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Research Article

Relative Rarity of Autoimmune Liver Disease Compared with Viral Hepatitis in A Sub-Saharan Black Population

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Abstract

The study aimed at determining the roles and contributions of autoantibodies, alcohol and viral agents in various liver diseases among Nigerians. 126 patients with liver diseases and 82 apparently healthy controls were studied. Antinuclear Antibodies (ANA), Antimitochondrial Antibodies (AMA), perinuclear Antineutrophil Cytoplasmic Antibodies (pANCA), anti-Soluble Liver Antigen/Liver Pancreas (anti-SLA/LP) and anti-Liver–Kidney Microsomal-1 (anti-LKM-1) as well as Hepatitis B surface antigen (HBsAg), Hepatitis Be Antigen (HBeAg), antibody to HBeAg (anti-HBe), antibody to hepatitis B core antigen (anti-HBc) and antibody to hepatitis C virus (anti-HCV), as well as HBV-DNA were analysed using ELISA.

About 75% were males with mean age of 47.5±14.4 yrs. Cases consisted of 61% Hepatocellular Carcinoma (HCC), 25.4% Liver Cirrhosis (LC), 7.9% Chronic Hepatitis (CH), 3.2% Acute Viral Hepatitis (AVH) and 1.6% Primary Biliary Cirrhosis (PBC). 51.3% consumed significant alcohol. No difference in percentage or level of ANA among cases and controls. AMA was detectable in 60.3% of cases compared to 43.9% of controls, and one case and one control were positive for anti-LKM-1 while all subjects were negative for anti-SLA/LP and pANCA. Anti-HBc was detected in 93.7% of cases and 73.2% controls. HBsAg, HBeAg anti-HBe and anti-HCV and HBV-DNA were significantly higher in cases than controls. AMA was significantly higher in CH and HCC compared with controls. HBsAg was significantly higher in HCC compared to controls and other liver cases. HBeAg, anti-HBe, anti-HBc, anti-HCV and HBV-DNA were significantly higher in CH, LC and HCC compared to controls ($p < 0.05$). There were 53 genotype E and two genotype A in cases, with genotype E in one control.

Prevalence of autoantibodies to liver antigens is similar in individuals with or without liver disease and, therefore not reliable in predicting autoimmune liver disease. HBsAg, anti-HBcAg, anti-HCV and HBV-DNA were strongly associated with liver disease. HBV genotype E is predominant in Nigeria.

keywords: *Autoantibodies; Viral Markers; Liver Disease Nigeria*

Introduction

Viral hepatitis, alcohol, Non-alcoholic fatty liver disease and autoimmune phenomena top the list of acquired aetiological agents of liver disease worldwide [1-3]. Several studies in the developed world have delineated the contributions of each of these agents in the causation of liver disease [1-3]. However, in sub-Sahara Africa and in Nigeria in particular there have been only a limited number of studies evaluating in a composite manner, the direct contribution of these identified major aetiologic agents of liver disease. Most of the studies considered isolated aetiological agents of liver disease with more emphasis on viral hepatitis B and C, and specifically none has yet examined the contribution of autoimmune liver diseases [4,5], save for an isolated case report of an autoimmune liver disease in a Nigerian woman [6].

In developing countries, most of the studies on viral hepatitis are limited to hospital based serological markers conducted on few sample sizes due to cost, unavailability of manpower and sufficiently equipped laboratories, thus making it challenging to study the molecular markers of viral hepatitis. Knowledge of the individual and combined contributions of the various risk factors of liver diseases in our environment will certainly help clinicians and epidemiologists to direct scarce resources and energies in the right direction. Our study evaluated individual and combined contributions of viral hepatitis, alcohol and liver-related autoantibodies among patients with a spectrum of liver diseases and controls, in order to establish the significance of these risk factors in the aetiogenesis of liver diseases in our environment.

Materials and Methods

A prospective, cross-sectional case-control study was carried out over a twenty-four-month period after an ethical approval (IRC Protocol number UI/IRC /07/0027) and informed consent among patients with liver diseases and apparently healthy control at the University College Hospital, Ibadan, Nigeria.

One hundred and forty-five consenting adults aged 18 years and above diagnosed with acute or chronic liver disease at the University College Hospital, Ibadan, Nigeria were recruited into the study. Liver disease was diagnosed in patients using a combination of clinical, biochemical, radiological and when possible histological features.

Eighty-two control subjects were drawn from apparently healthy relations of patients, patients with diseases unrelated to the liver, such as hypertension, unremunerated blood donors, administrative, medical and nursing staff of the hospital with no known clinical or biochemical features of liver disease.

