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PITFALLS IN DIAGNOSIS OF HEPATITIS B VIRUS INFECTION AMONG ADULTS NIGERIANS

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ABSTRACT

Objective: Hepatitis B virus infection is common in Nigerians and its diagnosis is necessary for effective treatment and eradication. This study is aimed at highlighting the serological factors jeopardizing the diagnosis and treatment of the infection among Nigerians adults.

Patients and Methods: Three studies were carried out. The first study involved 56 Nigerian adults and it compared the assay of HBsAg by Haemagulation Method (HMA) with Enzyme linked immunoassay (ELISA). The second study was a comparison of Glaxo Welcome HB rapid test(GWHB) with ELISA in sero-assay of HBsAg and HBeAg among 25 Nigerian subjects while the third study was on the assay of the sera of HBsAg positive patients for HBeAg and anti-HBe in forty two Nigerian patients by ELISA.

Results: The sero - prevalence rates of HBsAg were 41.8% and 61.8% by HM and ELISA respectively with false HBsAg sero-positives and sero-negatives by HM of 5.4% and 25.5% respectively. Similarly, there was sero-detection of HBsAg in 84% and 80% by ELISA and GWHB respectively in 25 Nigerian adults. In addition, 19% and 64% of the 42 patients with HBsAg sero-positivity were also positive for HBeAg and anti-HBe respectively, while 31% of the patients were both HBeAg and anti-HBe sero-negative.

Conclusion: Sero-diagnosis of HBsAg and other serological markers of infectivity in patients with HBV should be carried out by ELISA rather than HMA among adult Nigerians. Furthermore, high infectivity of the virus abounds among Nigerian with HBV infection.

Key Words: Pitfalls, diagnosis, HBV, Adults Nigerians, ELISA. *(Accepted 31 July 2008)*

INTRODUCTION

Hepatitis B virus occurs globally although with varying sero-prevalence rates in different regions and countries[1]. In Nigeria, just as in other African countries, it is endemic causing significant morbidity and mortality[2,3]. Although, the virus has many serological markers for its detection[4], the most commonly assayed marker is the hepatitis B surface antigen (HBsAg). However, various methods of sero-detection of HBsAg are available, each with different sensitivity, specificity, cost and technology of assay[5]. For routine diagnosis of HBV infection among Nigerians, both Haemaglutination Method (HMA) and Enzyme linked immunoassay (ELISA) are commonly utilized. These practices give rise to varying prevalence rates of the infection among the population because of the differences in the specificity and sensitivity of the diagnostic tests. A commonly used rapid test for HBsAg detection is Glaxo Welcome HB rapid test kit (GWHB) - AMRAD ICT, Australia.

Hepatitis B viral activity could also be determined by assaying hepatitis Be antigen (HBeAg), antibody to HBeAg (anti-HBe) and viral DNA. Positvity of these markers could be significant in the determination of subjects that would require antiviral therapy[6]. Values between 11.9%- 23.1% of HBeAg have been reported among Nigerian patients with chronic liver diseases[6,7] but there are no data on anti-HBe. Furthermore, scarcity of facilities for detailed assay of HBV serological markers in Nigeria could lead to the institution of antiviral therapy in some patients with chronic hepatitis B infection once they are discovered to be HBsAg sero-positive. In order to reduce these possible pitfalls in the diagnosis and treatment of HBV infection among Nigerians, we present the result of our study comparing sero-assays for HBsAg by HMA, GWHB and ELISA. In addition, we report the results of sero-analysis of HBeAg and anti-HBe in Nigerian patients that are HBsAg sero-positive by ELISA.

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MATERIALS AND METHODS

Comparison of HMA with ELISA in the sero-assay of HBsAg: Fifty-five Nigerian adult patients who consented to participate in the study had their blood and sera analyzed for HBsAg by HMA (Biotec Latex

Kit, UK) and ELISA (Wellcozyme Kit Murex, UK) at the Blood Bank of the Department of Haematology and Virology Department, University College Hospital, Ibadan, Nigeria; respectively.

