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Evaluation of Hepatitis B viral Serological Patterns and HBV DNA levels in Hepatitis B subjects at Nauth Nnewi, Southeastern Nigeria

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ABSTRACT

Nigeria is one of the countries with the highest incidence of Hepatitis B Virus (HBV) infection worldwide. The inaccessibility and unaffordability of HBV DNA quantification (viral load) assay which is the key laboratory test for therapy initiation, and monitoring is a challenge to HBV management. This study aimed at evaluating the HBV DNA viral load differences across the serological markers of HBV in order to develop a more cost-effective diagnostic algorithm for Hepatitis B management. Cross sectional study design was used with a total of 264 subjects comprising of 88 HBsAg seropositive treatment naïve subjects, 88 HBsAg seropositive subjects on antiviral therapy as case subjects and 88 age-matched apparently healthy HBsAg seronegative individuals were recruited as control subjects. Hepatitis B Virus DNA assay was performed using real time PCR technique, Hepatitis B core Antibody Immunoglobulin M and Hepatitis D Virus Immunoglobulin G assay. Immunochromatography was used for HBV Panel, Hepatitis C Virus assay and Human Immunodeficiency Virus testing. The non-treatment group has higher viral load (M=805.50 IU/ml) compared with treatment group (M = 65.50 IU/ml) (p<0.001). There was a significant difference in the HBV DNA levels of the four serological patterns observed in the study (P< 0.001). Among the four serological patterns observed, the second pattern with positive surface antibody, positive envelope antigen and negative envelope antibody showed highest viral load (M= 46850189.50 IU/ml) followed by the third pattern with negative surface antibody, negative envelope antigen, and negative envelope antibody (M=46555 IU/ml). The first pattern which has negative surface antibody, negative envelope antigen and positive envelope antibody has the lowest viral load (M=21.00 IU/ml) followed by the fourth pattern with positive surface antibody, negative envelope antigen and positive envelope antibody (M=493.00 IU/ml). This study showed that HBV serological markers can predict viral load and should be used as its alternative in resource poor settings.

Keywords

Hepatitis B Viral DNA, Hepatitis B Surface Antigen, Hepatitis B Surface Antibody, Hepatitis B envelope Antigen, Hepatitis B envelope Antibody, Hepatitis B core Antibody.

Introduction

Hepatitis B is an infectious disease of great public health importance caused by hepatitis B virus (HBV). It is an enveloped DNA virus that infects the liver and causes hepatocellular necrosis and inflammation [1]. This virus belongs to the family hepadnaviridae and genius orthohepatodnavirus and it is the only hepadnavirus that causes infection in humans [2]. It is 100 times more infectious than the most dreaded human immunodeficiency virus Infection and 10 times more infectious than hepatitis C virus [3]. Hepatitis B virus is one of several viruses known to cause viral hepatitis and continues to be the major cause of viral hepatitis in the developing and underdeveloped world. In addition to causing chronic liver disease and cirrhosis, it has a formidable record of accomplishment of being linked to primary hepatocellular carcinoma. It is estimated that HBV and HCV are the root cause of about 80% of all hepatocellular carcinomas (HCC) by promoting cirrhosis, which significantly reduced the life expectancy of the infected patients [4].

About 257 million people are chronically infected annually and about 2 in 3 people with Hepatitis B do not know they are infected [5]. Recent statistics indicate that not less than 23 million Nigerians are estimated to be infected with the HBV, making Nigeria one of the countries with the highest incidence of HBV infection in the world [6]. A national study done in Nigeria in 2016 shows a prevalence rate of 12.4 percent [7]. This worldwide burden of hepatitis B mandates accurate and timely diagnosis of patients infected with HBV and the use of treatment strategies derived from evidence-based guidelines. Most hepatitis B patients are asymptomatic in the early stage as specific clinical symptoms often occur at advanced disease stages, which are usually irreversible. Hence, the prognosis of the infection to liver disease is very crucial. The presence of derangement in specific laboratory analytes at the early stage of infection may signal a risk of fibrosis, cirrhosis and ultimately HCC.