Biodata, history of hepatitis B immunization, tribe, smoking, alcohol and HBV-specific drug history, educational level, past medical history of jaundice and family history of liver disease were collected by researcher- administered questionnaire.

Sera of the patients and controls were transported on dry ice during an overnight flight to the Institute of Immunology, Laboratoire de Sante, Luxembourg, where they were analysed for HBsAg, total Anti-HBc, Anti-HBe, HBeAg with third generation ELISA kits (ABBOT Murex, Germany) while IgG Anti-HCV was analysed using third generation ELISA kit (Ortho-Clinical diagnostics, Inc. UK), HBV-DNA and HCV-RNA were detected according to the manufacturer's protocols. Briefly, HBV DNA extraction was carried out from 200ul of serum with the QIAamp spin column (QIAamp viral DNA minikit, QIAgen Inc. Germany) according to the manufacturer's specification. The extracted nucleic acid was eluted in 100 ul elution buffer and the extracted DNA was kept at –25°C till used for molecular characterization. Quantification of viral DNA was carried out by 5' nuclease real time polymerase chain reaction on an ABI 7500 SDS Fast Real-Time PCR system (Applied Biosystems). Amplification of positive and quantifiable samples on the preS and S regions was done on Opticon Engine2 (Biorad) as described previously by Olinger., *et al*. [7].

Similarly, antinuclear antibodies (ANA), Antimitohondrial antibodies (AMA), perinuclear Antineutrophil cytoplasmic antibodies (pANCA), Liver Kidney Microsomal antibodies (Anti-LKM-1) and anti-soluble liver antigen (Anti-SLA-LP) were assayed, using the ELISA technique (Aeskulisa GmBH) according to the manufacturer's protocol. Sera of the control subjects were similarly analysed except for antinuclear antibodies (ANA) for which only 107 cases and 67 controls were analysed due to insufficient volume of some samples. Nineteen of the liver disease patients' samples were not suitable for analysis and were discarded. The report of this study was therefore based on the 126 patients and 82 controls. To determine the Hepatitis B genotype, phylogenetic trees were constructed with the MEGA 3.1 software, using the neighbour-joining and Kimura 2 parameter method and including reference strains of genotypes A–G and all known A and D subtypes. Sequences were submitted to GenBank/ EMBL/DDBJ under accession numbers AM110794–AM110915 for the pre-C/C gene and AM180623–AM180628 for the complete genome and preS segment sequences. Data were analysed using relative frequency, odds ratio, Pearson's Chi square, Fisher's exact test and Students' t-test at 5% level of significance.

Results

The 126 patients consisted of 91 (72.2%) males and 35 (27.8%) females, while the control group was made of 59 (72%) males. The mean ages of the cases and the controls were 47.5 ± 14.4yr vs 39.6 ± 16.5yr respectively.

There was no statistically significant difference in the sex distribution between the cases and control subjects ($p > 0.05$). The patients consisted of HCC 77 (61.1%), liver cirrhosis 32 (25.4%), chronic hepatitis 10 (7.9%), acute viral hepatitis 4 (3.2%), alcoholic cirrhosis 1 (0.8%) and primary biliary cirrhosis 2 (1.6%).

Fifty-two (41.3%) subjects with liver disease consumed significant alcohol. Significant alcohol consumption was 50g of alcohol per day for five years in men and 40g in women. In order to standardize and align with SI unit, many authorities have recommended conversion to grammes of alcohol consumed. To convert concentrations of alcohol, usually listed in volume percent (equivalent to the volume of solute/volume (%v/v) was multiplied by the specific gravity of alcohol, 0.79 g/ml [8,9,10]).

Of the five autoimmune serologic markers tested, an only antimitochondrial antibody (AMA) was found to be significantly higher among cases compared with controls, Table 1. Antimitochondrial antibodies were present in 76 (60.3%) of the cases compared with 36 (43.9%) controls ($p < 0.05$), while antinuclear antibodies (ANA) were present in 42 (39.3%) of cases compared with 27 (39.7%) controls ($p = 0.68$). Anti-soluble liver antigen (anti-SLA/LP) and perineutrophil cytoplasmic antibodies (pANCA) were completely absent among cases and controls Table 1. Anti-mitochondrial antibody was more prevalent in males (68.4%) compared with females (31.6%) among the cases.