Comparison of the Product # GWHB rapid test with ELISA in sero-assay of HBsAg: Twenty-five consecutive Nigerian subjects (18 males and 7 females) attending the Medical Clinic of the University College Hospital, Ibadan, Nigeria were studied. Whole blood specimens collected by capillary tube of the test kits were assayed consecutively for HBsAg and HBeAg following the manufacturer's protocol. Results were read immediately and recorded. Blood taken at the same time of assay by GWHB were also analysed for HBsAg by ELISA.

Assay of sera of HBsAg positive patients for HBeAg and anti-HBe: Forty-two Nigerian patients who were HBsAg sero-positive by ELISA had their sera tested for HBeAg and anti-HBe using ELISA technique. The ethical approval was sought and obtained from the Joint UI/UCH Ethical Review Board. Results of all the assays including calculations for sensitivity, specificity, Positive and Negative Predictive Values were documented, and the data were analysed using appropriate statistical instruments with significant difference specified at $p < 0.05$.

RESULTS

The subjects studied for assay of HBsAg by HMA and ELISA consisted of 14 healthy adults, 13 patients with chronic hepatitis and 14 patients each with liver cirrhosis and hepatocellular carcinoma. The sero prevalence rates of HBsAg in all the subjects were 41.8% and 61.8% by HMA and ELISA respectively ($x^2 = 4.41$ $P < 0.05$).

False HBsAg sero-positives and sero-negatives by HMA compared to ELISA were 5.4% and 25.5% respectively (Table I). There was no gender difference in the sero prevalence rates of HBsAg by either HMA or ELISA. Comparing sero assay of HBsAg by HMA with ELISA, HMA gave sensitivity, specificity, positive and negative predictive values of 59% , 86% , 87% and 56% respectively. This showed that sero-assay of HBsAg by ELISA was a better diagnostic method than HM (Mc Nemar test, $x^2 = 7.116$, $P < 0.01$).

There was sero-detection of HBsAg in 84% and 80% by ELISA and GWHB respectively in 25 subjects studied, Table 2. False negative result was 4% by GWHB when compared with ELISA and the sensitivity, specificity, positive and negative predictive values were 95%, 100%, 100% and 80% respectively. The HBeAg was not detected in any subject by GWHB. Table 3 shows that 19% and 64% of the 42 patients with HBsAg sero-positivity were also positive for HBeAg and anti-HBe respectively. Furthermore, 14% and 31% of the patients were Sero-positive and negative respectively for both HBeAg and anti-HBe. In addition, 69% of the patients were sero-positive for HBeAg and / or, anti-HBe. Table 4 shows that HBsAg sero-positive subjects in all the three studied subgroups had a male : female ratio of about 3:1 and their distribution spread through the different adult age groups irrespective of their genders differences.

Table 1: **Assay of HBsAg by ELISA and Haemaglutination Method in Nigerian Adults.**

	ELISA		
HMA	True	False	62%
	34	21	
Positive	True Positive	False Positive	PPV
23	20	3	87%
Negative	False Negative	True Negative	NPV
32	14	18	56%
42%	Sensitivity	Specificity	
	59%	86%	

HMA - Haemaglutination Method
ELISA -Enzyme Linked Immunoassay
PPV - Positive Predictive Value
NPV - Negative Predictive Value
HMA versus ELISA (Mc Nemar test) $X^2 = 7.186$, $p < 0.01$

Table 2: **Assay of HBsAg by GWHB and ELISA in 25 Nigerian adults patients.**

	ELISA		
GWHB	True	False	84%
	21	4	
Positive	True Positive	False Positive	PPV
20	20	0	100%
Negative	False Negative	True Negative	NPV
5	1	4	80%
80%	Sensitivity	Specificity	
	95%	100%	

ELISA- Enzyme Linked Immunoassay
GWHB - Glaxo Welcome HB rapid test
PPV- Positive Predictive Value
NPV- Negative Predictive Value