The diagnosis of HBV is not only imperative but also complex because of different viral antigens, which bring about varying serological profiles in different stages of the disease [8]. The ability of HBV to induce chronic hepatic inflammation gives rise to these intricate serological profiles. Serological markers are used routinely as diagnostic and prognostic indicators of acute and chronic HBV infection [9].

Hepatitis B Virus DNA quantification has been in use for diagnosis and monitoring of patients who are being treated for chronic hepatitis B (CHB). This diagnostic method is molecular-based and expensive; thus, less complex and cheaper laboratory tests as surrogate diagnostic markers might simplify hepatitis B management [4]. Serological markers of hepatitis B – HBsAg, HBsAb, HBeAg, HBeAb and HBcAb, could show some relationship with HBV DNA quantification. Establishing this relationship might simplify and reduce the cost of hepatitis B diagnosis and management.

The study was done to determine the relationship between HBV DNA and HBV Serological markers in hepatitis B subjects at NAUTH Nnewi.

Materials and Methods

Study Area

The study was carried out at the Gastroenterology unit of Nnamdi Azikiwe University Teaching Hospital (NAUTH), a tertiary institution in South Eastern Nigeria, which is a referral center for Hepatitis B care in Nigeria. NAUTH, Nnewi provides a wide range of Medical, Surgical, Diagnostic, outpatient, rehabilitative and support services to a catchment population of about 30,994,559. It has a total bed capacity of 368 with 8 wards: female medical, female surgical, male medical, male surgical, pediatrics ward, obstetrics ward, accident and emergency ward and children emergency ward. It also has a functional Accident and Emergency Unit and provides 24-hour emergency services all year round. The multi-disciplinary approach to service plays an important role in the development and delivery of the Health services [10]. NAUTH serves patients of high, moderate and low socio-economic status.

On the other hand, Nnewi has an area dimension of 72 km^2 and an approximate population of 155,443 (77,517 males and 77,926 females) with average population density of 2159 people per km^2. Igbo language is the vernacular though English is widely spoken. There are about 64 registered hospitals at Nnewi, 2 missionary hospitals, 1 tertiary (Nnamdi Azikiwe University Teaching Hospital, NAUTH) and 24 primary health centers [11].

Study Design

Cross sectional study design was used in the study. A total of 264 subjects (in the ratio of 5 males: 4 females) were recruited which comprised of 88 HBsAg seropositive treatment naïve subjects, 88 HBsAg seropositive subjects on antiviral therapy (Tenofovir 300mg daily/Entecavir 0.5mg daily or Pegylated interferons 180µg weekly) as case subjects and 88 age-matched apparently healthy HBsAg seronegative individuals were recruited as control subjects.

Sample Size Determination

Using G* Power software version 3.0.10, power analysis for a one-way ANOVA with three groups was conducted to determine a sufficient sample size using an alpha of 0.05, a power of 0.96, and a medium effect size (f=0.25). Based on the aforementioned assumptions, the calculated sample size of 264, with 88 subjects per group has 96% power to detect a difference of 0.25 at significance level of 0.05 [12].

Sampling Technique

Purposive sampling technique was employed in selecting the participants based on the inclusion criteria. Patients that gave their consent who also met the selection criteria were recruited as they come to the clinic until the sample size was completed.

Sample Collection

After obtaining informed consent, 10ml of venous blood was collected from the fore arm of each subjects using a disposable syringe; 7ml was dispensed to a sterile K2-EDTA vacutainer (1.2mg/ml concentration) for hepatitis B DNA Viral load, hepatitis B panel, complete blood count, retroviral screening (RVS) and anti HCV screening while 3ml was added to a sterile plain container for HBsAg quantitation, HBcAb IgM titer quantitation, HDV IgG assay, Gamma Glutamyl Transferase (GGT) and liver function tests. The plasma and serum samples were separated into sterile plastic containers and preserved at –86°C.

Ethical Approval

Ethical approval was sought and obtained from the Ethics Committee of Nnamdi Azikiwe University Teaching Hospital Nnewi before the commencement of this study (Reference Number: NAUTH/CS/66/VOL.10/187/2017/096).