Markers	Cases N= 126	Controls N= 82	X^2	p-value
ANA	42(39.3)	27(39.7)	0.75	0.68
AMA	76(60.3)	36(43.9)	5.37	0.02
LKM-1	1(0.8)	1(1.2)	0.09	Fischer's exact 1.0
pANCA	0	0	-	-
Anti-SLA/LP	0	0	-	-
HBsAg	103(81.7)	49(59.8)	12.21	0.000
HBeAg	27(21.4)	1(1.2)	17.41	0.000
Anti-HBe	60(47.6)	20(24.4)	11.32	0.001
Anti-HBc	118(93.7)	60(73.2)	16.88	0.000
Anti-HCV	45(35.7)	8(9.76)	17.63	0.000
DNA	58(46%)	1 (1.2%)	19.58	0.000
PreS PCR	53 (42.1%)	1 (1.2%)	16.24	0.000

Table 1: *Prevalence of viral markers and autoantibodies among cases and controls.*

NB: Percentage in parenthesis; ANA analysed: cases = 107, controls = 67.

**P values less than 0.05 ($p < 0.05$) in front of data show that there are significant differences between cases and controls.*

Similarly, among the controls, AMA was more prevalent (83.3%) in males compared with females (16.7%). However, among cases, the proportion of females positive for AMA was higher when compared with the control group. Fifty-two (57.1%) of the 91 males and 24 (68.5%) of the 35 females among cases were AMA positive but they constitute 68.4% and 31.6% of the 76 positive for AMA (Table 2). Among the controls, 30 (50.8%) of the 59 males and 6 (26.0%) of the 26 females were AMA positive. One positive anti-LKM-1 each occurred among the cases and the controls, and both were males, Table 2. The highest positivity for AMA among cases was recorded in the

age group 30-39 years in contrast to the controls which was in age group less than 30 years. The result presented in Table 3 also showed that the least occurrence of AMA was found in the age group 70 years or more, in both cases 5(6.6%) and controls 2(5.6%). Anti-LKM was recorded positive in one sample each among cases and controls, and both were below 30 years of age.

Parameter	Total positive		Total positive (Cases)		Total positive (Controls)		
	Cases	Control	Male	Female	Male	Female	X^2 P-value
ANA*	42 (39.3)	27(39.7)	37(88.1)	5(11.9)	21(77.8)	6(22.2)	1.31 0.253
AMA	76(60.3)	36(43.9)	52(68.4)	24(31.6)	30(83.3)	6(16.7)	2.77 0.096
LKM-1	1(0.8)	1(1.2)	1(100)	0	1(100)	0	- -
HBsAg	103(81.7)	49(51.8)	75(72.8)	28(27.2)	34(69.4)	15(30.6)	0.19 0.661
HBeAg	27(21.4)	1(1.2)	20(74.1)	7(25.9)	1(100)	0	0.35 0.556
Anti-HBe	60(47.6)	20(24.4)	44(73.3)	16(26.7)	11(55)	9(45)	0.35 0.128
Anti-HBc	118(93.6)	60(73.1)	88(74.6)	20(25.4)	43(71.7)	17(28.3)	2.16 0.141
AntiHCV	45(35.7)	8(9.7)	37(82.2)	8(17.8)	6(75.0)	2(25.0)	0.23 0.630

Table 2: *Sex distribution of subjects with positive autoantibodies and viral markers.*

Cases =126; Controls = 82

*NB: Percentage in parentheses; *ANA=107 cases; 67 controls.*

** P values less than 0.05 ($p < 0.05$) in front of data show that there are significant differences between cases and controls.*

Age grp (yrs)	AMA		Anti-LKM-1		ANA	
	Cases	Control	Cases	Control	Cases	Control
< 30	10(13.2)	15(41.7)	1 (100)	1 (100)	6(14.3)	8(29.6)
30 - 39	19(25)	7(19.4)	0	0	8(19.0)	5(18.5)
40 - 49	14(18.4)	3(8.3)	0	0	12(28.3)	2(7.4)
50 - 59	15(19.7)	6(16.7)	0	0	9(21.4)	4(14.8)
60 - 69	13(7.1)	3(8.3)	0	0	4(9.5)	2(7.4)
≥ 70	5(6.6)	2(5.6)	0	0	3(7.1)	6(22.2)

Table 3: *Age group distribution of subjects positive for autoimmune markers.*

NB: Number with percentages in parenthesis.

All the tested serologic viral markers were significantly different and higher between the cases and control subjects. Hepatitis B surface antigen was positive in 103 (81.7%) cases compared with 49 (59.8%) controls ($p < 0.05$). Similarly, HBeAg, Anti-HBe, anti-HBc (total) and anti-HCV were significantly higher among cases compared with controls (Table 1). Among the cases, HBsAg and anti-HBc had the highest frequencies, being present in 81.7% and 93.7%, respectively. These parameters were also noted to be high among control subjects, though at a relatively lower rates Table 1. Also, all the markers of HBV and anti-HCV were higher in the male gender, but did not reach statistical significance as depicted in Table 2.

