International Scholars Journals

International Journal of Medicine and Medical Sciences ISSN: 2167-0404 Vol. 6 (5), pp. 340-344, May, 2016. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Hepatitis B serologic markers among individuals with hepatitis B surface antigen seropositivity in Makurdi, Nigeria

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Accepted 30 April, 2016

Hepatitis B Viral DNA load determination in our environment is still difficult to attain for general diagnostic and treatment purposes. Available serologic markers of hepatitis B virus (HBV) infection are complex in interpretation. We determined the pattern of HBV serologic markers in HBV surface antigen (HBsAg) seropositive patients between June 2011 and March 2015. HBsAg seropositive patients were screened for other HBV serologic markers namely, antibody to HBsAg (HBsAb), HBV e antigen (HBeAg), antibody to HBeAg (HbeAb) and antibody to HBV core antigen (HBcAb). Liver enzymes (AST, and ALT), age, sex and complaints of subjects were also documented. Sixty-three HBsAg seropositive patients consisting of 65.1% males and 34.9% females with age ranging from 15 to 80 years were enrolled. Majority (70%) was between 20-40 years old. All patients with HBsAg seropositivity were sero-negative for HBsAb. HBeAg seropositivity was seen in 4.7% (n=3/63). Among the 8% (n= 5/63) which were seropositive for HBeAb, 2 of them were also HBeAg seropositive. HBcAb seropositivity was 95.2% (n=60/63). Serum transaminase enzymes level was normal in 80% of patients. Commonest complaint (over 80%) was upper abdominal pain. We conclude that in absence of Hepatitis B Viral DNA load determination, understanding of serological markers of HBV is important in the management of patients with chronic HBV infection.

Key words: Hepatitis B Virus, sero-markers, laboratory diagnosis, Nigeria.

INTRODUCTION

Hepatitis B virus (HBV) is the most common cause of chronic liver disease and hepatocellular carcinoma in the world today, and is a major cause of necrotizing vasculitis (Polyarteritis) (Chloe & Claudia, 2015). The complete infectious virion (Dane particle) is a 42nm spherical particle consisting of HBsAg, HBcAg, HBeAg, DNA and DNA polymerase (Chloe & Claudia, 2015). Laboratory diagnosis of HBV includes detection of HBsAg, HBsAb (anti-HBs), HBcAb (anti-HBc), HBeAg and HBeAb (anti-HBe) in the serum. Polymerase chain reaction (PCR) is useful for amplifying and quantifying serum DNA (Chloe & Claudia, 2015; Thompson & co-workers, 2010). HBsAg is the serological hallmark of HBV infection which is seen within 10 weeks in the serum following fresh exposure to HBV (Okocha *et al.,* 2012). Continued presence of HBsAg for a duration of longer than 6 months may be explained as chronic infection (Okocha *et al.,* 2012).

Antibody to Hepatitis B surface antigen (HBsAb), a neutralizing antibody, suggests the recovery and protective immunity against HBV infection. It is also detectable in response to successful hepatitis B immunization (Chen, Liang & Hu, 2012).

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On the other hand, presence of serum Hepatitis B e Ag is associated with active Hepatitis B Virus replication and transmission of infection (Mast, Weinbaum & Fiore, 2006). Remission of disease is associated with sero-conversion from HBeAg to HBeAb and serum disappearance of HBV DNA (Mast, Weinbaum & Fiore, 2006). However, patients with precore/core promoter HBV genome mutations have a continued HBV replication and active liver disease with a low production of HBeAg (Mast, Weinbaum & Fiore, 2006; Jiang –Horng, 2008). Again, mutations in polymerase gene confer drug resistance to adefovir, dipivoxilentecavir dipivoxil, entecavir and lamivudine (Jiang–Horng, 2008). Hence, some HBV mutants may pose a problem in the diagnosis, pathogenesis and therapy of HBV infection.

Although, Hepatitis B core antigen is not found in serum because it is an intracellular antigen, the serum antibody to Hepatitis B core antigen (anti-HBc or HBcAb), symbolizes an earlier contact with HBV (Jiang –Horng, 2008). In the presence of early infection by HBV, the antibody IgM anti-HBc, first appears in the serum, which is usually detected within 1 month after appearance of HBsAg (Jiang –Horng,2008; Kao, 2007). A presence of high level of HBcAb (IgM anti-HBc), strongly suggests a recent HBV infection and lasts within a duration of 6 months. However, in exacerbated chronic hepatitis B infection, a detectable level of the IgM-HBcAb is seen. Therefore, there is a need to raise the cut-off point of serum concentration of IgM anti-HBc for acute hepatitis B infection in order to differentiate it from chronic hepatitis B infection with exacerbation, especially in HBV endemic societies such as in Africa and Asia (high endemicity, defined as HBsAg greater than 7% in an adult population) (Jiang –Horng, 2008) . On the other hand, the presence of IgG anti-HBc, which is not a neutralizing antibody remains for life in both acute and chronic hepatitis B cases. Therefore, in the absence of circulating HBsAg, an isolated IgG anti-HBc in the serum, may suggest an occult HBV infection in persons positive for serum HBV DNA irrespective of other HBV serologic markers (Kao, 2007).