Inclusion Criteria

Participants included in this study were hepatitis B seropositive subjects aged 18 to 65 years attending Gastroenterology Clinic of NAUTH, Nnewi and apparently healthy HBsAg seronegative individuals (NAUTH staff, students and others) who gave their consent.

Exclusion Criteria

Those patients who were co-infected with HIV, HDV and HCV including HBsAg seronegative subjects who have received HBV vaccination were excluded from the study with those less than 18 years and above 65 years.

Laboratory Diagnosis

HBV DNA Viral Load using Cobas Ampliprep/Taqman Real Time PCR Technique (Roche Molecular System Inc, USA as described by Ireogbu and Nwajiobi-Princewill (2016) [1].

Principle

Molecular biology technique based on polymerase chain reaction (PCR) for simultaneously amplification and quantification of target HBV DNA. The test permits automated specimen preparation followed by automated PCR amplification and detection of HBV target DNA and HBV Quantitation Standard (QS) DNA. The HBV DNA in the plasma sample is extracted in the Cobas Ampliprep using Magnetic Glass Particle technology, which is based on the affinity of DNA for silica gel-covered magnetic beads. The extracted HBV DNA is transferred to Taqman analyzer alongside Master Mix reagent, which contains primer pairs and probes specific for both HBV DNA and HBV QS DNA. The detection of amplified DNA is performed using a target-specific and a QS-specific dual-labeled oligonucleotide probes that permit independent identification of HBV amplicon and HBV QS amplicon. The quantitation of HBV viral DNA is performed using the HBV QS added to each specimen at a known copy number and is a non-infectious DNA construct that contains HBV sequences with identical primer binding sites as the HBV target DNA and a unique probe binding region that allows HBV QS amplicon to be distinguished from HBV amplicon. The COBAS TaqMan Analyzer calculates the HBV DNA concentration in the test specimens by comparing the HBV signal to the HBV QS signal for each specimen and control.

Procedure

The frozen plasma was placed at room temperature until completely thawed before use. The High Positive, Low Positive and Negative controls were removed from 2-8°C storage and brought to room temperature before use. All reagent cassettes were removed from 2-8°C storage loaded immediately onto the COBAS AmpliPrep Instrument and allowed to equilibrate to ambient temperature on the instrument for at least 30 minutes before the first specimen was processed. The appropriate number of reagent cassette racks, sample racks with Input S-tubes, SPU racks, K-tip racks, K-tube racks and K-carriers on K-carrier racks were loaded onto the respective rack positions of the COBAS AmpliPrep Instrument. Sample rack was prepared by attaching a barcode label clip to each sample rack position where a specimen (S-tube) was to be placed. The specific barcode label clips for the controls were attached to each sample rack position where the controls (S-tube) were to be placed. One Input S-tube was placed into each position containing a barcode label clip.

The Amplilink software was used to create specimen orders for each specimen and control in the Orders window Sample folder and HBV test definition file was selected and saved. The specimen and controls were vortexed for 5 seconds and 650 µL added to the S-tubes. The tubes were then inserted in the sample rack and loaded to the Ampliprep. The Amplilink software was used to start the COBAS Ampliprep. At the completion of the COBAS TaqMan Analyzer run, the Results Report was validated and printed [13].

Determination of Hepatitis B serological Markers (HBV Panel Test) using The OnSite HBV Combo Rapid Test Kit (CTK Biotech, Inc. San Diego, CA, USA as described by Magnius and Espmark 1972) [14].

Principle

This is a lateral flow chromatographic immunoassay for the qualitative detection of HBsAg, HBsAb, HBeAg, HBeAb, and HBcAb in human serum or plasma. The technique measures HBsAg, HBeAg in serum with dual-antibody sandwich method, and measures HBsAb with dual-antigen sandwich method, and measures HBeAb and HBcAb with neutralization competitive inhibition method.