The higher prevalence of HBsAg was in the age ranges 30 - 39, 40 - 49 and 50 - 59 years among the cases, while the highest prevalence among the control group was in the age group below 30 years Table 4. Lower prevalence of HBsAg was found in the ages below 30 years and above 70 years among cases, while the lowest prevalence among the controls was in the age 60 years and above. The HBeAg was most prevalent in the age 30-39 years among the cases. Anti-HBc was more prevalent in the age range 30-39 years among cases, similar to the

pattern of HBsAg, but more controls were positive for anti-HBc in the age group less than 30 years Table 4. The prevalence of anti-HCV was highest among the cases in the age ranges 40 to 49 years.

Age grp (yrs)	HBsAg		HBeAg		Anti-HBe		Anti-HBc		Anti-HCV	
	Cases	Control	Cases	Control	Cases	Control	Cases	Control	Cases	Control
<30	10(9.7)	18(36.7)	3(11.1)	0	8(13.3)	3(15.0)	12(10.2)	19(31.7)	4(30.8)	2(6.5)
30 - 39	23(22.3)	7(14.3)	9(33.3)	1(100)	14(63.3)	4(20.0)	26(22.0)	10(16.7)	10(33.3)	0
40 - 49	23(22.3)	8(16.3)	3(11.1)	0	14(23.3)	3(15)	26(22)	11(18.3)	12(46.2)	2(16.7)
50 - 59	23(22.3)	6(12.2)	6(22.2)	0	9(15)	5(25)	26(22)	8(13.3)	10(37)	0
60 - 69	16(15.5)	5(10.2)	4(14.8)	0	10(16.7)	4(20)	20(16.9)	6(10)	5(22.7)	3(37.5)
≥70	8(7.8)	5(10.2)	2(7.4)	0	5(8.3)	1(5)	8(6.8)	6(10)	4(50)	1(16.7)

Table 4: *Age group distribution of subjects positive for viral markers.*

NB: Number with percentages in parenthesis.

Total anti-HBc had the highest prevalence among the different types of liver diseases, except for PBC in which it was negative. Total anti-HBc was positive in all cases with acute viral hepatitis and alcoholic liver disease. It was also present in 31(96.9%) of liver cirrhosis, 73(94.8%) of HCC, and 9(90%) of chronic hepatitis (Table 5). Prevalence of Hepatitis B surface antigen was highest among cases with HCC, 70 (90.9%) of the 77 cases. This was followed by chronic hepatitis 8 (80%), acute viral hepatitis 3 (75%) and liver cirrhosis 21(65%). Prevalence of HBeAg, anti-HBe and anti-HCV and percentage frequencies of viral markers and autoantibodies among cases are shown in Figure 1.

Type of liver disease	No tested	HBsAg	HBeAg	Anti-HBe	Anti-HBc	Anti-HCV	ANA	AMA	Anti-LKM-1
Acute hepatitis	4(3.2)	3(75)	1(25)	2(50)	4(100)	1(25)	1(25)	2(50)	0
Chronic hepatitis	10(7.9)	8(80)	4(40)	6(60)	9(90)	6(60)	4(40)	9(90)	0
Liver cirrhosis	32(25.4)	21(65.6)	5(15.6)	14(43.8)	31(96.9)	11(34.4)	11(34.4)	15(46.9)	0
Alcoholic cirrhosis	1(0.8)	0	0	1(100)	1(100)	0	0	1(100)	0
PBC	2(1.6)	1(50)	1(50)	1(50)	0	1(50)	0	1(50)	0
HCC	77(61.1)	70(90.9)	16(20.8)	36(46.8)	73(94.8)	26(33.8)	26(61.9)	48(62.3)	1(1.3)
Total Cases	126	103	27	60	118	45	42	76	1
p-value		0.07	0.55	0.86	.00	0.60	0.65	0.210	0.99

Table 5: *Frequency of viral and autoimmune markers among liver cases.*

Percentage in parenthesis.

Anti-SLA and pANCA were negative in all.

