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Molecular Quantitation of Hepatitis B Virus DNA and Alanine Aminotransferase Levels as Marker of Disease Progression in Adult Patients with Chronic Hepatitis B Infection in Northwestern Nigeria

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Abstract
Background: *The elevated levels of HBV DNA and alanine aminotransferase levels (ALT) remain major risk factors for progression of chronic HBV infection which is associated with serious, long-term complications.*
Nucleic acid amplification tests (NAATs) measures viral genome in body fluids and are now widely used for estimating patient's infectivity, assessment of prognosis, efficacy of antiviral treatment and identifying emergence of drug resistance.
Methods: *The study was carried out on 136 participants. Blood specimens was obtained for quantitative PCR assay at the NCDC Molecular diagnosis laboratory using commercial reaction mix with an analytic sensitivity of 20-50 copies/ml of HBV DNA and serum was obtained for ALT assay. Data analyzed using Statistical Package for Social Sciences (SPSS) version 20 and results presented as tables*
Results: *A prevalence rate of 28.7% for Hepatitis B envelop antigen was established among subjects, while 71.3% were negative. The mean ALT level was 34.5IU/ml, 84.6% had normal ALT values, 1.5% were mildly elevated and up to 14% were markedly elevated. No strong association was found between HBeAg status and raised ALT levels.*
The mean HBV DNA quantity was 37,094,093 copies/ml. About 85.3% had detectable levels of HBV DNA while 4.7% had levels below the lower limit of detection.
Conclusion: *HBV DNA level and serum ALT are important biomarker of disease progression in patients with chronic hepatitis B infection and imperative for consideration in its effective management.*
Keywords: *Hepatitis B, Hepatitis C, HBV DNA, HBeAg, alanine aminotransferase ALT.*

Introduction

Worldwide, an estimated population of 350-400 million people are infected chronically by Hepatitis B virus (HBV),[1] with complications such as liver cirrhosis, liver failure and hepatocellular cancer (HCC). Thus, HBV infection is a veritable global health problem.

Persistently elevated levels of HBV DNA (>10,000copies per ml) and in some patients raised alanine aminotransferase levels (ALT) (>1.5 times above upper limit of reference range) remain major risk factors for progression of chronic HBV infection.[2]

Alanine aminotransferase ALT, is commonly used in evaluating liver damage due to chronic HBV infection. Increase in serum ALT level has been

reported to be significantly associated with liver related mortality.[3] However, the this is in contrast with other studies.[4]

The recently developed nucleic acid amplification tests (NAATs) can measure the concentration of viral genome in patients body fluids and are now widely used not only in diagnosis of HBV infection, but also for estimating patient's infectivity, assessment of prognosis, monitoring of efficacy of antiviral treatment and for identifying emergence of drug resistance.

Chronic HBV infection is often associated with serious, long term complications including liver cirrhosis, liver failure and HCC[5,6] and despite the huge burden of the disease in Africa, there is paucity of studies and data on molecular quantitation of HBV DNA, ALT levels and other markers of disease progression. A cross sectional study of this type will update current information and help in guiding critical decisions in improving patient management.

Methods

This is a cross sectional study carried out at Aminu Kano Teaching Hospital, Kano. One hundred and thirty-six consenting participants were recruited from gastroenterology and hepatology clinic, the participants were asymptomatic chronic HBV positive (at least two HBsAg positive results, minimum of 6 months apart). They must be 18years and above and must not be on any antiretroviral drug. All those with chronic hepatitis B infection who are already on antiretroviral drug were exempted from the study.

Sample collection, storage and laboratory analysis

Under aseptic conditions, about 8 milliliters of venous blood was collected using a hypodermic needle and syringe. 4mls of the sample was transferred into EDTA bottle for quantificative PCR assay, while the remaining 4mls was put into plain bottle to obtain serum for ALT assay. The EDTA samples were promptly transported to the NCDC Molecular diagnosis laboratory of the Aminu Kano Teaching Hospital, Kano and separated into plasma by centrifugation of the anticoagulated whole blood at 2,000rpm for 20minutes at room temperature within 6 hours of sample collection. Plasma obtained was stored at -20^{o}C for subsequent use for PCR analysis. The second aliquots of whole blood in plain bottles were allowed to clot and serum separated by centrifugation at 2,000rpm for 20minutes at room temperature and stored at -20^{0}C for subsequent ALT assay.

