Hepatocellular Carcinoma infectious of hepatitis B, C and D viruses?

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Abstract

Primary HepatoCellular Carcinoma (PHCC) has been strongly associated with HBV and HCV infections among other aetiological factors. However; do the patients still spread the viruses? This study involved forty one Nigerian adult patients with PHCC and 45 controls who were tested for HBsAg, H₃eAg, Anti-HBe, Anti-HBs, anti-HCV IgM and IgG, anti-HDV and HDV antigen using ELISA. Statistical analysis was carried out with the student – t – test and Mc Nemar test at $p < 0.05$. The subjects consisted of male:female ratio of 3:1 for both the PHCC patients and controls. Evidence of exposure to hepatitis B, C and D viruses was detected in 95.1%, 44% and 0% of the patients respectively while the respective values of 24%, 11.1% and 0% were obtained for the controls. Indication for high (HBeAg) and low (anti HBe) HBV viral replication, and acute HBV infection were detected in 12.5%, 92.7% and 2.2% respectively among the patients while only 35.6% of the controls had low HBV viral replication. Acute and chronic infections of HCV were also found in 26.8% and 24.4% of the patients respectively compared to the respective values of 2.2% and 11.1% of the controls. Occult HBV infection occurred in equal proportions (11%) of both the patients (31.7%) and controls (35.6%). In conclusion, infectious HBV and HCV particles are present among Nigerian patients with PHCC while HDV infection is uncommon. Hence, safe medical care should be practised for all patients with PHCC while relatives should be screened for these viruses.

Keywords: *HBV, infectious, hepatocellular carcinoma patients*

Résumés

La Carcinome hépatocellulaire primaire (CHCP) a été fortement associée à des infections par le VHB et le VHC parmi d'autres facteurs étiologiques.

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Cependant, les patients continuent-ils de transmettre le virus? Cette étude a été menée sur 41 patients adultes nigériens malades du CHCP et 45 sujets pilotes qui ont été testés pour le HBsAg, HBeAg, l'anti-HBe, les anti-BU anti-VHC IgM et l'IgG anti-HDV et l'antigène faisant usage de l'ELISA. L'analyse statistique a été réalisée par « student - t - test et test de Mc Nemar à $p < 0,05$. Les patients formaient un rapport hommes: femmes de 3:1 autant pour les malades de CHCP que pour les groupes pilotes. La preuve d'une exposition au virus de l'hépatite B, C et D virus a été détectée chez 95,1%, 44% et 0% des patients, respectivement, tandis que les valeurs respectives de 24%, 11,1% et 0% ont été obtenues pour les essais. Les indications de réplication virale pour le haut (HBeAg) et bas (anti-HBe) du VHB et une infection aiguë par le VHB ont été détectées dans 12,5%, 92,7% et 2,2% respectivement chez les patients tandis que seulement 35,6% des témoins avaient une faible réplication virale VHB. Les infections aiguës et chroniques du VHC ont été retrouvées chez 26,8% et 24,4% des patients, respectivement, par rapport aux valeurs respectives de 2,2% et 11,1% des groupes pilotes. L'infection VHB occulte eu lieu dans les mêmes proportions (11%) à la fois des patients (31,7%) et les groupes pilotes (35,6%). En conclusion, les particules infectieuses du HBV et du HCV sont présentes chez les patients nigériens malades du CHCP alors que l'infection du HDV est rare. Par conséquent, la sécurité des soins médicaux devrait être pratiquée pour tous les patients atteints de CHCP tandis que les parents doivent être testés pour ces virus.

Introduction

Primary HepatoCellular Carcinoma (PHCC) occurs worldwide and is common in the developing countries particularly in Nigeria [1]. Majority of the patients present late at the hospital with attendant complications when the little intervention that could be offered is mainly palliation [2]. Hence the definition of the major risk factor of the tumour such as chronic hepatitis B or C virus infection or co infection, alcoholic cirrhosis, non-alcoholic steatohepatitis, diabetes mellitus (metabolic syndrome is the likely risk process) and liver cirrhosis from different causes. There is higher risk of development of PHCC in patients infected with HBV and having high viral load,

of male gender and older age, who had progressed to chronic infection with or without presence of cirrhosis and have had exposure to aflatoxins. Similarly, the tumour had been linked to preceding HCV infection and cirrhosis in combination with concurrent alcohol abuse in a milieu of obesity especially in association with insulin resistance and previous or concurrent infection with HBV [3]. The high prevalence of PHCC

HBsAg, HBeAg, Anti-HBe, Anti-HBs, anti-HCV IgM and IgG, anti-HDV and HDV antigen.