Procedure

The test board and testing samples were brought to room temperature. When ready to test, the pouch was opened at the notch and the test device removed and placed on a clean, flat surface and labelled with specimen's identification number. The right side of the test board was kept horizontally from left to right- HBsAg, HBsAb, HBeAg, HBeAb HBcAb and pipette dropper was used to add 70µl of plasma sample into the 5 sample wells avoiding air bubbles. Timer was set immediately the sample was added and the result was read at 15mins.

Result Interpretation for HBsAg, HBsAb and HBeAg (Sandwich method)

The test was interpreted as positive when purple bands appeared on both the control (C) window and the test (T) window (regardless of the colour intensity at the test window). The test was negative when one purple band appeared on the control window of the strip and no purple band appeared on the test window. When there was no purple band on the control window even when a purple band appeared on the test window, the test was interpreted as invalid and was repeated.

Result Interpretation for HBeAb and HBcAb (Competition method)

The test was interpreted as negative when purple bars appeared on both the control (C) window and the test (T) window. It was positive when one purple bar appeared on the control window and no purple bar appeared on the test window. The test was invalid when there was no purple bar in the control window and even when a purple bar appeared on the test window, the result was interpreted as invalid and was repeated.

Statistical Analysis

Data obtained were analyzed using Statistical Package for Social Sciences (SPSS) version 20) software. Data were expressed as mean ± SD and median. The significance of differences in mean values among groups were analyzed using one-way ANOVA for normally distributed variables, while Kruskall Wallis was used to analyze the significant differences in median values among different groups for variables not normally distributed. Mann-Whitney was also used to analyses significant differences between groups. Spearman's correlation coefficient was used to assess the levels of relationship between two variables. Regression analysis and receiver Operators curve were also used appropriately. The level of significance was considered at $p < 0.05$.

Results

Frequency of Pattern of HBV Serological Markers among Hepatitis B Subjects.

Table 1 shows the frequency distribution of HBV serological patterns observed in the study. Pattern 1-HBsAg+ve HBsAb-ve HBeAg-ve HBeAb+ve HBcAb+ve had the highest number of occurrences with frequency of 118 out of 264(45%), Pattern 2-HBsAg+ve HBsAb-ve HBeAg+ve HBeAb-ve HBcAb+ve had a frequency of 73 with 27.69% of occurrence. Pattern 3- HbsAg+ve HBsAb-ve HBeAg-ve HBeAb-ve HBcAb+ve had a frequency of 56 with 21.16% of occurrence. Pattern4- HBsAg+ve HBsAb+ve HBeAg-ve HBeAb+ve HBcAb+ve had a frequency of 17 with 6.15% of occurrence.

Table 1: Frequency of HBV Serological Markers among HBV seropositive Subjects.

SN	Serological Patterns Observed	Frequency (% Occurrence)
1	HBsAgpos HBsAbneg HBeAgneg HBeAbpos HBcAbpos	118(45%)
2	HBsAgpos HBsAbneg HBeAgpos HBeAbneg HBcAbpos	73(27.69%)
3	HBsAgpos HBsAbneg HBeAgneg HBeAbneg HBcAbpos	56(21.2%)
4	HBsAgpos HBsAbpos HBeAgneg HBeAbpos HBcAbpos	17(6.1%)

Key: Pos = positive, Neg = negative.

Viral Load in Different Serological Patterns

Table 2 shows comparison of median values for viral load in different serological patterns. There was a significant difference in the median values of Viral load among all the serological patterns presented ($P < 0.005$). Multiple comparison also revealed that the median value of Viral load in Pattern 1(21.00) is significantly lower compared with Patterns 2, 3 and 4 (46850189.50, 46555 and 493.00 respectively ($P < 0.05$).The median value for Pattern 3 (46555) is significantly higher compared with pattern 4 (493) and significantly lower compared with Pattern 2 (46850189.50). Also, the median value for pattern 2 is significantly higher compared with Pattern 4 ($P < 0.005$).

Table 2: Viral Load in Different Serological Patterns.