Laboratory Analysis of Biochemical Parameters: ALT levels were measured using commercially available assay kits (Randox Bio-diagnostics, Crumlin United Kingdom) according to manufacturer's instructions. In order to ensure sufficient analytical quality control, in each batch of tests, a set of standards (level 1, level 2 and level 3) and a set of positive and negative controls was included in each run. The controls and calibrators supplied with the kit was checked for validity according to manufacturer's recommendation i.e., mean absorbance for the negative controls and calibrators. Test sample results were acceptable only when these criteria are met. A reference range of 0-40 IU/L was used and values that are 1.5 times above upper limit of normal were considered significantly high (i.e.,> 60 IU/L).

Quantitative PCR was carried out using commercial reaction mix (Liferiver Bio-Tech, San Diego California U.S.A.) with an analytic sensitivity of 20-50 copies/ml of HBV DNA was used. The kit contains HBV primer set and DNA extraction reagents that were used in the PCR mastermix. The primers targeted the S gene and their sequences were 5´ GTG TCT GCG TTT TAT CAG (sense/forward primer) and 5´ GAC AAA CGG GCA TAC CTT (antisense/reverse primer) designed to amplify a 98 base pair product from position 379 to 476 of the HBV genome. PCR was carried out using Applied Biosystems Real time PCR machine, model ABI 7300 at the Molecular diagnostic laboratory (NCDC) Aminu Kano Teaching Hospital, Kano.

Data processing and statistical analysis

All data generated were collated, checked and entered into a database design using MS Excel spreadsheet and analyzed using Statistical Package for Social Sciences (SPSS) version 20. Results was presented as tables. Two by two tables were used and χ^2 test applied to the association between HBV DNA levels, ALT levels and other patient characteristics. Using a null hypothesis, the association was accepted or rejected based on the χ^2 test for each variable. Probability values of <0.05 were considered statistically significant.

Results

Sociodemographic Characteristics

One hundred and thirty-six (136) eligible subjects participated in this study. The mean, median and range of their age were 33, 35 and 19-68 years respectively. As shown in Table 1, 102(75%) males and 34(25%) females participated in the study giving a male to female ratio of 3:1, subjects were predominantly young with 105(77.2%) aged below 45 years. Majority 108(79.4%) of subjects were Hausas by ethnicity while the remaining 28(30.6%) composed of people from other ethnic groups. Most of subjects 92(67.6%) had tertiary education, while 42(30.9%) had primary or secondary education. In total only 33(24.3%) of subjects had never been married, Civil servants 38(27.9%) constituted the largest occupational group among subjects, while medical personnel comprised only 6(4.4%) of subjects.

As highlighted in Table 2, majority 110(80.9%) of subjects were asymptomatic without any significant complaint of ill health while the remaining 26(19.1%) had complaints possibly related to the infection and its sequelae. The rates of co-infections with HIV and HCV were found to be 2(1.5%) and 4(3%) respectively. Only 12(8.8%) of participants volunteered a history of cigarette smoking currently or in the past while 3(2.2%) admitted to ingestion of alcoholic beverages. Previous history of blood transfusion of at least one pint of blood was established in 19(14%) of participants. All participants 136(100%) volunteered to a heterosexual orientation while none admitted to intravenous drug usage.

Seroprevalence of HBeAg among participants

A prevalence rate of 39(28.7%) for Hepatitis B e antigen was established among subjects, while the remaining 97(71.3%) were negative for the antigen.

Distribution of Alanine aminotransferase levels

The mean, median and range of ALT levels were 34.5, 18 and 4-412 IU/ml respectively while the standard deviation was 52.20 IU/ml. In the study, 115(84.6%) had ALT values within the reference range while 2(1.5%) of subjects had mildly elevated values above the reference range and up to 19(14%) had markedly elevated ALT levels (more than 1.5 times higher than the upper limit of the reference range).

