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Sensitivity comparison between rapid one-step test strip and ELISA methods in detection of HBsAg among selected chronic liver disease patients in University of Calabar Teaching hospital, Calabar, Nigeria

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ABSTRACT

Background: Hepatitis B virus (HBV) is the eloquent hepatic infection that leads to hepatocellular carcinoma. This study aims to determine the frequency of HBV by screening selected chronic liver disease (CLD) patients alongside few apparently healthy subjects who might be carriers and pose a high risk in HBV transmission. This study also aims to compare the sensitivity between two diagnostic tests; one step rapid test strip device (RIA) and Enzyme Linked Immunosorbent Assay (ELISA). **Methodology:** Serum of 111 subjects comprising of 76 CLD patients and 35 apparently healthy (control) subjects was screened for Hepatitis B surface Antigen (HBsAg) using one step rapid test strip device and Enzyme Linked Immunosorbent Assay (ELISA). **Results:** Sero-positivity of the two methods of detecting HBsAg was higher in age group 26-35 years and the male folk was found higher in number than the females. Seropositivity of ELISA method for detecting HBsAg was higher (81.1%) compared to one step rapid test method (28.8%). All the CLD patients 76(100%) and 14 out of 35 of the apparently healthy subjects were positive for ELISA test method. **Conclusion:** In conclusion, comparing the two methods, ELISA test is more sensitive than one step rapid test strip device for detecting HBsAg among CLD patients as well as apparently healthy subjects in University of Calabar Teaching Hospital, Nigeria.Therefore, we recommend strongly, that ELISA method be used to confirm test results obtained from the one step rapid test strip, when screened for CLD.

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Key words: Chronic liver disease, ELISA methods, Hepatitis B, Rapid test strip

INTRODUCTION

Hepatitis B Virus, which causes serious liver damage is one of the WHO's target for global eradication by 2020.[1] It is a major public health problem world-wide and is more prevalent in the developing countries.[2,3] More than 2 billion people are infected with HBV world-wide while some 280 million are chronic carriers, harbouring the virus in their liver.[4] Nigeria is one of the countries with the highest incidence, with a prevalence of 10 – 15%. In Nigeria, there is a strong relationship between HBV infection and various forms of chronic liver disease [CLD], including chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.[5] Blood is considered one of the important causes of transmission of diseases including hepatitis B and Hepatitis C[6,] and most of the diseases can be diagnosed.[7] The presence of HBs Ag in serum or plasma is an indication of active Hepatitis B infection either acute or chronic. There are currently about 350 million people worldwide who are chronically infected with hepatitis B virus (HBV),[8,9] 15 to 40% of whom will develop serious sequelae during their lifetime.[10] Clinically, HBV

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infection is indistinguishable from other viral hepatitis. Accordingly, its diagnosis relies on a specific laboratory tests for distinguishing it from such viruses.[11,12] Hepatitis B surface antigen (HBsAg) is detectable in serum prior to the development of symptoms, and remains detectable during clinical convalescence. The serological presence of HBsAg beyond 6 months defines chronic hepatitis B. However, some people, referred to as "carriers", may have little or no damage toliver at all, albeit they are continuously making as well as transmitting such viruses for years.[12,13] There is neither seasonal trend for HBV infection nor high predilection for any age group. However, definite high risk groups were identified, among them parenteral drug abusers, institutionalized persons, those dealing with health care (such as surgeons, dentists, nurses, pathologists, and blood bank personnel), multiply transfused patients, organ transplanted and hemodialysed patients.[14,15]

Following Blumberg's discovery of hepatitis B surface antigen (HBsAg), many attempts have been made to develop several in vitro diagnostic techniques for the detection of this antigen and its homologous antibody, including a wide-range of different serological, immunological and molecular-biological techniques.[16] According to the financial facilities as well as technical feasibility (i.e. trained personnel and the availability of advanced laboratory equipment) one should have the choice to select and apply one or more of these laboratory tests.[17] The recommendations for the screening of Hepatitis B also call for testing the serum or plasma specimens by ELISA tests.[18] Elisa tests are generally costly as well as the instruments and chemicals are concerned. However, in the locality, many different and mostly rapid one step kit test devise (commercial diagnostic kits) has been used continuously for the screening of HBsAg in patient due to cost (inexpensive). Occasionally, the results are deranged from those obtained using the different rapid test kit. Such observations raised the need for intra-laboratory and inter-laboratory quality control evaluation to rationalize uses of rapid test kits to gratify the reliable benchmark of highly sensitive, specific and reproducible tests. Therefore, the aim of this study was to assess the comparison between rapid one-step test strip and ELISA methods in detection of HBsAg among selected chronic liver disease patients in University of Calabar Teaching Hospital, Calabar, Nigeria.