	Median Viral load (IU/ml)	Multiple Comparison	Mann-Whitney U test	*P -Value*
Pattern 1	21.00	Pattern 1vs 2	1056.0	0.000*
Pattern 2	46850189.50	1 vs 4	370.50	0.021*
Pattern 3	46555.00	1 vs 3	211.50	0.000*
Pattern 4	493.00	2 vs 3	1098.0	0.000*
Kriskal Wallis	135.544	2 vs 4	355.0	0.000*
P-Value	0.000*	3 vs 4	69.000	0.000*

Key: Pattern 1 = HBsAg positive HBsAb negative HBeAg negative HBeAb positive HBcAb positive; Pattern 2 = HBsAg positive HBsAb negative HBeAg positive HBeAbnegative HBcAbpositive; Pattern 3 = HBsAg positive HBsAb negative HBeAg negative HBeAbnegative HBcAb positive; Pattern 4 = HBsAg positive HBsAb positive HBeAg negative HBeAb positive HBcAb positive.

Mean Age and Sex Distribution of Test Subjects

Figure 3.3 shows the total number of female test subjects was 76 (43.2%) while their male counterparts were 100 (56.8%). The female subjects with ages less than 20 had the lowest frequency of 3 (3.9%), this was followed by those female subjects greater than 50 years of age. Those female within the age bracket of 21-30 had the highest frequency of 32 (42.9). Those females within the ages of 31-40 were second to the highest with the frequency of 20 (26.9%) while those within ages 41-50 were 13 (16.9%). The male subjects with ages less than 20 also had the lowest frequency of 4 (3.9%), this was followed by those male subjects greater than 50 years of age with frequency of 12 (11.8%). Those male subjects within the age bracket of 31-40 had the highest frequency of 42 (43.1%). Those within the ages of 21-30 were second to the highest with the frequency of 26 (25.5%) while those within ages 41-50 were 16 (15.7%).

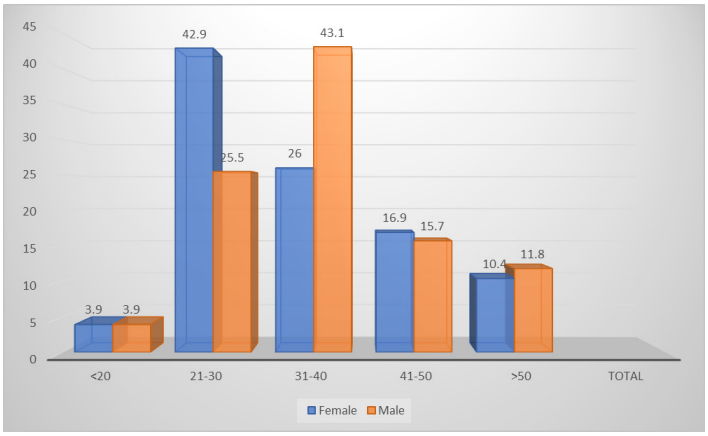

Figure 1: Mean Age and Sex Distribution of Test Subjects (N=176); X-Axis (Age in years), Y-Axis (% Frequency).

Frequency of Hepatitis B Subjects in Acute and Chronic Stage of HBV Infection

Figure 3.4 shows Hepatitis B Subjects in Acute and Chronic Stage of HBV Infection using their HBcAB IgM results. A total of 22(12.22%) HBV seropositive subjects were positive for HBcAg IgM showing that they were in acute stage while 156(86.67%) subjects were negative showing chronic stage.