Association of ALT levels and sociodemographic characteristics

As shown in Table 3, ALT levels were elevated in only 11(10.5%) of those aged below 45 years and in 10(32.3%) of those above 45 years. In the same vein 6(17.6%) of the females had elevated ALT while 15(14.7%) of the males had elevated levels of ALT. Marital status relative to raised ALT levels showed 21(20.6%) of those who had ever been married had elevated levels of ALT while none of those who were single showed elevated ALT levels (X^2 = 8.27, P value=0.001).

Association of ALT levels and clinic-laboratory parameters

As shown in Table 4, ALT levels were elevated in 8(20.5%) of subjects who were HBeAgpositive, as opposed to only13(13.45%) of HBeAg negative subjects with elevated ALT levels, however the difference was not statistically significant and hence no strong association was found between HBeAg status and raised ALT levels(X^2=1.077, P-value=0.216).

There was no statistically significant association between raised ALT levels and HIV status, HCV status or previous episode of blood transfusion.

Distribution of HBV DNA levels

The mean, median and range of HBV DNA quantities were 37,094,093, 9450 and 52-360,000,000 copies/ml respectively. The standard deviation was 92,085,251 copies/ml. Overall, 116(85.3%) had detectable levels of HBV DNA while the remaining 20(14.7%) subjects had HBV DNA levels below the lower limit of detection (50 copies/ml).

Association of HBV DNA and sociodemographic characteristics

In terms of age, HsBV DNA levels were above 10,000copies/ml in 54(56.2%) of subjects below 45 years, while a higher percentage 62.5% (25) of those aged 45 years and above demonstrated high levels of HBV DNA above 10,000 copies/ml. However, this difference observed was not statistically significant. (X^2=0.169, P-value=0.422).

There was also no statistically significant association found between HBV DNA levels and other sociodemographic characteristics

Association of HBV DNA levels and some clinic-laboratory parameters

Among subjects who were HBeAg positive, 25(64.1%) had HBV DNA levels above 10,000 copies/ml compared with 54(55.7%) out of those who were negative for the antigen. However, the difference observed was not statistically significant. (X2=0.812, P-value= 0.24)

Also, there was no statistically significant association found between HBV DNA levels and HIV status, HCV status or previous blood transfusion.

Bivariate linear regression analysis for ALT AND HBV DNA levels

The obtained values for ALT and HBV DNA levels were subjected to a bivariate linear regression analysis to test the association between HBV DNA levels (independent variable) and ALT levels among subjects. The calculated regression coefficient R was 0.05 suggesting a near absent association that was not statistically significant.

Table I: Frequency distribution of subjects based on their sociodemographic characteristics

Baseline Characteristic		Frequency n (%) Total N=136
Sex	Male	102 (75)
	Female	34 (25)
Age (Yrs)	18-44	105 (77.2)
	45-64	29 (21.3)
	65 and above	2 (1.5)
Ethnicity	Hausa	108 (79.4)
	Fulani	8 (5.9)
	Yoruba	5 (3.7)
	Igbo	3 (2.2)
	Others	12 (8.8)
Marital status	Married	102 (75)
	Single	33 (24.3)
	Divorced	1 (0.7)
Education	Tertiary	92 (67.6)
	Secondary	23 (16.9)
	Primary	19 (14)
	Informal/none	2 (1.5)
Occupation	Civil servant	38 (27.9)
	Business person	25 (18.4)
	Medical personnel	6 (4.4)
	Others	35 (25.7)
Cigarrete smoking	No	124 (91.2)
	Yes	12 (8.8)
Drinks alcohol	No	133 (97.8)
	Yes	3 (2.2)
IVDU		0 (0.0)
MSM		0 (0.0)
Heterosexual		136 (100)

Table II: Frequency distribution of participants based on clinico-laboratory parameters