Hepatitis B Viral DNA load determination in our environment is still difficult to attain for general diagnostic and treatment purposes. The serum markers of hepatitis B virus (HBV) infection which are now available seem complex to interpret in the midst of mutant factors. We hereby report the pattern of serologic markers seen among individuals with HBV surface antigen (HBsAg) seropositivity in Makurdi, Nigeria for an improved diagnosis and treatment of the condition in this locality.

SUBJECTS AND METHODS

Study area

This study was conducted in Makurdi, Nigeria. Makurdi is the capital city of Benue state, Nigeria. Benue state is located within the North central geographical zone of Nigeria.

Subjects

This is a prospective study conducted in Delight Specialist Clinic and maternity, Makurdi Nigeria between June 2011 and March 2015 involving 63 patients. The inclusion criterion was patients with HBV surface antigen (HBsAg) seropositivity. A questionnaire for demographic data including, age, sex, marital status, occupation, history of blood transfusion, needle prick, previous medical history, complaints of the subjects and other variables was completed by each patient. Informed consent was obtained from each subject and each assured of maximum confidentiality.

Methods

Blood sample was obtained from each subject, centrifuged to separate the serum and analysed in the laboratory. The serum samples were coded and screened for HBV serologic markers namely, HBsAg, antibody to HBsAg (HBsAb), HBV e antigen (HBeAg), antibody to HBeAg (HBeAb) and antibody to HBV core antigen (HBcAb). HBV combination immuno-chromatographic rapid kits manufactured by Acumen Diagnostics Incorporated (Lot SAG90808; expiry date 08/2015) were used for the analyses. Manufacturer's instructions were strictly followed. Other investigations conducted included liver enzymes (AST, and ALT). Results were appropriately interpreted.

Analysis

The results were analyzed using SPSS 11.0 statistical software; chi-square (X^2) was used to compare association between proportions and P-values <0.05 was considered significant at 95.0% confidence level.

RESULTS

Table 1 showed the age and sex distribution of subjects. A total of 63 HBsAg seropositive patients consisting of 41 (65.1%) males and 22 (34.9%) females with age range from 15 years to 80 years were included in the study. Majority (69.8%) aged between 20 years and 40 years old, only about 9.5% and 20.7% were aged below 20 and above 40 years respectively.

Among the patients with HBsAg seropositivity, all (100%) were HBsAb seronegative and 3 were HBeAg seropositive. A total of 60 (95.2%) were HBcAb seropositive (Table 2).

Serum AST levels was normal in 80% of the patients. About 50% of the patients have no clinical symptoms. Among symptomatic patients; the commonest complaint

Tables 1. Age and sex distribution of subjects.

Age (years)	Male	Female	**Total (%)**
<20	4	2	**6 (9.5)**
21-30	13	10	**23 (36.5)**
31-40	16	5	**21 (33.3)**
41-50	7	3	**10 (15.9)**
>50	1	2	**3 (4.8)**
Total (%)	**41 (65.1)**	**22 (34.9)**	**63 (100)**

Table 2. Serologic pattern seen among subjects.

	HBsAg		**HBsAb**		**HBeAg**		**HBeAb**		**HBcAb**	
	Pos.	*Neg.*	*Pos.*	*Neg.*	*Pos.*	*Neg.*	*Pos.*	*Neg.*	*Pos.*	*Neg.*
Male	41	0	0	41	3	38	38	3	IgG=35,IgM=5	1
Female	22	0	0	22	0	22	20	2	IgG=18,IgM=2	2
Total (%)	63	0	0	63	3	60	58	5	60 (95.2)	3 (4.8)

(over 80%) was upper abdominal pain or heat like feelings around the right upper quadrant, followed by weakness.

DISCUSSION

The present study of HBsAg sero positive individuals in a general population in Makurdi recorded 95%(n=58/63) prevalence of HBcAb (anti-HBc) and 4.7% (n=3/63) prevalence of HBeAg. The HBeAg prevalence of 4.7% recorded in this study is higher than a 3.1% prevalence in a previous study in 2013 in Makurdi, North Central Nigeria (Odimayo, Nwokedi & Nwadioha, 2013). However, it is lower than the 8.2% prevalence in blood donor clinics across two teaching hospitals in Lagos, S.W Nigeria (Akimbami *et al.,* 2012), 8.6% reported in Enugu, S.E Nigeria (Ijeoma & co-workers,2010), and 8.8% in Benin City, South Central Nigeria (Abiodun *et al.,*1994). Lesi and co-workers recorded a much higher prevalence of 25% in subjects with HBV/HIV co-infections. It is evident therefore that only a little percent 4.7% (with serum Hepatitis B e Ag sero positivity) of the study population had active Hepatitis B Virus replication with high infection transmissibility.