Table 3: **Assay of HBeAg and Anti-HBeAg in HBsAg Sero-positive Nigerian Adult Patients With Chronic Hepatitis B**

Serological Markers			
Sex	HBsAg+	HBeAg+	Anti-HBe+
Male	34(81)	7(17)	21(50)
Female	8(19)	1(2)	6(14)
Total	42(100)	8(19)	27(64)
Pattern of Serological Markers			
HBsAg	HBeAg	Anti-HBe	Total
+ve	+ ve	-ve	2 (5)
+ve	+ve	+ve	6 (14)
+ve	- ve	+ve	21(50)
+ve	- ve	-ve	13(31)
Total (100)	(19)	(64)	42(100)

Parenthesis -percentage

Table 4: Age Distribution in all HBsAg Sero-positive Nigerian Adults by ELISA.

	SUBJECTS			SEX		Total (%)
Age (years)	A	B	C	Male	Female	
≤20	2	3	6	9	2	11 (9.0)
21-30	12	6	19	29	8	37 (30.3)
31-40	11	6	7	18	6	24 (19.7)
41-50	8	4	2	11	3	14 (11.5)
51-60	16	6	6	21	7	28 (23.0)
>60	6	-	2	5	3	8 (6.5)
Total	**55**	**25**	**42**	**93**	**29**	**122 (100)**

KEY
A = Comparison of haemaglutination method with ELISA in the sero-assay of HBsAg
B = Comparison of the Glaxo Welcome HB rapid test(GWHB) with ELISA in sero-assay of HBsAg
C = Assay of sera of HBsAg positive patients for HBeAg and anti-HBe HBsAg.

Table 5: **Algorythm for the serological diagnosis of HBV infection among Nigerians.**

A. Determination of HBsAg sero-status by ELISA.
B. Determination of infectivity status of those who are HBsAg sero-positive by assay of HBeAg, anti-HBe and HBV DNA polymerase chain reaction (detection of HBV pre-core mutants). This may require antiviral therapy if chronic B.
C. Determination of co-infection(s) - anti-HCV, anti-HDV and anti- HIV- I & II by ELISA in those who are with chronic B.
D. Determination of presence of anti-HCV, anti-HDV and anti- HIV- I & II by ELISA in HBsAg sero-negative blood for donation.
E. Determination of the presence of antibody to hepatitis B core antigen (IgM & IgG anti-HBc) and antibody to HBsAg (anti-HBs) in HBsAg sero-negative subjects in order to detect those that will require immunization against HBV (anti-HBc sero-negative subjects).
F. 6-monthly monitor of subjects who are only HBsAg sero-positive (HBsAg carrier) for sero-conversion to anti-HBs or amplification of the disease by presence of HBeAg, anti-HBe and / or HBV DNA.