MATERIALS AND METHODS

Subjects

This study included a total of one hundred and eleven (111) subjects aged 19 – 76 years. Seventy six (76) of them i.e. a cohort study, were selected chronic liver disease (CLD) patients visiting the University of Calabar Teaching Hospital (UCTH) and randomly selected 35 apparently healthy were control subjects. This study was carried out from September 2012 to March 2013. The bio-data of the subjects were obtained prior to specimen collection from file with the help of a clinician and verbal consent from each of the patient was sought for this study in order to fulfil the ethical guidelines of research conducted on humans. The inclusion criteria for the selection of the 76 CLD patients used for this study were; presence of jaundice, ascites, hepatomegaly and edema. Patients without the above features were excluded from this study. The subjects used as control were also subjected to clinical examination to confirm that they were normal (not having CLD). Those who declined participation were excluded from the study.

The 111 sera samples were screened using Wondfo one step rapid test for the detection of hepatitis B surface antigen (HBsAg) (manufactured by Guangzhou wondfo Biotech Co. Ltd; Wondfo Scientech Park, South China University of Technology, Guangzhou, P.R, China). It is a rapid immunochromatographic assay designed for qualitative determination of HBsAg in human serum. It is for in vitro diagnostic use with sensitivity of 96.2% and specificity of 99.3%. The test was performed and interpreted according to manufacturer's specification.

Detection of HBsAg using ELISA method

The 111 subjects (76 CLD patients and 35 apparently healthy) were also screened using ELISA method for hepatitis B. The ELISA test is a solid-phase microtiter plate coated with monoclonal antibodies to human IgM which is based on sandwich principle. ELISA for in-vitro qualitative detection of HBsAg test kit in human serum (Catalog number KAPG4SGE3, DIAsource ImmunoAssay, Belgium) was used.

Statistical analysis

PRIMER version 17 was used for statistical analysis in this study. The X^2 (Chi-square) test was performed for quantitative variables to check for relationship in detecting HBV infection. Percentages were calculated directly for HBV infection. $P = 0.05$ was used as the accepted significance level.

RESULTS

One hundred and eleven (111) subjects were recruited in this study. Seventy six (76) were selected patients attending Medicine out-patient Department (MOPD) of the

University of Calabar Teaching Hospital (UCTH). These seventy-six patients were chronic liver disease patient and were all positive for Hepatitis B surface antigen (HBsAg) while thirty-five (35) were apparently healthy control subject and were all negative.

From Table 1, the age range of the 111 subjects was between 19 – 76 years with a mean of 37.7±1.32 years. It was observed in this study that majority of the subjects were in the age group 26 – 35 years. Seropositivity of ELISA method for detecting HBsAg was higher (81.1%) compared to one step rapid test method (28.8%).

Among the one hundred and eleven (111) subjects used in this study that were screened using rapid test strip device, 32 (28.8%) were positive and 79 (71.2%) were negative, whereas, using ELISA test method, 90 (81.1%) were positive and 21 (18.9%) negative (Figure 1). All 76 CLD patients and 14 apparently healthy subjects were seropositive for ELISA test method while only 21 apparently healthy subjects were seronegative (Figure 2). Figure 3 shows the frequency of seropositive and seronegative HBsAg using rapid strip test device method according to sex in CLD patients. It shows that using the method, 24 were male and 8 were female positive CLD patients while 22 males and 22 females were negative. In 76 CLD patients that were positive for ELISA test, 46 were males and 30 females. However, 14 of the apparently healthy subjects that were positive for ELISA showed that 9 were males and 5 were females (Figure 4).