The study was carried out after being granted ethical clearance by the UI-UCH Ethical Review Committee. Data obtained from the study were processed confidentially and statically analysed using the student - t -test and Mc Nemar test at a significant level of $p < 0.05$.

Table 1: Prevalence of Serological markers of HBV, HCV and HDV in Nigerian patients with PHCC and Controls

Groups (number)	HBsAg	HBeAg	Anti-HBe	Anti-HBs	HBV total	Anti-HCV IgM	IgG	total	Anti-HDV	HDV Ag	HBV+HCV
HCC (41)	63.4	12.5	92.7	7.3	95.2	24.4	26.8	43.9	0	0	41.5
Control (45)	0	0	35.5	26.7	35.6	11.1	2.2	11.1	0	0	4.4
P value<	0.000	0.022	0.00	0.023	0.000	0.155	0.001	0.001	-	-	0.000
Odd Ratio		35.34(7.53-165.95)				6.55(2.14-20.04)			-	-	60.00 (7.56-476.44)

in Nigerians has been largely secondary to HBV with or without associated HCV infection. Nigerian patients with PHCC have been reported to have 54.8% and 14.3% of HBV (HBsAg) and HCV (anti-HCV) respectively [4]. The prevalence of HBV will be higher if other serological markers of the infection are assayed since these markers feature depending on the clinical phase of the presentation of a patient in the virus's natural history. The markers such as HBeAg, anti-HBe, Anti-HBe, Anti-HBs are of different clinico-serological significances with HBeAg and anti-HBe indicating high and low infectivity states of HBV respectively [5]. Although, co-infections as well as triple infections of HBV, HCV and HDV both acute and chronic forms have been reported [6, 7], these have been scantily described in Nigerian patients with PHCC [8]. However, the infectivity status of the viruses remained undefined.

Hence, our study among Nigerian patients with PHCC is to determining the serological markers of HBV, HCV and HCV infections and defining their infectivity status

Materials and methods

This prospective case controlled study involved forty one adult Nigerian patients with PHCC and 45 Controls (apparently healthy adult without liver diseases) who were enrolled after obtaining informed consent from them. The patients were diagnosed by clinical features suggestive of PHCC, use of ultrasonography of their liver, elevated serum alpha-fetoprotein and confirmed histologically. The Controls and the patients were sex and age matched. The sera of blood collected from the subjects were assayed by enzyme linked immunoassay method for

Results

The subjects consisted of 31 male and 10 female patients as well as 33 male and 12 female controls at a gender ratio of 3:1 for each of both groups. The patients were 48.9 ± 13.8 years (mean \pm SD) while the controls were 49.4 ± 13.7 years. Evidence of exposure to hepatitis B, C and D viruses were observed in 95.2%, 43.9% and 0% of the patients with the respective values of 35.6%, 11.1% and 0% for the controls. Acute and chronic infections of HCV were also found in 24.4% and 26.8% of the patients respectively compared to the respective values of 2.2% and 11.1% among the controls (Table 1). Indication for high (HBeAg) and low (anti-HBe) HBV viral replication, and acute HBV infection were detected in 12.2%, 80.5% and 2.4% respectively among the patients while only 35.6% of the controls had low HBV viral replication. Possible HBV infectivity or early acute HBV infection (HBsAg alone) was detected only among the patients (2.4%). Low infectivity of HBV is common than high infectivity 80.5% vs 12.7%, in Nigeria patients with PHCC. Patients infectious of HBV also had 90% and 100% of the acute and chronic HCV infections found among PHCC subjects while the controls that were infectious of HBV also had the only chronic HCV and 40% of the acute HCV infections among all the controls. Patients with PHCC are more infectious of HBV than HCV ($p=0.0001$ using Mc Nemar test) and also more infectious of either HBV 95.2% vs 35.6% $p=0.0001$ or HCV 43.9% vs 11.1% $p=0.001$ than the healthy subjects (Table 2). Occult HBV infection occurred in equal proportions of both the patients (31.7%) and controls (35.6%). Co infection