DISCUSSION

Generally, most of the assays for HBsAg status are carried out by HMA in resource poor populations such as Nigeria because of its simple technology, availability and cheaper cost. Screening for HBV infection is particularly important when there is need for blood transfusion, immunization against the infection, determination of occupational risk exposure and involvement in some medical procedures or treatment. Furthermore, many patients present in the hospital with HBsAg sero-positive results with mixed feelings, worries and uncertainty about the consequences. Apart from the heavy work load that this would present on medical personnel, absenteeism from work by patients, the socioeconomic and emotional burden of a positive report on the patient and the relations is enormous. Amelioration of this burden is by eliminating false results through the use of other more sensitive and specific methods of assaying HBsAg such as the ELISA, radio-immunoassay tests - RIA and the gold standard - viral DNA studies. Our study showed that HMA had a negative predictive value of 56% (indicating a high false negative result) when compared with the ELISA method for the detection of HBsAg. This report corroborates previous comparison of the two methods that HM gives false positive results[8]. Declaring false positive results by HM to patients would cause unnecessary fear to the ignorant and innocent patients, an unpalatable situation that could be avoided if only the patients had been retested by ELISA. In addition, false HBsAg sero-negative results by HM (compared to ELISA) in 25.5% of Nigerian adults is highly significant[9]. Informing an infected healthy adult as uninfected (and vice versa), has serious medico-legal implications. The high percentage of false results of HBsAg status (positive -5.5% and negative - 25.5%) of about 31% among Nigerians by HMA compared with ELISA is a clear indication that there is no further justification for using only HMA in screening blood for HBsAg prior medical treatment. This calls for an urgent action by the various sections of the Ministry of health in the local, state and federal levels of Nigerian government to make available to all Blood Bank Units the necessary equipment and wares needed for regular screening of all donated blood for HBsAg by using a better method such as ELISA. This will provide a safer blood transfusion service in the nation and reduce significantly the spread of HBV infection. In addition, management of the infection among Nigerians should never be dependent on screening for HBsAg only by HMA but by ELISA. The second study showed that GWHB is comparable to ELISA in the detection of HBsAg in Nigerian subjects, however, the presence of false sero-negative rate of 20% by the former calls for a parallel routine test by ELISA in those requiring the rapid test. GWHB seems rather poor in the detection of HBeAg status when compared with ELISA. Our result is however lower than the HBeAg rate of 10.8% obtained in a previous study among Nigerian HBsAg sero-positive blood donors[10] and is unlike the result from our study group with chronic hepatitis. The findings reinforce the need to screen all patients with ELISA or other tests such as RIA and polymerase chain reaction for HBV DNA assay in order to identify subjects that may require anti-viral therapy.

Nigeria being an endemic nation for HBV infection, the carriage of the infection occurs among the various age groups of the population particularly the adults[10-12] where it predominate among the 21-30 year group as documented in the Americans[13]. However, the presence of our subjects with HBsAg sero-positivity in all the age groups and the higher proportion of male gender among them is similar to earlier reports among Nigerians[7,10,12,13]. However, the preponderance of the infection among the reproductive and workforce of the nation re-emphasizes the importance of accurate diagnosis of the infection by highly sensitive and specific assay methods in order to eradicate its reservoir through effective treatment of infected subjects. The detection rate of HBeAg in our subjects is lower than the value of 45% obtained among similar group of Indian subjects studied by Chandra et al[14] but similar to the value of 11.9% reported among Nigerians with chronic liver diseases[7]. In contrast, the anti-HBe detection rate of 64% in our subjects (first report among Nigerians) is similar to 53% reported among the Indian subjects[14].

The high percentage of our subjects with chronic HBV infection who are either HBeAg or anti-HBe sero-positive shows that majority of them are respectively of high or low level of infectivity for HBV. This could be the reason for the endemic nature of the infection among Nigerian population of whom were the patients. This situation is further worsened because the subjects who were sero-negative for HBeAg but anti-HBe positive, could be carrying HBV pre-core mutant and thus are negative secretors of HBeAg[15]. Hence, there is the need for the definition of actual presence of the HBV pre-core mutant among Nigerian subjects by HBV DNA studies. From the above, an assay of serological markers of HBV using

ELISA has become imperative among Nigerians in order to determine the National burden of the infection so as to ensure effective management and planning in combating the HBV endemicity. An algorithm for the serological diagnosis and treatment[16, 17] of HBV infection among Nigerians as shown in table 5 is hereby suggested.

In conclusion, our studies have shown that serological diagnosis of HBV infection is best carried out by ELISA rather than by the HMA method. Furthermore, though GWHB is comparable to ELISA in the assay of HBsAg during emergency situations, a parallel assay by ELISA will be needed to detect false sero-negative result. GWHB seems to be poor in the detection of the infectivity status of HBsAg sero-positive subjects; hence these patients should be further assessed for HBeAg and anti-HBe by ELISA technique. In addition, HBV infectivity is high among adult Nigerian subjects with HBV infection and would demands accurate diagnosis of the infection among Nigerian population in order to ensure its effective treatment and prevention

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