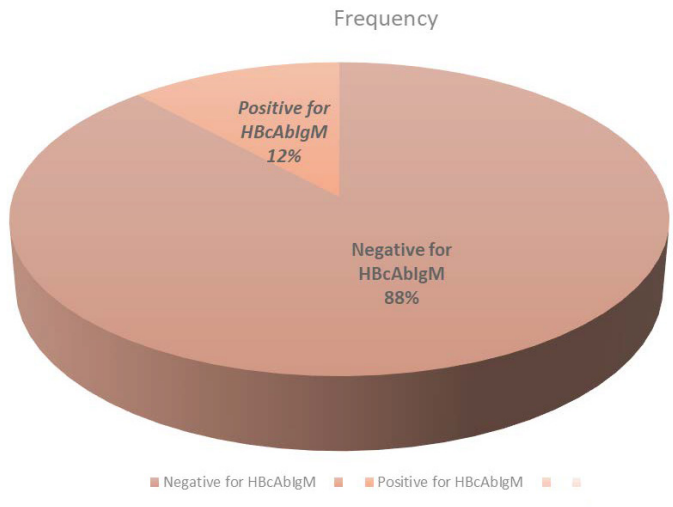

Figure 2: Frequency of HBV Seropositive Subjects in Acute and Chronic Stage of HBV Infection based on HBcAb IgM positivity.
Key: Negative = Chronic Stage, Positive = Acute Stage.

Discussion

Hepatitis B infection has five serological markers, which are HBsAg, HBsAB, HBeAg, HBeAb and HBcAb. These markers can take different patterns depending on the stage of infection as well as the viral infectivity. In the present study, four different serological patterns were observed. Interestingly, all the four patterns had significant correlation with HBV viral load. Also, viral load levels among these patterns were significantly different. Pattern number 1 which had positive HBsAg, negative HBsAb, negative HBeAg, positive HBeAb and positive HBcAb has the highest observed frequency among the four patterns (45%) and correlated with the lowest viral load level. This could be due to HBeAg seronegativity and HBeAb seropositivity observed in the group. This finding is in keeping with previous works on HBeAg negative HBV patients [1,2].

Pattern 2 with positive HBsAg, negative HBsAb, positive HBeAg, negative HBeAb and positive HBcAb, with frequency of 27.7% correlates with the highest level of viral load. This finding is in tandem with the report of Price *et al.,* [15] done in Uganda. The HBeAg positivity could be the reason for very high viral load among these subjects. Pattern 3 with positive HBsAg, negative HBsAb, negative HBeAg, negative HBeAb and positive HBcAb had a frequency of 21.2% correlated with high viral load. The high viral load observed in this pattern could be due to the presence of precore and basal core promoter mutants present in the HBeAg-ve subjects in this group. Precore mutants are variants of HBV which does not produce HBeAg while basal core promoter mutants cause reduction in HBeAg [16]. HBV infections caused by these are difficult to treat and can cause prolonged duration and higher risk of liver cirrhosis. Hence, HBV patients with this pattern should be monitored with viral load and those with high viral load should be monitored specifically for liver cirrhosis.

Pattern 4 with positive HBsAg, positive HBsAb, negative HBeAg, positive HBeAb and positive HBcA,had the lowest frequency of 6.1% and correlated with low viral load. The presence of atypical HBsAg among the group with this pattern could be the explanation to the low viral load observed among this group. This finding is in consonance with the findings of Liu *et al*. [17] who studied the clinical and virological characteristics of HBV patients with coexisting HBsAg and anti-HBs in China. HBsAb is the major neutralizing antibody protecting against infection and reflects immunity against HBV infection [18]. In this study, the patients with positive anti-HBs had lower HBV viral load, which indicate that the HBsAb could partly neutralize HBsAg and clear HBV particles in the circulation. HBV infection is characterized by slow progress and no obvious symptoms. By the time the apparent liver-related symptoms appear, the infection would have already reached the advanced stage.

This study recorded 100 percent positivity for HBsAg among the test subjects given that it was only HBV seropositive subjects that were recruited. HBsAg is the earliest serologic marker of HBV infection. The appearance of the neutralizing antibodies against HBsAg (HBsAb) generally indicates recovery and immunity to HBV infection however, in this study, 17(6.4%) of HBV, seropositive subjects with positive HBsAg were HBsAb Positive. HBsAb coexistence with HBsAg is a very rare and atypical serologic profile in patients with chronic HBV infection. Actually, this antibody detected could not be directed to the particular HBV strain causing the infection but a similar strain of the virus due to HBV mutation [19]. Hence, its presence does not confer immunity to the subjects and does not mean that HBV has ceased to replicate. This coexistence has also been reported among Chinese population by Chen *et al.,* 2018 [20] and Liu *et al.,* 2016 [17].