Table 1: Age distribution of 111 subjects of the different methods used in the detection of HBsAg

Age Group	Subjects tested (%)	Subjects tested positive (%) for one step rapid test	Subjects tested positive (%) for ELISA
19-25	19 (17.1)	5 (15.6)	16 (17.8)
26-35	44 (39.6)	15 (46.9)	36 (40.0)
36-45	28 (25.2)	9 (28.1)	21 (23.3)
46-55	13 (11.7)	1 (3.1)	10 (11.1)
56-65	3 (2.7)	1 (3.1)	3 (3.3)
>76	4 (3.6)	1 (3.1)	4 (4.4)
Total	111 (100)	32 (28.8)	90 (81.1)

DISCUSSION

It is estimated by the World Health Organisation that there are about 350 million chronic carriers of hepatitis

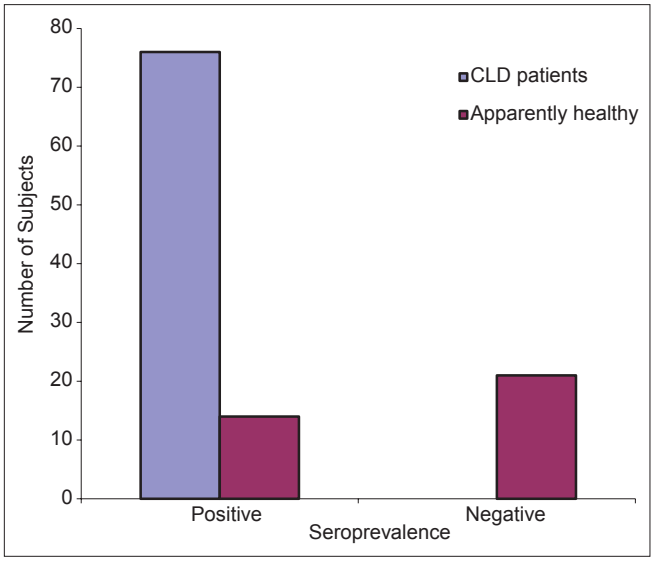

Figure 2: Frequency of CLD patients and apparently healthy patients that are seropositive and seronegative for ELISA test

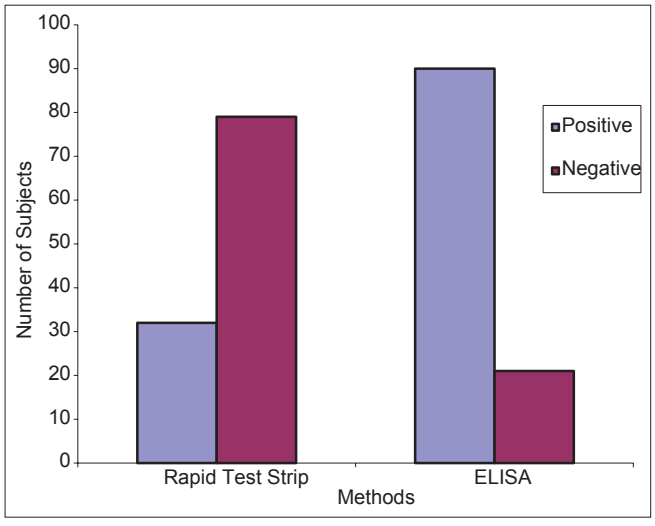

Figure 1: Frequency of Seropositivity and seronegativity of 111 subjects according to the different methods use for HBsAg detection

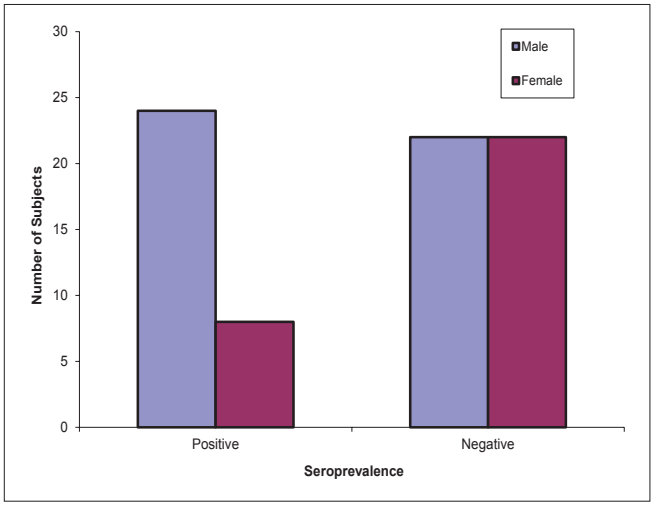

Figure 3: Frequency of seropositive and seronegative HBsAg using rapid test strip according to sex in CLD patients