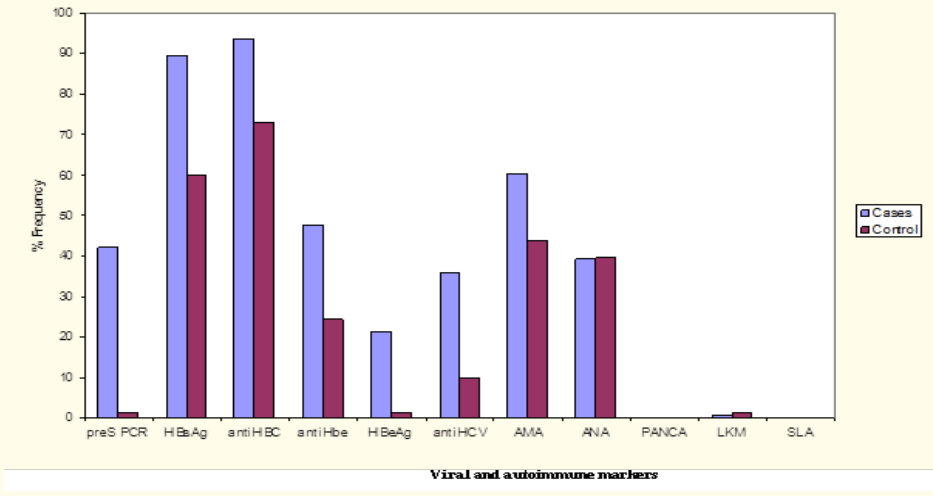

Figure 1: *Percentage frequency of viral and autoimmune markers.*

Chronic hepatitis had the highest frequency of AMA, being positive in 9 (90%) of the 10 cases, this was followed by HCC, 48(62.3%) of the 77 cases tested were positive for AMA. The only patient with alcoholic cirrhosis was also positive for AMA.

PreS PCR was positive in 53 (42.1%) vs. 1 (1.2%) and HBV-DNA in 58 (46%) vs. 1 (1.2%) among test and control subjects respectively. Overall, HBV-DNA was detected in the sera of 58 (46.0%) of 126 patients with liver diseases compared with only one among the 82 (1.2 %) controls (X2=51.53, p = 0.000). Thirty-five (60.3%) of the 58 cases that were HBV-DNA positive were concomitantly AMA positive. The only LKM-1 positive case was also HBV-DNA positive in contrast to the HBV-DNA negative LKM-1 positive found in the control group (Table 6). Among the liver cases, HBV-DNA was found to be positive in all the anti-SLA and pANCA negatives, similarly, among the control group the only HBV-DNA positive was found among the pANCA and anti-SLA negative.

	AMA+	
Viral markers	**Cases N=76**	**Control N=36**
HBsAg+	62(81.6)	21(58.3)
HBeAg+	16(21.1)	0
Anti-HBe+	41(53.9)	8(22.2)
Anti-HBc+	70(92.1)	25(69.4)
Anti-HCV+	27(60)	5(62.5)

Table 6: *Prevalence of viral markers in samples significantly positive for AMA autoantibodies.*

Among the cases, HBsAg positive samples had the highest level of HBV-DNA positivity, with 57(98.3%) of the 58 DNA positives occurring in HBsAg positives, compared with 1 (1.7%) among HBsAg negatives. This showed that, of the 103 HBsAg positives 57 (55.3%) had HBV-DNA, while of the 23 HBsAg negatives, 1 was HBV-DNA positive, suggesting the incidence of occult HBV infection of 4.3%. The only HBV-DNA positive sample among the controls was also HBsAg positive. Twenty-three (85.2%) of the HBeAg positive cases however were HBV-DNA positive with a lower HBV-DNA positivity (35.4%) being recorded among cases who are negative for HBeAg (Table 7). The DNA positivity was however too low to be compared statistically. It is obvious that there is a stronger association of HBeAg with HBV-DNA.

	Cases			Controls		
	DNA+	**DNA-**	**Total**	**DNA+**	**DNA-**	**Total**
HBsAg+	57(98.3)	46(67.6)	103	1(100)	48(59.3)	49
HBsAg-	1(1.7)	22(32.4)	23	0	33(40.7)	33
Odds ratio	27.26	-	-	-	-	-
Total	58	68	126	1	81	82
P	0,00			1.00		
HBeAg+	23(39.7)	4(5.9)	27	0	48(59.3)	49
HBeAg-	35(60.3)	64(94.1)	99	1(100)	33(40.7)	33
Odds ratio	10.51					
Total	58	68	126	1	81	82
P	0.00			1.00		
Anti-HBe+	32(55.2)	28(41.2)	60	1(100)	19(23.5)	20
Anti-HBe-	26(44.8)	40(58.8)	66	0	62(76.5)	62
Odds ratio	1.76					
Total	58	68	126	1	81	82
P	0.12 1.0					
Anti-HBc+	56(98.2)	62(91.2)	118	1(100)	59(72.8)	60
Anti-HBc-	2(1.8)	6(8.8)	8	0	22(27.2)	22
Odds ratio	2.71					
Total	58	68	126	1	81	82
P	0.22 Fisher's			1.00		
Anti-HCV+	2(1.8)	2(2.9)	4	-	-	-
Anti-HCV-	56(98.2)	66(97.1)	122	1(100)	81100)	82
Odds ratio	1.20					
Total	58	68	126	1	81	82
P	0.03			1.00		

Table 7: *Frequency of HBV-DNA positivity compared with viral markers*

**P < 0.05 is considered statistically significant.*

High frequency of HBV-DNA positives were also observed among anti-HBe (53.3%), anti-HBc (47.5%), and anti-HCV (50%) positives but lower than that that in HBsAg positives. The corresponding frequencies of HBV-DNA in anti-HBe (39.4%), anti-HBc (25%) and anti-HCV (45.9% negatives were relatively lower with significant statistical difference attained only for anti-HCV (Table 8). There was also no statistically significant difference in the HBV-DNA positivity among cases positive for Anti-HBe and anti-HBc (p=0.12 and 0.22 respectively), (Table 7).