Baseline Characteristics		Frequency n (%) Total N=136	ALT range IU/ml	HBV DNA range copies/ml
Asymptomatic		110(80.9)	4-65	52- 7.91x10^7
Mildly symptomatic		19 (14)	12-72	395- 219,000,000
Markedly symptomatic		7(5.1)	92- 412	220,000
HIV Status	**Negative**	134(98.5%)	12- 113	41,800- 2.3x 10^7
	Positive	2(1.5%)	35- 204	28,120- 80,200
HCV Status	**Negative**	132(97.1%)	21-65	3350- 50,200
	Positive	4(2.9%)	27-148	28,120- 80,200
Previous Blood Transfusion	**No**	117(86%)	17-45	22,120- 3x 10^7
	Yes	19(14%)	9-81	418- 1.97x 106
HBeAg	**Negative**	97(79.3%)	10-65	81,500- 1.34x10^8
	Positive	39(28.7%)	14-79	3350- 5.05x10^7

Table III: ALT levels and its association with baseline socio-demographic characteristics

Baseline Characteristic	ALT IU/ml n(%) N=136 0-40	>40	X^2	P value
Age(yrs) <45	94(89.5)	10(10.5)	**8.697**	**0.006***
≥45	29(78.5)	11(27.5)		
Gender Male	87(85.3)	15(14.7)	**0.169**	**0.433**
Female	28(82.4)	6(17.6)		
Ethnicity Hausa	92(85.2)	16(14.8)	**1.58**	**0.443**
Others	23(82.1)	5(17.9)		
Marital Status				
Ever Married	81(79.4)	21(20.6)	**8.27**	**0.001***
Never Married	34(100)	0(0)		
Cigerrete Smoking No	105(84.7)	19(15.3)	**0.015**	**0,584**
Yes	10(83.3)	2(20.6)		
Drinks Alcohol No	112(84.2)	21(15.8)	**0.56**	**0.602**
Yes	3(100)	0(0)		

*Statistically significant by Fischers exact test.
ALT reference range is 6-40 IU/ml

Table IV: ALT levels and its association with some clinico-laboratory parameters

Baseline Parameter		ALT IU/ml n(%) N=136 0-40	≥40	X^2	P value
HIV Status	**Negative**	114(85.1)	20(14.9)	**1.857**	**0.286**
	Positive	1(50)	1(50)		
HCV Status	**Negative**	113(54.6)	19(15.4)	**3.77**	**0.113**
	Positive	2(50)	2(50)		
Previous Blood Transfusion					
	No	99(84.2)	18(15.4)	**0.002**	**0.595**
	Yes	16(84.6)	3(15.8)		
HBeAg	**Negative**	84(86.8)	13(13.4)	**1.077**	**0.216**
	Positive	31(79.5)	8(20.5)		

ALT reference range is 6-40 IU/ml

Table V: HBV DNA levels and their association with baseline sociodemographic parameters

Baseline Characteristics		HBV DNA copies/ml n(%) N=136 <10,000	≥10,000	X^2	P-value
Age(Yrs)	**<45**	45(42.9)	60(57.1)	**0.169**	**0.422**
	≥45	12(38.5)	19(61.3)		
Gender	**Male**	41(40.2)	61(59.8)	**0.493**	**0.307**
	Female	16(57.1)	18(52.9)		
Ethnicity	**Hausa**	41(38)	67(62%)	**3.36**	**0.053**
	Others	16(57.1)	12(42.9)		
Marital Status					
Ever Married		46(45.1)	56(54.9)	**1.701**	**0.135**
Never Married		11(32.4)	23(67.6)		
Smokes cigarette	**No**	53(42.7)	71(59.4)	**0.398**	**0.378**
	Yes	4(33.3)	8(66.6)		
Drinks alcohol	**No**	54(40.6)	79(59.4)	**4.252**	**0.071**
	Yes	3(100)	0(0)		

Table VI: HBV DNA levels and their association with clinico-laboratory parameters

