ORIGINAL ARTICLE

Comparative Evaluation of Enzyme Linked Immunosorbent Assay Method and Immunochromatographic Rapid Test Strip in the Diagnosis of Hepatitis B Virus Infection Among Human Immunodeficiency Virus Infected Patients in North Western Nigeria

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ABSTRACT

Background: This study aimed at comparing results of the rapid test strips and ELISA method in the detection of HBsAg among HIV infected patients.

Methods: The study was a cross sectional in which 180 treatment naïve adult HIV infected patients were screened for HBsAg using rapid test strips and these were re-tested for HBsAg with ELISA method. The $CD4^+$T cell counts was performed with Cyflowcounter machine. Interviewer administered questionnaire technique was adopted in obtaining information about the study participants.

Results: Eighteen (10%) and thirty seven (20.6 %) patients were positive for HBsAg using HBsAg rapid test strips and HBsAg ELISA kit respectively. The false positive and false negative of the rapid test strips with ELISA as a gold standard were 11.11 % and 12.96 % respectively. The sensitivity and specificity of rapid test strips with ELISA as a gold standard were 43.24 % and 98.60 % respectively. About 22(59.5 %) of those positive for HBsAg with ELISA method were severely immunosuppressed.

Conclusion: Rapid test strips were inferior compared to ELISA in the detection of HBsAg among HIV infected patients and severe immunosuppression might impair the performance of rapid test strips. Manufacturing companies need to improve on their rapid test strips. Validation of rapid test strips prior to their usages should be ensured. WHO and member states should come up with standard protocol for the screening and diagnosis of HBV infection and there is need to step up HBV immunization strategies.

KEYWORDS: HIV/HBV co-infection, Rapid test strip, ELISA

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Introduction

Both Human Immunodeficiency Virus (HIV) and Hepatitis B Virus (HBV) infections have similar routes of transmission, therefore their co-infection is prevalent. Individual infected with HIV is prone to developing Acquired Immunodeficiency Syndrome (AIDS) either due to depletion of $CD4^+$ T cell (which plays a key role in the fight against a foreign body) counts to less than 200 cell/mm,[3] or opportunistic infection(s).[1] Both HIV and HBV infections are prevalent in African region.[2,3] Nigeria had HIV prevalence of 3.4 % , while Sokoto State had a prevalence of 6.4 %.[4] About 2 billion people have been infected globally by HBV infection while the prevalence of HBV infection in Nigeria is about 13.6 %.[5,6]

The hepatotropic nature of HBV infection results in chronic liver diseases such as chronic

hepatitis, liver cirrhosis, and hepatocellular carcinoma and the risk of liver cirrhosis and end stage liver disease is increased in HBV infected patients that are co-infected with HIV infection.[2,7]

The serological diagnosis of HBV infection is made by the demonstration of HBsAg, however other HBV serological markers that are also important in the management include: Anti HBs, Anti HBc, HBeAg, Anti HBe, and HBV DNA[8].

Rapid diagnostic test strips for HBsAg which are less sensitive compared to Enzyme Linked Immunosorbent Assay (ELISA) are commonly used in resource limited countries for the detection of HBV infection in target groups, such as HIV infected patients, suspected cases of HBV infection, patients from surgical units, blood donors and general population. The rapid kits are cheap, do not require special skill and are readily available.

Material and Methods

This was a cross sectional prospective study conducted in Specialist Hospital Sokoto (SHS) and Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto from March 2014 to October 2015. Ethical committee of the two Hospitals approved the study. Blood samples were collected from 180 adult HIV infected patients after getting their informed consent. Treatment naive patients were included in the study, while those who had HBV vaccination were excluded. Questionnaire was used for the collection of data about the study participants and interviewer administered technique was adopted.

The blood samples were initially screened for HBsAg with Egens rapid test strips, following the manufacturer's instructions. The test is a lateral flow immunochromatographic screening test, based on the principle of sandwich immunoassay with monoclonal antibodies for the detection of HBsAg. All the blood samples were re-tested for HBsAg with HBsAg ELISA kit (Fortress Diagnostic UK) following the manufacturer's instructions. Sensitivity and specificity of the ELISA kit are 99.75 % and 99.87 % respectively. The $CD4^+$ T lymphocyte counts estimation was done with Cyflow counter Machine (PARTEC, Germany).

Data was entered into Statistical Package for Social Sciences (SPSS) Version 20 for analysis, while results were presented in simple proportions, frequencies and percentages. Sensitivity and specificity of the rapid test strips were determined using the formulae below:

Sensitivity = True positive All with disease,

Specificity = True negative All without disease.[20]

Results

One hundred and eighty subjects comprising 71(39.6%) males and 109(60.6%) females participated in the study. The age (Mean ± SD) of the study participants was 32 ± 9. Of the 180 HIV infected patients 18(10 %) were positive for HBsAg when screened with rapid test strips, while 37(20.6 %) patients were positive for HBsAg when re-tested with ELISA method. Table 1 shows comparison of HBsAg detection between rapid test strips and ELISA method.

Table 1: Comparison of HBsAg detection with rapid test strips and ELISA method among HIV study participants

Type of assay	HBsAg positive n(%)	HBsAg negative n(%)	Total
HBsAg (EGENS rapid strip)	18(10)	162(90)	180
HBsAg (Fortress ELISA Kit)	37(20.6)	143(79.4)	180

Screening test outcome of HBsAg, using rapid test strips with ELISA test as a gold standard Table 2 shows the test outcome and indicates that 2 and 21 of the HIV infected patients that were positive and negative respectively for HBsAg using rapid test strips were negative and positive respectively for HBsAg when re-tested with ELISA method. These values correspond to 11.11 % and 12.96 % as false positive and false negative respectively for the rapid test strips. The validity of rapid test strips in terms of sensitivity and specificity are 43.24 % and 98.60 % respectively.