	Cases			Controls		
Autoantibodies	**DNA+**	**DNA-**	**Total**	**DNA+**	**DNA-**	**Total**
AMA+	35	41	76	1	35	36
AMA-	23	27	50	0	46	46
Total	58	68	126	1	81	82
P	0.00			1.294		
LKM+	1		1	0	1	1
LKM-	57		125	1	80	81
Total	58		126	1	81	82
Fischer's exact test	0.460			1.000		
pANCA+	0	0	0	0	0	0
pANCA-	58	68	126	1	81	82
Total	58	68	126	1	81	82
Anti-SLA+	0	0	0	0	0	0
Anti-SLA-	58	68	126	1	81	82
Total	58	68	126	1	81	82

Table 8*: Prevalence of HBV-DNA positivity compared with autoimmune markers among subjects.*

**P < 0.05 is considered significant.*

Hepatitis B virus DNA detection varied in the various classes of liver disease. HBV-DNA detection was highest among cases with acute viral hepatitis (75%) with none found among cases with alcoholic liver disease and primary biliary cirrhosis. Hepatocellular carcinoma and liver cirrhosis showed a serum HBV-DNA prevalence of 50.6% and 40.6% respectively (Table 9). Only 3 of the 10 patients with chronic hepatitis had detectable HBV-DNA.HCV-RNA was negative in all subjects who were positive for anti-HCV and in those who were negative for anti-HCV. Out of the 56 samples sequenced for genotyping, there were 53 (94.6%) genotype E, and 2 (3.6%) genotype A among cases and one genotype E among controls.

Clinical diagnosis	**HBV DNA+ (%)**	**HBV DNA- (%)**	**Total**
Alcoholic cirrhosis	0	1(100)	1
HCC	39 (50.6)	38(49.4)	77
Liver cirrhosis	13(40.6)	19(59.4)	32
PBC	0	2(100)	2
Chronic hepatitis	3(30)	7(70)	10
Acute hepatitis	3(75)	1(25)	4
Total	58	68	126

Table 9: *Frequency of HBV-DNA detection in clinical diagnosis group.*

Discussion

Our study showed that the spectrum of liver diseases in hospital setting are mostly HCC (61.1%) and liver cirrhosis (25.4%), with autoimmune liver diseases being uncommon constituting only about 1.6%. This confirms the age long suspicion that autoimmune liver diseases are rare among Nigerians, compared with Caucasian populations [11]. There were no readily available data among other Afri-

can countries to compare our findings with because there are no published data on autoimmune liver diseases in Africa. This study also showed male preponderance in liver diseases in keeping with findings in previous studies [12-14]. This gender difference in prevalence of liver diseases is thought to be multifactorial as the male gender is more affected with hepatitis viruses and are more likely to indulge in alcohol consumption, even though, women die more of alcohol-related liver disease [15,16], this being because women are less resistant to the damaging effect of alcohol on the liver [17].

Alcohol did not seem to play a major role in the causation of liver disease among our cohort of patients, in spite of over half of the patients having imbibed significant alcohol. According to the guidelines on alcoholic liver diseases by the American College of Gastroenterology (ACG) and other studies, alcohol consumption is significant if amounts consumed is up to 80g of alcohol per day for ten years in men [18], and 60g or more per day for women [19,20].

Antimitochondrial antibodies (AMA) were the only autoantibodies found to be statistically significantly higher among the patients with liver disease (60.3%) compared with the apparently normal control group (43.9%). The enigma of this finding is in the fact that even those who were apparently healthy also had a relatively high percentage of AMA though its presence is supposed to be diagnostic of primary biliary cirrhosis [21]. Similarly, the lack of significant difference in the levels of ANA and LKM-1 in liver cases compared to controls would suggest that there is a mechanism responsible for erratic production of liver-related autoantibodies in the Nigerian population. This is contrary to what obtains among Caucasians in whom the presence of ANA correlates well with systemic or organ specific autoimmune disease, being the most common autoantibodies in autoimmune hepatitis [22]. In the same population, ANA has been found to be predictive of autoimmune diseases [23,24], while LKM-1 is a useful laboratory tool in the diagnosis of type-2 autoimmune hepatitis, which is more common among children and young adults [25].