Baseline parameter		HBV/DNA copies/ml n(%) N=136 <10,000	≥10,000	X^2	P-value
HIV Status	**Negative**	57(42.5)	77(57.5)	**1.465**	**0.336**
	Positive	0(0)	2(100)		
HCV Status	**Negative**	56(43.2)	76(56.8)	**2.974**	**0.11**
	Positive	0(0)	4(100)		
Previous blood transfusion					
	No	48(41)	69(59)	**0.27**	**0.391**
	Yes	9(47.4)	10(52.6)		
HBeAg	**Negative**	43(44.7)	54(55.7)	**0.812**	**0.24**
	Positive	14(35.9)	25(64.1)		

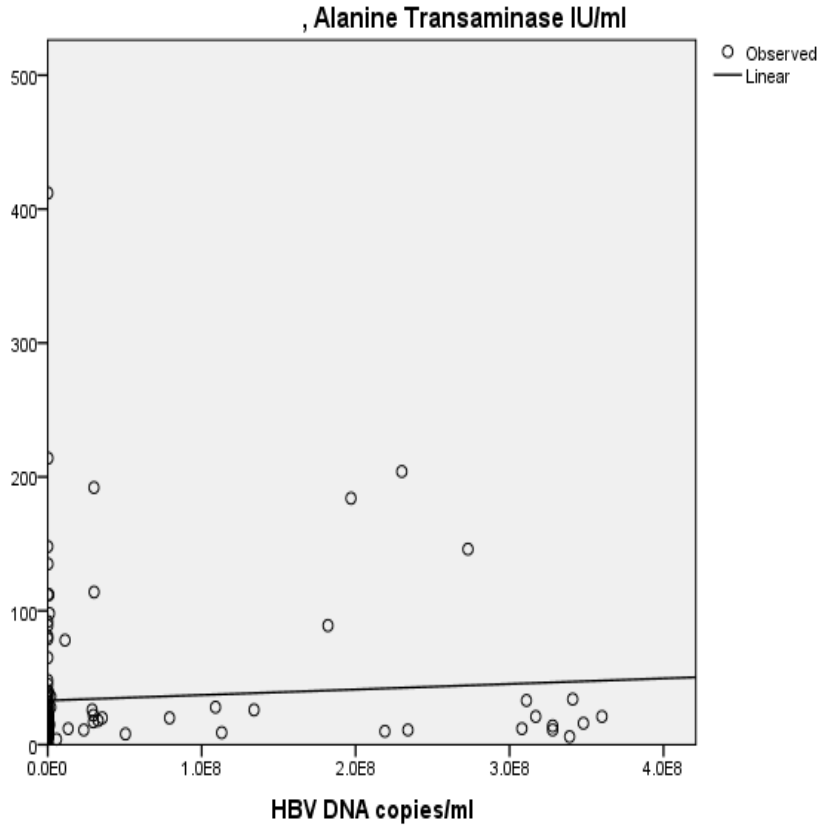

Figure I: Bivariate linear regression analysis plot

Discussion

In this study, among 136 subjects with chronic HBV infection, the proportion of males was significantly higher than females (3:1). Similar finding is documented in previous studies; Iregbu *et al.*[7] in Abuja (2:1), Ndububa *et al.*[8] in Ile-Ife (3:1 in asymptomatic patients), Akinbami *et al.*[9] in Lagos (97.8% males). Okwuraiwe *et al.*[10] reported a ratio of 2.9:1 and suggested it could be due to increased financial resources available to males to access medical care. Another reason could be the socio-cultural and religious practice in our study area where most women stay home taking care of the family while the men often engage in occupations, businesses, schooling and pre-employment blood test in the course of which they are opportunistically screened for the infection.