Table 2: HBsAg screening test outcome using rapid strip with ELISA test as a standard.

	Disease state		
Screening Test results	**HBV co-infection**	**No HBV co-infection**	**Total**
Positive	16	2	18
Negative	21	141	162
Total	**37**	**143**	**180**

Severe immunosuppression among HIV study participants that were positive for HBsAg with ELISA method Among the 37 HIV and HBV co-infected study participants, 22(59.5 %) were severely immunosuppressed ($CD4^+$ T lymphocyte counts > 200 cells/mm^3) while 15(40.5 %) had $CD4^+$ T lymphocyte counts $\geq$ 200 cells/$mm.^3$ These results are shown in Table 3.

Table 3: Prevalence of severe immunosuppression among HIV subjects that were positive forHBsAg with ELISA

Severe immunosuppression ($CD4^+$ T lymphocyte counts < 200 cells/mm^3)	n(%)
Yes	22(59.5)
No	15(40.5)

Discussion

In May 2016, World Health Organization launched global strategies for the elimination of HBV infection as a public health problem by the year 2030.[9] Increasing access to HBV infection diagnosis is one of those strategies. World Health Organization (WHO) recommends Anti-Retroviral Therapy (ART) at any level of $CD4^+$ T cell counts in a patient with HIV and HBV co-infection with evidence of active liver disease.[5] Therefore accurate HBV infection diagnosis in HIV infected patients is required for proper management of the HIV and HBV co-infected patients.

This current study recorded lower HBsAg seropositivity (10 %) with rapid test strips compared to ELISA method (20.6 %). Similarly majority of the studies among blood donors,

suspected HBV infected cases, general population and patients mainly referred from surgical unit documented lower HBsAg seropositivity with rapid test strips compared to ELISA method, though with varying degrees of accuracy.[10, 11, 12, 13, 14, 15] However Maity *et al.*, (2012), in their study in India among volunteer attendees of the integrated counseling and testing centre, found that the rapid test strips from 3 different companies were able to identify all the individuals with HBsAg seropositivity as identified by different ELISA kits.[16]

False negativity of HBsAg rapid test strips (12.96 %) obtained in the current study among HIV infected patients is comparable to (9.0 %) recorded among blood donors in the same study area, however it is higher compared to what was documented (3.0 % and 6.30 % as mean and highest value respectively with rapid test strips from different companies) by Dogbe and Arthur (2015) in their study areas in Ghana.[11,12]

The sensitivity of the rapid test strips recorded in the current study was 43.24 % and this implies that the rapid test strips were not able to correctly identify HIV patients who have HBV co-infection compared to ELISA method. The implication of sensitivity of 43.24 % in the current study was that, more than half of the HIV infected patients with HBV co-infection were misdiagnosed as not having HBV infection and this may interfere with the proper management of these misdiagnosed patients. The specificity of the rapid test strips observed was high (98.60 %) and this is not unexpected due to the nature of high specificity of monoclonal antibodies used in the rapid test strips.[17] These results pointed out that the test strips were able to correctly identify HIV infected patients who have no HBV co-infection to a large extent.

Similarly a study done among HIV infected patients in Guinea-Bissau by Honge *et al.*, (2014) reported that among 77 samples positive for HBsAg using chemiluminescence assay (which is a variation of a standard enzyme linked immunosorbent assay), only 48 samples reacted for HBsAg when re-tested with rapid test strips (HBsAg strip Ref 2034**:** VEDA-LAB, Alencon France) and this accounts for about 62.3 % as the sensitivity of the HBsAg strips they used.[18] The specificity (99.2 %) of HBsAg strip Ref 2034 obtained by these researchers is comparable to what was documented in the current study (98.6 %).

Similarly Gerett*iet al.*, (2010) in their study in Ghana documented lower sensitivities in the detection of HBsAg among HIV infected study participants when they used two different categories of rapid test strips.[19] These researchers obtained sensitivities of 69.3 % and 70.7 % respectively for Determine and Vikia rapid test strips for HBsAg compared to what was obtained by enzyme immunoassay (Manual Murex version 3 plate enzyme immunoassay: Abbott Diagnostics, with 98.6 % sensitivity) and chemilumiscent immunoassays (Architect HBs Ag: Abbott Diagnostic, Maidenhead, UK, with 97.9 % sensitivity; Liaison HBs Ag Ultra: Diasorin, Blacknell, UK, with 97.1 % sensitivity). However 100 % specificity was documented for the two categories of rapid test strips used.

Factors that may impair the performance of rapid test strips include: severity or stage of the disease, mutation of HBsAg determinant, operational problems and unexpected technical issues not revealed by early validation tests.[20, 21, 22] We suggest that severe immunosuppression may also impair the performance of rapid test strips and this point may explain why the HBsAg rapid test strips used in the current study couldn't identify more than half (of the HIV infected study participants that were positive for HBsAg by ELISA method) as having HBV co-infection, because more than half of the HIV and HBV co-infected were severely immunosuppressed.

In conclusion, the rapid test strips for HBsAg were found to be inferior compared to ELISA method in the diagnosis of HBV infection among HIV infected patients, however the test strips had high specificity. Immunosuppression might impair the performance of rapid test strips. We supported the idea recommended by some researchers of ensuring validation of rapid test strips prior to their usage in the laboratories. Manufacturing companies need to improve on their rapid test strips and subsequent researches may probably come up with platform which manufacturing companies may utilize to improve their rapid test strips.

This study also recommends that WHO should work with member states in the formulation of standard protocol in the screening and diagnosis of HBV infection among various target groups and also step up HBV vaccination strategies, so as to ensure its target of elimination of HBV infection from being a public health problem by the year 2030.

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