The ninety-five per cent (n=60/63) prevalence of HBeAg negativity in the study marks chronicity. However, it is worthy of note that there may be presence of HBeAg negative mutant variants which have been found to be more prevalent in Asia and Far East (Chan *et al.,* 2000; Funk, Roseberg & Lok, 2007). 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 (Laras, Koskinas & Avigidis, 1998). The sero-prevalence of anti-HBe (HBeAb) in the present study was recorded as 92% (n=58/63). Seroconversion from HBeAg to anti-HBe is usually associated with serum HBV DNA undetectable by hybridization technology and remission of liver disease. Nevertheless, a certain proportion of anti-HBe-positive patients continue to have HBV replication and active liver disease (Laras, Koskinas & Avigidis 1998). These patients usually harbour precore/core promoter mutations in the HBV genome that prevent or decrease the production of HBeAg (Laras, Koskinas & Avigidis,1998). Chronic HBeAg-negative hepatitis is usually a late phase in the natural history of chronic HBV infection, rather than a result of de novo infection with a mutated variant (Akimbami *et al.,* 2012).

Hepatitis B core antigen is an intracellular antigen that is not detectable in serum. Its antibody, anti-HBcore (HBcAb), indicates a prior exposure to HBV (Chloe & Claudia, 2015). The presence of IgM anti-HBc with high index value usually indicates a recent HBV infection. However, the index value of IgM anti-HBc may increase to levels usually detectable in acute infection in 10-20% hepatitis B (Laras, Koskinas & Avigidis,1998; Chloe &

Claudia, 2015). Immunoglobulin G anti-HBcore is not a neutralizing antibody and remains detectable throughout the patient's life. It can be found in patients who have recovered from acute hepatitis B and in those with chronic HBV infection (Chloe & Claudia, 2015). In our study, we recorded 95% (n=60/63) sero prevalence of anti HBc. Immunoglobulin G fraction constituted 88% of the anti HBcore which may represent patients that recovered from acute infection and in those with chronic HBV infection, while the remaining 12% was the IgM anti-HBc may show an active or recent HBV infection. The twelve per cent sero prevalence of IgM anti-HBcore recorded in this study is comparable to 10.1% in Lagos South western Nigeria (Akimbami *et al.,* 2012), 10% in Pakistan (Sheikh & co-workers, 2011).However, a lower prevalence of 4% was reported in Italy (Lavarani *et al.,*1983) amongst HBsAg positive individuals.

A proportion of hepatitis B surface antigen negativity in HBV endemic population, may test positive to anti-HBcore, this phenomenon is described as 'Isolated' anti-HBc hepatitis. HBV infectivity in such cases of isolated hepatitis is questionable, however, HBV DNA has been detected in the serum. Therefore, combined screening for HBsAg testing along with anti-HBcore is invaluable (Antar *et al.,* 2010).

In this study, we observed a male to female ratio of 2:1 in HBsAg and anti-HBcore reactivity, in keeping with the preponderance of HBV infection globally. Various studies locally and internationally have recorded a male preponderance in individuals infected with hepatitis B virus (Ijeoma & co-workers, 2010; Abiodun *et al.,*1994; Kurien *et al.,* 2005). The age group 21-30 years and 31-40 years accounted for 69.8% of the study population. This is comparable to an Indian study with 43% of the study group in the age range 30-39 years (Kurien *et al.,* 2005). The study population had a bias for the youths as these have the potentials for HBV risk factors.

Recruitment into the study was strictly on subjects with hepatitis B surface antigen sero positivity. Hence, the systematic selection done in this study did not guarantee the detection of individuals with 'Isolated' anti-HBcore Hepatitis (ie hepatitis B surface antigen negativity with anti-HB core reactivity). Again, non-use of genotypic method excluded the population that would have measured for HBeAg negative mutant variants (these patients usually harbor precore/core promoter mutations in the HBV genome that prevent or decrease the production of HBeAg in the phase of active HBV replication with presence of detectable HBV DNA). In consideration of this phenomenon, the actual number of active HBV infection might have been underestimated in this study. In order to capture the exact number and staging of the HBV patients, there is need for viral DNA load determination.

In conclusion therefore, the HBV serologic markers screening in an HBV-endemic area in a resource poor country, such as Nigeria, is particularly important for early diagnosis and effective treatment of this condition in order to keep in check the development of advanced liver disease. In the absence of Hepatitis B Viral DNA load determination, understanding of the serological markers of HBV is particularly important in the management of patients with chronic hepatitis.

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