Majority of subjects were under 45 years of age (mean age of 33±15 years), this is comparable to the study in Lagos,[9] which reported highest prevalence of chronic HBV infection within the 30-39 years age group, Ndububa *et al*[8]in Ile-Ife reported mean age of 29.82 years in asymptomatic patients as well as Lesi *et al.*[11] who also documented an average age of 44.1 years in patients with chronic liver diseases. Similarly, Mendy *et al.*[12]reported from Gambia that majority of subjects in their study were below 45 years. This can be attributed to the established fact that most cases (80%) of hepatitis B infection in Africa are acquired early in life.[10]

Most of the subjects (84.5%) had formal education, with the dominant occupational categories being civil servants and students (27.9% and 23.5% respectively). This can be explained by required pre-employment and pre-school admission health screening in most formal institutions in the country leading to incidental discovery of the hepatitis B infection in these patients. In this study, only 4.4% of subjects were healthcare workers, this low percentage could be due to improved compliance with hepatitis B vaccination and practice of standard precautions among health personnel in our centre. This contrast with a high infection rate of 39% reported by Olubuyide *et al.*[13] in Lagos, among their Surgeons and Dentists. They speculated that this could be due to low vaccination and poor practice of standard precautions.

The finding of 14% of subjects with previous history of blood transfusion is significant because it is an established risk factor for acquisition of the hepatitis B virus. Akinbanmi *et al.*[9] reported 31.1% subjects with history of previous blood transfusion. Multimer *et al.*[14] reported that blood transfusion clearly increased the risk of HBV infection in their study as shown by significantly higher markers of the infection in subjects who had been transfused. Oluyinka *et al.*[15] reported a high frequency (17%) of occult hepatis B infection among blood donors in western Nigeria, these subjects had no detectable HBsAg with positive HBV DNA confirmed by nested PCR, the authors recommended that potential blood donors in Nigeria should be pre-tested for occult infection by nucleic acid testing (NAT) and/or anti HBc to minimize risk of HBV transmission.

None of the subjects volunteered to a homosexual orientation or intravenous drug usage. However, this cannot be ruled out due to the sensitive nature of such information. This is similar to Akinbami *et al.*[9] in Lagos who reported that all participants were heterosexual, while 4.1% admitted to intravenous drug usage.

A frequency of only 1.5% was found in our study for co-infection with HIV and HBV among our subjects and a rate of 2.9% for hepatitis B and C co-infection. Although reports from Jos[16] and Gombe[17] showed a higher frequency of hepatitis B and HIV co-infection (11.8% and 26.5% respectively), while Idoko *et al.*[18] reported a frequency of 16.7% in Jos, this disparity was probably due to the fact that in our centre, cases of HIV and HBV co-infection are managed by physicians at a dedicated Antiretroviral therapy (ART) centre rather than the gastroenterology clinic where our study subjects were co-opted. Unfortunately, approval to include patients from this special clinic was not obtained.

Cigarette smoking and ingestion of alcohol are

important risk factors for disease progression and long-term complications of HBV infection. Among the study subjects, 8.8% admitted cigarette smoking and 2.2% to ingestion of alcoholic beverages. Trichopoulos *et al.*[19] reported in a European cohort study that among 115 subjects with chronic HBV infection who developed HCC, both alcohol ingestion and smoking were identified as important risk factors for malignancy (O.R of 1.77 and 1.90 respectively).

Majority of subjects (81%) were asymptomatic, while 14% had general nonspecific symptoms of poor appetite, body weakness and low-grade fever, these can also be caused by other common illnesses like malaria and respiratory tract infections. However, 5% had complaints related to overt liver disease such as jaundice, leg swelling, abdominal swelling and bleeding diasthesis. This is similar to the report by Akinbami *et al*.[9] of 5.2% symptomatic. In contrast 50/70 of biopsy proven subjects in the Ile-Ife study[8] were symptomatic and up to 66% of subjects in Lagos, Lesi *et al*.[11]was symptomatic. Both the Lagos and Ile-Ife researches mostly studied patients who had established liver disease.

HBeAg is considered an important biomarker for viral replication and infectivity. In the index study, the prevalence rate of HBeAg seropositivity among participants was found to be 28.7%, however a lower prevalence rate of 19.2% was reported by Forbi *et al.*[16] in Keffi/Abuja, 8.6% by Ijeoma *et al*[21]in Enugu, 10.8% in Ibadan (Otegbayo *et al*),[22] 8.2% by Akinbami *et al.*[9](Lagos), while 8.3% was reported from Turkey,[23] 37.9% from Brazil,[4]and 13.6% by Sagnelli *et al.* This disparity in prevalence rates could be explained by variability in immunological responses to the virus in different populations.

