Original article

Evaluation of Three Rapid Diagnostic Tests for Detection of Hepatitis B Surface Antigens (HBsAg) in Yaounde-Cameroon.

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ABSTRACT

***Introduction*:** Hepatitis B Virus infection is a leading cause of liver disease worldwide. Rapid tests for its diagnosis are less costly, and easy to perform, hence can be used in areas with limited resources. ***Objectives*:** To evaluate three Rapid diagnostic tests for the detection of HBsAg in Yaounde. ***Methodology*:** Comparative cross sectional study. Using SD HBsAg ELISA as gold standard we evaluated Determine HBsAg, Acumen HBsAg, and Vikia HBsAg.The Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative predictive Value (NPV) of rapid tests were determined. A random sample of 360 patients, including 180 regular blood donors and 180 patients with history of viral hepatitis B was obtained. ***Results*:** The age of volunteers ranged from 17-60 years, and the mean age was 31.98±9.08 years. More males (62.5%) were represented than females (37.5%). All of the 180 regular blood donors were tested negative by SD HBsAg ELISA and all of the 180 patients with history of viral hepatitis B, were tested positive by SD HBsAg ELISA (100%). Sensitivity, PPV, Specificity, NPV of rapid tests, with respect to SD HBsAg ELISA, exceeded 96%. Vikia HBsAg test performed best in this study, with a sensitivity of 99.44%, specificity of 99.44%, PPV of 99.44% and NPV of 97.79%. There was no significant difference in the sensitivity and specificity of the rapid tests (P=0.63). ***Conclusion*:** Vikia HBsAg test appears to be the most suitable for use in areas with limited resources.

Key words: sensitivity, relative, positive predictive value, negative predictive value, rapid diagnostic tests, HBsAg.

RÉSUMÉ :

***Introduction* :** Le virus de l'Hépathite B et l'infection est la principale d'hépatopathie à travers le monde. Les tests rapides de dépistage de l'Ag HBs sont peu coûteux et faciles à utiliser, donc indiqués pour les régions à ressources limitées. ***Objectifs* :** Evaluer trois tests rapides de dépistage pour la détection de l'HBsAg à Yaoundé. ***Méthodologie* :** nous avons mené une étude transversale et comparative. Nous avons prélevés des échantillons de sang auprès de 180 donneurs de sang réguliers et 180 patients ayant des antécédents de sérologie Ag HBs positive. Les tests rapides suivants étaient évalués avec SD HBsAg ELISA comme référence : Determine HBsAg, Acumen HBsAg, et Vikia HBsAg. ***Résultats* :** L'âge de nos volontaires variait entre 17 et 60 ans avec une moyenne de 31.98±9.08 ans**.** Les hommes (62.5%) étaient plus nombreux que les femmes (37.5%). **L**es 180 donneurs de sang réguliers étaient négatifs au SD HBsAg ELISA et les 180 patients aux antécédents d'hépatite B étaient positifs au même test. La sensibilité, la spécificité, la VPP et la VPN étaient déterminés pour les tests rapides. Le test le plus performant était le Vikia HBsAg avec une sensibilité de 99.441%, VPP de 99.44% et VPN 97.79%. Il n'y avait pas différence significative entre la sensibilité et la spécificité des différents tests rapides (P=0.30). ***Conclusion* :** Le test Vikia HBsAg semble être le meilleur des tests rapides et pourrait être indiqué pour les régions à ressources limitées.

Mot clés : sensibilité, spécificité, valeur prédictive positive, valeur prédictive négative, tests rapides, Ag HBs.

INTRODUCTION

Hepatitis B virus belongs to the family of Hepadnaviridae. It is a double stranded DNA enveloped virus. Under the electron microscope, it is seen as aggregates of virus coat proteins [1]. Hepatitis B virus infects the liver and may progress to liver cirrhosis. Hepatitis B Virus have tubular structures, which in the serum of infected individuals, is found as spherical particles with diameters of 22 nm or filaments of similar diameter [2]. There are point mutations of the 'a' determinant of HBsAg, which is the major neutralizing epitope, and a double-loop structure projecting from the surface of the HBV particle [3, 4]. Nine serotypes of HBsAg have been described, and have been related to six genomic groups, A through F, based on sequencing of the S gene of isolates from different geographical regions [5]. Hepatitis B Virus (HBV) has mutant forms of HBsAg and rapid tests differ in their abilities to detect mutant forms of the HBsAg in clinical samples [3]**.**

HBsAg is one of the first serum markers to appear during the course of HBV infection, and can be detected 2 to 8 weeks before biochemical evidence of liver dysfunction and the onset of jaundice. HBsAg is cleared within a few months in self-limiting illness. If HBsAg persists for more than 6 months, spontaneous clearance is very improbable and the infected individual is considered a chronic HBV carrier [6].

Some of the techniques used in the diagnosis of Hepatitis B virus infection are: immunochromatography, immunoagglutination, ELISA, counter immuno electrophoresis, reverse passive hemagglutination, PCR [7]. Among the many commercially HBsAg assays available, enzyme-linked immunosorbent assays, rapid tests such as

Vikia HBsAg, Acumen HBsAg, and Determine HBsAg are currently used in Yaounde.