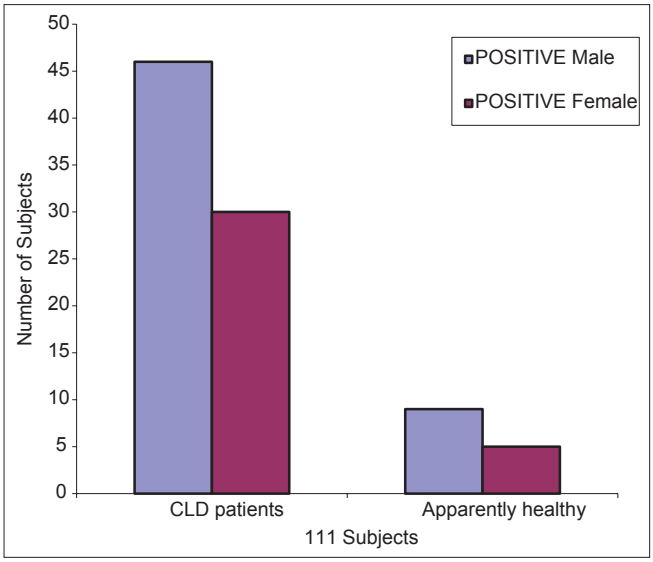

Figure 4: Frequency of seropositivity using ELISA test according to sex

B spreading in every continent in Asia, America, Europe and Africa.[10,11] In this study, two methods were used (rapid strip test device and ELISA test) to check for sensitivity in screening for hepatitis B surface antigen among chronic liver disease patients. A greater percentage of the subjects (81.1%) tested positive using ELISA method while only 28.8% tested positive using the rapid test strip. It is also interesting to observe that all chronic liver disease patients were positive for HBsAg using ELISA method including 14 (40.0%) out of 35 apparently healthy subjects used as control group. This study gives us the insight and awareness about the alarming condition of hepatitis B virus among CLD patients and most importantly the apparently healthy subjects (control group). In comparing the sensitivity of both methods, the data given indicates that the frequency of HBV is more when screened for HBsAg using ELISA than rapid test strip device. This is probably because of the absence of HBV vaccines in both rural and urban areas in our locality.

The result of this study has revealed a higher frequency of HBV in both test (rapid strip and ELISA) in younger males, compared to females. There was no significant difference in the two methods based on gender. Chronic liver disease patient were also a decade younger in age. Other researchers have reported similar findings. A number of them have suggested that increasing age is a higher risk to have the disease, probably due to longer exposure to multiple risk factors,[12-14] and higher in males than females.[15,16]Although, the sample size is not very large as compared to the report in earlier studies stated, but the results obtained are very important since this is the first study from Calabar, Nigeria, where a high frequency rate has been seen in ELISA method when compared to rapid strip test device. So, there is a great and urgent need for people in this locality and beyond to screen their blood for HBV and it should be screened using the ELISA technique which has been found to be more sensitive and accurate as compared to the rapid test strip device method. Moreover, the state and federal government should create a program where vaccination will be given to the people as well as educating the public on the virus and its modes of transmission in order to control the spread.

CONCLUSION

This study shows that frequency of HBV is high in Calabar, Nigeria, and the incidence is greater in males than the females. We also noted that in comparing both methods (ELISA method and the rapid test strip) for assessing the presence of HBsAg, ELISA test method was found to be more sensitive than the rapid test strip device. Therefore, we recommend strongly, that ELISA method be used to confirm test results obtained from the one step rapid test strip, when screen for CLD.

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Authors Contribution:
DCO and MKA designed the study, collected samples, wrote the protocol, and the first draft of the manuscript. **ZAO** performed all the serological assays. Authors **DCO, MKA, ZAO and VUN** managed the literature searches, analyses of the study and managed the experimental process. All authors read and approved the final manuscript.

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