Among the study subjects, 14.7% had undetectable HBV DNA levels which is similar to 9.3% reported by Iregbu *el al*.[7]in Abuja, Nigeria. The mean and median HBV DNA level in the study population were found to be 3.7 X 10^7 and 9405 copies per ml which is comparable to Koyuncuer *et al.*[23]who reported a mean value of 4.45x 10^7 copies/ml in Turkey and a median HBV DNA of 10,599 copies/ml reported in Lagos Nigeria.[10]

In accordance with widely quoted guidelines that proposed a threshold of below 10,000 copies/ml to define inactive disease. 58.1% of participants had HBV DNA levels above 10,000 copies/ml, in contrast with only 33.1% reported in Abuja Nigeria,[7] while 32.4% was reported by Nita *et al*.[4] in Brazil. This is important in view of its utilization as criterion for instituting chemotherapy in these patients due to higher risk of disease progression. Chen *et al.*[25] reported in the REVEAL-HBV study that serum HBV DNA level of ≥10,000 copies/ml is a major risk factor for hepatocellular carcinoma irrespective of HBeAg status, ALT level or presence of cirrhosis on histology. However, none of our subjects were evaluated for HCC.

There were notable variations between proportions of participants with elevated HBV DNA levels among categories of variables analyzed including; age, gender, marital status, ethnicity, HIV status, HCV co infection, previous blood transfusion, cigarette smoking and ingestion of alcohol but the differences observed were not statistically significant. This is similar to findings reported by Mendy *et al*.[12] from the Gambia.

The mean and median of ALT values in the study were 34.50±52.29 and 18 IU/ml respectively. Similarly, a study in Turkey[23] reported a mean ALT level of 32.6±21 IU/ml in similar category of patients, and by implication, majority of subjects in these studies had ALT values within the reference range of 6-40IU/ml. However, Nita *et al*.[4] in Brazil reported an elevated mean and median values of 67.7±201.8 and 34 IU/ml respectively in patients being evaluated for chronic hepatitis B infection majority of which had evidence of established liver damage.

An overall 14% of participants had significantly elevated ALT levels (i.e 1.5 above times the `upper limit of normal), while a much higher

percentage of 46.1% was reported from Brazil[4] using the same cut-off value. This difference is likely due to the fact that the participants in the index study were mostly asymptomatic carriers who were diagnosed with the infection incidentally as part of routine pre-employment or in some instances pre-school admission health screening.

Analysis of factors that might to be associated with ALT levels showed that marital status (never married) and younger age <45 years are significantly associated with lower ALT levels. This finding is likely a function of the fact that at younger ages the participants are in earlier phases of the chronic infection in comparison to older age group who might have had longer exposure to the virus thus having have higher chances of disease progression and liver related long term complications. Although there were some differences in proportions of ALT levels among different categories of the following variables; age, gender, history of previous blood transfusion, HIV status, HCV co infection, cigarette smoking and ingestion of alcohol, however there were no statistically strong associations found between them and elevated ALT levels. The HBeAg seropositive group appears to have higher proportions of participants with elevated levels of both ALT and HBV DNA when compared with the HBeAg negative group, although the difference observed was not statistically significant. In comparison, Nita *et al*.[4] reported that HBeAg positive patients had significantly higher ALT and HBV DNA levels compared to HBeAg negative patients (p value <0.0001 for both comparisons)

Conclusion

HBV DNA level is an important biomarker of disease progression in patients with chronic hepatitis B infection. It is imperative that physicians managing these patients use it periodically to monitor disease progression and select patients that will benefit most from available therapeutic interventions. However, access to HBV DNA assay needs to be increased in the wider Nigerian population to improve quality of care in these patients.

Ethical consideration

Ethical approval was obtained from the Health Research Ethics Committee and informed consent/assent from all participants before enrolment.

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