In the sixties, Greenwood postulated that the rarity of autoimmune disorders among Nigerians may be due to the presence of several environmental parasitic antigens stimulating the immune system. His study at the same centre, showed that diseases in which autoimmune processes were thought to be involved are uncommon in Western Nigeria [11] and suggested that the infrequent occurrence of autoimmune diseases in parts of tropical Africa is related to the immunological disturbance produced by multiple parasitic infections. He also described a low incidence of autoantibodies in rheumatoid arthritis and high incidence of rheumatoid factor among apparently healthy Nigerians [26]. Evidence abound that some of the immunological changes noted in apparently healthy Africans are related to infection with malaria [26,27]. Similar evidence of malaria infection affecting immunological response has also been documented in mice infected with malaria [26]. In 1995, Skalsky*., et al.* [28], in a study of chronic liver diseases in rural south-west Cameroun found that serum autoantibodies were frequently found and were not correlated with the presence of autoimmune liver disease. The complete absence of anti-SLA/LP in both the test and the control subjects further validates the rarity of type-1 autoimmune hepatitis among our patients with liver diseases, as these autoantibodies had been found to be 100% specific for AIH [29]. Similarly, pANCA were also completely negative in both cases and controls, further substantiating the rarity of autoimmune liver diseases in our patients. These findings are in contradiction to studies in Caucasian populations [29-32], and, in India [33], a developing country, where they have been found to be useful in the diagnosis of autoimmune liver disease; and sometimes used for prognostication [34].

Unlike the serological autoantibodies, serological and molecular viral markers were by far more common and significantly higher among patients with liver diseases. The finding of HBsAg in 81% of the liver cases compares favourably with previous studies in Nigeria and the rest of sub-saharan Africa [35,36]. Not surprisingly, the prevalence of HBsAg among the control group is rather high, this is likely due to the fact that most of the control group were recruited from hospital staff like ward maids, nurses, doctors and relations of patients on admission on the wards or clinics of the hospital. Such high prevalence among health workers have been previously documented at the same centre [37,38].

Hepatitis B core antibody was the most prevalent HBV marker among liver cases and controls. This is due to the high rate of exposure of individuals to the virus in an endemic area like Nigeria. Similar results were found in other hyperendemic areas of the world like

South Africa [39], Gambia [40], the rest of sub-sahara Africa. The higher prevalence of other HBV markers such as HBeAg and anti-HBe, in addition to anti-HCV among liver cases compared to controls shows their strong association with liver disease and are therefore likely pathogenic. In particular, HBeAg, a marker of viral replication, has been shown to be a surrogate marker for Hepatitis B x antigen (HBxAg), a transactivating protein implicated in the pathogenesis of liver cancer [41].

The higher prevalence of HBsAg in the older age groups of 30 - 59 years among cases compared to less than 30 years among controls would be in keeping with the established fact that HBV was transmitted horizontally within the family early in life [42,43] and become pathogenic, leading to liver diseases a decade or more later [44,45]. Persons aged above seventy years also had lower prevalence of the virus. This might suggest that the disease states caused by HBV are associated with mortality leading to premature deaths and reducing survival to old age in our environment.

Although HBeAg was generally low in both cases and controls, it was highest in the age group below forty years. This might be due to the loss of the antigen in the evolution of the infection. It has been observed that persons of African descent tend to have low HBeAg levels due to the development of hepatitis B pre-core and core mutants, a condition associated with inability to secrete the envelope antigen [46], and thus poor response to interferon therapy.

Cases that were positive for anti-HCV were found to be a decade or two older (< 50 years) suggesting a late pathogenetic effect of HCV compared to HBV. This observation has been previously documented by Okuda., *et al*. [47] and Shiratori., *et al.* [48], who found that patients with HBV-related liver disease were usually about ten years younger than those with HCV-related liver disease.

The number of patients diagnosed with primary biliary cirrhosis (PBC) {2 (1.6%)} and alcoholic liver disease {1 (0.8%)} were too few to draw any reasonable conclusion from. It could be said that PBC, a form of autoimmune liver disease and alcoholic liver disease are rare in the South-western part of Nigeria, in spite of a relatively high significant alcohol consumption as found in this study (50.3%). A study in the Middle belt and Northern Nigeria, however, showed higher prevalence of alcoholic liver disease [49]. The low incidence of alcoholic liver disease in spite of significant alcohol consumption confirms the assertion that HBV is the main causative agent of liver disease in our environment, with alcohol likely having an accelerating effect [50].