Table 2: Infectiousness of HBV and HCV in Nigerian PHCC patients and Controls

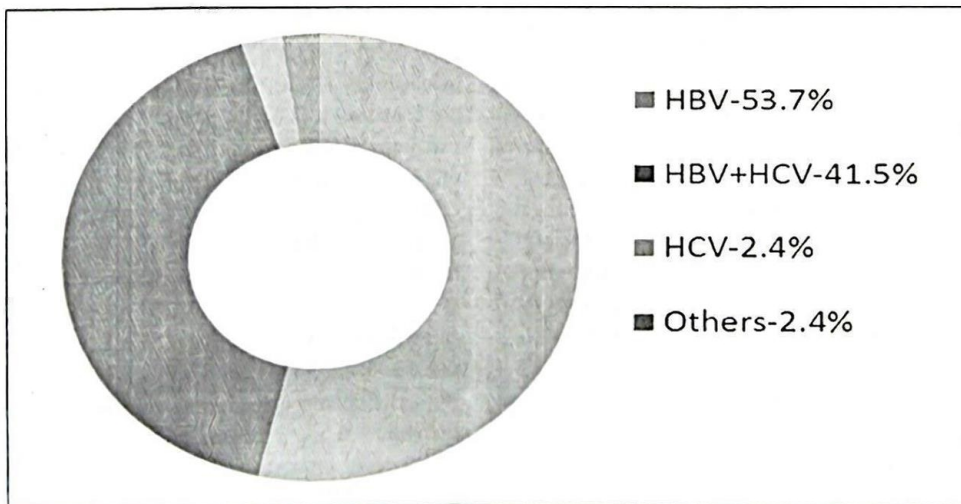
Degree of HBV infectiousness	HBV		HCV	
	PHCC	Control	PHCC	Control
High (HBeAg+)	12.2	0	9.8	0
Low (Anti-HBe+)	80.5	35.6	31.7	4.4
Possible (HBsAg alone)	2.4	0	0	0
NIL	4.9	0	2.4	6.7

the patient and controls respectively while lone HCV infection was identified in 2.4% and 6.7% of respective patients and controls. Only one patient (2.4%) with PHCC was neither infected with HBV nor HCV compared to 44.4% of the controls, Table 3. Possible aetiological factors for PHCC among our patients were principally secondary to HBV followed by HCV, co-infection of HBV and HCV and others in 95.2%, 43.9%, 41.5% and 2.4% respectively (Figure 1).

Table 3: Clinico-serological profiles of HBV, HCV and HDV infections in Nigerian PHCC patients and Controls

Stage of HBV Infection	HBsAg	HBeAg	Anti-HBs	Anti-HBe	PHCC	Control	HCV IgM	HCV IgG	HCV
Chronic/acute	+	+	+	-	5	0	2	2	4
Vaccination in chronic	+	-	+	+	1	0	0	0	0
Chronic	+	-	+	-	19	0	7	6	10
Early acute	+	-	-	-	1	0	0	0	0
Occult	-	-	+	-	11	11	(1)	3	4(1)
Resolving acute	-	-	+	+	2	5	(1)	(1)	1(1)
Vaccinated	-	-	-	+	0	7	(1)	0	1(1)
Susceptible	-	-	-	-	2	22	3(2)	0	3(2)
Total	63(0)	12(0)	93(36)	7(27)	41	45	15(4)	12(1)	23(5)

Parenthesis – Values for the Controls. HDV infection is 0% in either the Patients or Controls



of HBV and HCV was observed in 41.5% of HCC compared to 4.4% of controls ($p=0.0001$). Infection with only HBV was detected in 53.7% and 31.2% of

Discussion

Viral hepatitis such as HCV, HBV and HDV share similar routes of transmission, hence dual or triple viral infection can occur in a proportion of patients

at the same time. Those occurring secondary to HBV and HCV are important factors in the development of chronic hepatitis, liver cirrhosis (LC) and hepatocellular carcinoma (PHCC). In addition, chronic HDV infection also plays an important role in liver damage with oncogenic potential. Furthermore, dual infection with HBV and HCV could increase the risk for development of HCC and triple infection involving HBV, HCV and HDV show severe and progressive liver disease [7].