HBeAg is a marker of high replication and infectivity and in this study, 37(14.1%) were HBeAg seropositive. These HBeAg seropositive subjects might stand the chances of developing persistent liver disease leading to cirrhosis and even primary liver cancer in the absence of proper management. This agrees with the finding of Brunetto *et al.,* (2013) [21]. However, the 14.1 prevalence of HBeAg obtained in this study is higher than the 4.7% recorded in a previous study in 2016 in Makurdi, Nigeria. The disparity could be the smaller sample size used in the previous study (63 versus 262) and the stronger immune level of the former.

Moreover, in this study, 85.9% of the HBsAg positive subjects were HBeAg seronegative which is a marker of chronicity, however, this negativity may be because of the presence of HBeAg negative mutant variants [22]. In such individuals, the HBV is actively replicating with risk of transmission but is unable to produce HBeAg. The most frequent variant creates a stop codon in the precore region in the viral genome, which completely abolishes the production of HBeAg [23]. HBV viral load should be used in such case to check for active replication.

This study also showed 91.6% HBeAb seropositivity, which is in agreement with the 92% recorded by [22]. Seroconversion from HBeAg to anti-HBe is usually associated with serum HBV DNA undetectable level and remission of liver disease. On the contrary,

a certain proportion of anti-HBe-positive patients will continue to have HBV replication and active liver disease. These patients usually harbor precore/core promoter mutations in the HBV genome that prevent or decrease the production of HBeAg [23,15]. HBcAb had 100% positivity in the present study; HBcAg is an intracellular antigen that is not detectable in serum. Its antibody, anti-HBcore (HBcAb), indicates a prior exposure to HBV. Hence, the 100% seropositivity of HBcAb is expected since all the test subjects are HBV exposed patients.

The sex distribution of the subjects in this study showed that there were more males 100 (57.2%) males and 76 (42.8%) females. This shows that HBV infection is more prevalent in males than females and the finding is in keeping with the findings of Yewande *et al.* (2018) [24] which reported that males are 2.8 times more likely to get infected with HBV compared to their female counterparts. This could be due to the fact that males are more prone to risk factors and behaviors sexual intercourse, injection drug use, barbing et ce tera which predispose them to HBV infection. Regarding the age distribution, highest frequency of HBsAg seropositivity was found among the younger age group 21-30 followed by 31-40 group for both males and females. This agrees with the findings of Kolou *et al.,* 2017 [25] carried out among age range 20-30 years old in Lome, Togo. Also, another work done by Gheorghe *et al.,* (2013) [26] gave a similar report. Considering the modes of transmission of HBV, the high sexual activity of individuals within these age brackets might explain this high frequency. The age group <20 had the least frequency both among males and females (3.9 both). The >50 age group were the second to the least of the subjects (9% females and 12% for females). This finding is in agreement with that of Yewande *et al.,* (2018) [24]. The children (<20) and the aged (>50) are less prone to risky behaviors than the middle aged (21-30, 31-40).

The present study grouped the Hepatitis B subjects into acute and chronic infections using HBsAb IgM. Of the 176 hepatitis B subjects recruited in the study, 154 (86.6 %) had chronic infections while 22 (13.4 %) had acute infection. This is close to the finding of (Mbaawuaga *et al.,* 2013) [27] in Benue, Nigeria. The relative low number of subjects with acute infection compared with those chronically infected could be attributed to the fact that about 90 percent of primary adult HBV infections become acute, but most patients recover after a 4 to 8 week illness and only about one-third of these patients become chronic carriers. However, the acutely infected subjects if not properly managed could become chronically infected.

Conclusion

The study has developed a less complex, cost effective and reliable laboratory-testing algorithm that will go a long way to improve HBV management in resource poor settings. HBV patients should be screened first for the HBV serologic markers after which only those who presented with these patterns - positive HBsAg, negative HBsAb, positive HBeAg, negative HBeAb and positive HBcAb associated with very high viral load and positive HBsAg, negative HBsAb, negative HBeAg, negative HBeAb and positive HBcAb should proceed for HBV viral load. Those patients who present with the patterns other than the above may not need HBV viral load assay at the initial stage.

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