When viral markers were evaluated among cases and controls that were AMA positive, all the HBV markers were equally high in both groups. This would further strengthen the suspicion that the measured autoantibodies do not really have any pathogenic role, since viral markers have been strongly associated with liver diseases among cases.

Apart from HBsAg, anti-HCV and AMA, another marker of HBV found to be significantly higher among cases compared to controls and strongly associated with liver disease is the HBV-DNA. This molecular marker of HBV is one of the markers of replication of HBV besides HBeAg and has been found to be strongly associated with liver diseases such as acute and chronic hepatitis's, liver cirrhosis and hepatocellular carcinoma [51,52] and higher HBV-DNA has been associated with more severe liver disease as was found in this study.

The occurrence of HBV-DNA in the serum in one case of HBsAg negative among cases would suggest a low incidence of occult HBV among our patients with liver diseases in this study. Occult HBV, a phenomenon in which HBV-DNA is present in the serum in the absence of serum HBsAg, is a recognized occurrence in the complex biology of HBV, and has been found to be as high as 3.8% to 30% or even higher [53,54] in some studies both in Nigeria and other parts of the world. The condition has however been associated with liver diseases [55,56]. High frequency of HBV-DNA, similar to that found in HBsAg was also found in cases positive for anti-HBc and also in the only one subject among controls that had detectable HBV-DNA. The finding of only one case (4.3%) of detectable HBV-DNA among controls would suggest that HBV-DNA levels were generally low among subjects with HBsAg without liver disease, further strengthening the strong association of HBV-DNA with liver disease. It would also suggest that development of liver diseases in HBV infection is directly proportional to serum HBV-DNA. Therefore, serial measurement of HBV-DNA in the serum of normal individuals with HBsAg would be a useful tool in monitoring development of liver disease, and thus early treatment.

Monitoring of serum HBV-DNA should be the standard practice in the surveillance of subjects with HBsAg in addition to liver ultrasonography and serum alpha-fetoprotein [57,58]. A previous study in Ibadan, however showed a prevalence of 7.2% for occult hepatitis B among the patients with viral hepatitis, though the sample size was small relative to that of this study [53].

Our study showed that subjects with HBV-DNA are about thirty times as likely to develop liver disease (Odds ratio 27.1, Table 11) compared to those without HBV-DNA, while those positive for HBeAg are about ten times as much compared to those who are negative for HBeAg. Such strong association was not however found with anti-HCV and other measured viral markers in this study. Some studies have found odds ratio lower than our finding; Chan., *et al.* [51] in Honk-Kong found an odds ratio of 11.4%, while a relative risk of 60.2% was documented for those who were HBeAg positive in addition to HBsAg. In the Gambia, Mendy., *et al*. [52] found a seventeen to thirty-nine-fold increase in the risk of cirrhosis and hepatocellular carcinoma for patients who were positive for serum HBV DNA. Our findings, therefore, makes it reasonable to check for HBV-DNA, HBsAg and HBeAg among patients with liver disease in our environment.

Quantitative analyses of HBV-DNA, showed that patients with liver cirrhosis, a pre-malignant liver disease, had the highest HBV viral load titre, followed by acute viral hepatitis, then HCC and chronic hepatitis in that order. This suggests that HBV viral replication tends to be high, in cirrhosis, before the development of HCC. Therefore, a rising trend in the titres of HBV DNA in patients with CH might be a pointer to development of advanced complications of liver disease.

In contrast to the finding of HBV-DNA in a significant number of subjects with HBV infection, all the subjects who were positive for anti-HCV were negative for HCV-RNA on polymerase chain reaction. This could be explained in part by false positive results, recovery from HCV infection or the suppressive effect of HBV on HCV which has been observed in some studies. This may however be a phenomenon peculiar to our patients with HBV infection or due to the recognized high rate of false positivity to anti-HCV screening in populations with low prevalence of HCV infection, therefore, requiring RIBA and RNA PCR for confirmation [59,60]. Further studies are required in our environment to unravel the phenomenon. Phylogenetic analysis of HBV in our study showed results in keeping with previous finding by Odemuyiwa., *et al.* [61], who found hundred percent of the twenty patients with HBV to be genotype E, showing the predominance of the E genotype.

Conclusion

In conclusion, autoimmune liver diseases are uncommon in Ibadan, Nigeria and, the prevalence of autoantibodies to liver antigens is equally high in individuals with or without liver disease. Antimitochondrial antibodies (AMA) were significantly higher among cases with liver disease compared to controls. Antimitochondrial antibodies which are the hallmark of primary biliary cirrhosis, is a condition associated with intense fibrosis of the bile ducts. Since most of the patients in this study had HCC and liver cirrhosis which are also associated with fibrosis, there may be a link between serum AMA and fibrosis in the liver.

Conflict of Interest

None declared by authors.

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