Detection of these viral infections is thus very important. The results of this study showing prevalence of 63.4% and 95.1% by assay of HBsAg alone and combination of HBsAg with anti-HBe demonstrates the value of assaying HBV markers other than just HBsAg as currently practiced in most health facilities in Nigeria [9]. This observation was also consequent to the assay of both anti-HCV, Ig M and Ig G for acute and chronic HCV infections which gave a higher prevalence of HCV infection among the patients. However, the absence of acute and chronic HDV infections corroborates the rarity of HDV infection among Nigerians and indicates that the virus might be contributing an insignificant role in the pathogenesis of PHCC in the population [8].

Our study has not only confirmed that HBV more than HCV are of major aetiological factors for PHCC among Nigerians but shows that HCV is of increasing role compared to previous reports [4,10]. This is further highlighted by the presence of combined HBV and HCV infection of either the acute or chronic forms in higher proportions of our PHCC patients, a new observation of HCV infection among Nigerians. The presence of occult HBV infection in high percentages among both groups of our subjects, the healthy adults and patients with PHCC shows that this type of the infection could have previously been missed because of assay of only HBsAg for the presence of HBV infection. In addition, this study also demonstrated that HBV seronegative for HBeAg (HBV mutants) is commoner among Nigerians than the wild type [5], an increasing event in Asia [11]. The presence of only HBsAg with the absence of other HBV markers in one of our patients suggest the patient is in the early phase of acute HBV infection [11].

Hence, patients with PHCC are susceptible to acute HBV infection possibly more accentuated by the reduced immunity caused by the cancer or a reactivation of a latent infection. This will require determination of HBV DNA for further elucidation.

Since seropositivity of HBeAg and anti-HBe suggest high and low HBV replication respectively and thus infectivity levels, the high rate infectivity (both low and high) of HBV (92.7%) among our

patients is very pertinent to note. Furthermore, these subjects also have high rate of both acute and chronic HCV infection (41.5%). These suggest that PHCC is a part of the spectrum for the natural history of HBV and HCV infections [12] and as such, patients with PHCC are infectious which could be very high as in some of the patients but mostly of low infectivity. Furthermore, both HBV and HCV infections seem synergistic in their onco-potential as shown by the very high odd ratios for the co-infection among our patients. Hence, patients with PHCC should be treated as potentially infectious until proven otherwise. This infectivity state could be possibly due to reduced immunity of the patients created by the malignancy thus encouraging reactivation of hitherto latent virus (es) or making the patient susceptible to new infection(s).

The higher prevalence of anti-HBs among the controls compared to the patients supports the protective role of HBV vaccination in the prevention of PHCC. Other preventable measures such as safe medical practice, screening of donated blood, patients and their relations, practicing safe sex, avoidance of sharing razors, syringes, tooth brushes, nail clippers, or single use and safe disposal of needles when getting a manicure, a tattoo, or having any body part pier should become routine practice both in healthcare and non-medical settings [7].

Considering the aetiological factors for our patients through the assay of serologically markers for HBV, HCV and HDV infections and their respective odd ratios for PHCC, this study has shown that PHCC in Nigerians might be principally due to co-infection of both HBV and HCV followed by lone infection of HBV than HCV and possible factors yet unidentified. The role of HDV infection needs to be further defined by assay of HDV RNA in the blood of Nigerian patients and adults with viral infection. Other hepatitis such as HEV infection and other possible aetiological factors will also be needed to be researched upon among Nigerians.

In conclusion, this study has shown that HBV more than HCV acting singly or in concert is the major risk factor for PHCC among Nigerians. These patients with PHCC are also infectious (low infectivity more than high) of both HBV and HCV and should be handled as potential sources for the transmission of the viruses. However, HDV is uncommon among our patient but further works will be needed to unravel the other factors contributing to PHCC among Nigerians. Since PHCC seems to be principally a preventable disease, safety measures should be applied when patients with the disease are undergoing medical care and relations should be screened.

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