There are three possible explanations of false-negative results in commercial assays. In chronic HBV carriers, the HBsAg level may be below the detection limit, that is, a high proportion of individuals with antibodies against HBV core antigen (anti-HBc) as the only serological marker of infection are low-level chronic carriers of the virus [8]. Another explanation as to why in chronic carriers, the HBsAg level may be below the detection limit, is that virus variants yield sequences that are not recognized by the antibodies employed in the assays. In different geographic locations, vaccine-escape mutants are emerging under the selective pressure of active immunization, and there is a danger that they will become dominant strains as vaccination becomes universal [9]. Vaccine-escape mutants within the 'a' determinant of the S gene are not recognized as effectively by conventional diagnostic tests as the wild-type particle [10]. A third possible explanation is that there are variants in other parts of the genome that down regulate the production of HBsAg [10]. Therefore, to reduce the residual risk of transfusion-associated hepatitis B, the sensitivity of HBsAg screening assays is continuously improved.

Transmission of Hepatitis B virus can be sexual, parenteral, or perinatal. The concentration of HBsAg is highest in blood, serum [11]. It is estimated that approximately 350 million people worldwide have chronic HBV infection**.** Hepatitis B virus infection is high (43%) of global population and lifetime risk of infection is above 60%. It is intermediate in 43% of global population (life time risk of infection is between 20%-60%), and low (12% of global population and life time risk of infection is below 20%), [11].

A study carried out in 2005 in Cameroon, by Fuat and colleagues [12], to determine a new subtype (genotypes) Ac (A3) of hepatitis B virus and recombination between genotypes A and E(among pigmies and Bantus), showed a prevalence of 9.4%, 17.3%, and 86.8%, for HBsAg, anti-HBs and anti-HBc respectively.

Diagnosis of Hepatitis virus is carried out serologically, to determine acute and chronic infections. HBsAg is used as a general marker of infection [13]. HbsAb is used to document recovery and/or immunity to HBV infection. AntiHBcIgM is a marker of acute infection. HbeAg, indicates active replication of virus and therefore infectiveness. Anti Hbe, indicates that virus is no longer replicating. However, the patient can still be positive for HbsAg, which is made by integrated HBV [14]. HBV DNA indicates active replication of virus, more accurate than HbeAg, especially in cases of active mutant. It is used mainly to monitor response to therapy [15].

Prevention of HBV infection can be by; Vaccination, or Hepatitis B Immunoglobulins (HBIG). Other measures include; screening of blood donors, blood and body fluids precautions [16].

Treatment of HBV infection is by Interferon (for HbeAg positive carriers with chronic active hepatitis); Lamivudine (most patients will respond favorably). Successful response to treatment will lead to the disappearance of HBV DNA, and seroconversion to HbeAg [11].

The aim of this study was to evaluate three rapid diagnostic tests used in Yaounde-Cameroon. This investigation has been conducted with a view to determine the relative sensitivities and relative specificities of Vikia HBsAg, Determine HBsAg, and Acumen HBsAg using SD HBsAg ELISA as gold standard. This is important because HBV screening is done routinely in hospitals, especially in blood transfusion practice. It it is an appropriate preventive measure to use the most sensitive rapid detection test by our district hospitals.

PATIENTS AND METHODS

Study Location-Type

The study was a comparative, cross sectional study, carried out in three institutions; samples were collected at the Yaoundé Central Hospital, Clinique de L'Espoir Essos, and Centre hospitalier d'Essos-Yaounde. These centres were chosen because of the large number of Hepatitis B infected patients and also large the number of voluntary blood donors that attend the hospitals. The samples were analyzed in the blood bank unit of the CNPS Hospital Yaoundé.

Sampling procedure and selection of Subjects:

A total of 360 serum samples were collected during the period from May to September 2008 and preserved at -20ºC until tested. Out of this number, 180 Samples were randomly selected from patients with a history of hepatitis B (which include patients who came to the hospital for consultation and blood donation and were diagnosed positive for HBsAg), and 180 samples randomly selected from regular blood donors between the ages of 18 and 50 years.

Principles and Procedure of Tests:

All reagents was brought to room temperature before testing [17,18,19].

If specimen was not immediately tested, they were refrigerated at 2-8 degree for storage periods greater than three days, or frozen [20].

A) The principle of Determine HBsAg rapid test is based on immunochromatography, which is a qualitative test based on the detection of HBsAg in blood and blood products. It was done essentially according to the manufacturers (Abbott, Japan) [17].

B) The principle of Vikia HBsAg is a qualitative test based on the association of monoclonal and

polyclonal antibodies specific to HBsAg. This test uses the principle of lateral immunochromatography for the detection of circulating HBs antigen in blood and blood products. It was performed essentially as recommended by the manufacturers (Biomerieux, Brazil).

C) The principle of the Acumen HBsAg is also a qualitative lateral flow, immunoassay for the detection of HBsAg in serum or plasma. It was performed as recommended by the manufacturers (Nafdac) [18].

D) SD HBsAg ELISA, is an assay, which contains a microplate pre-coated with anti-HBsAg on wells It was done essentially as recommended by the manufacturers (Standard diagnostics, Korea) [20]

Sensitivity and Specificity were calculated according to the formula given by Marcillat and colleagues [21.

RESULTS:

A total of 360 samples were examined by three rapid tests for detection of HBsAg. Of the 360 samples, 180 were positive samples (patients with history of HBsAg) and 180 were negative samples (regular blood donors), and examined by Determine, Acumen, and Vikia, using SD ELISA 3.0 as gold standard. As shown in table 2: Invalid results were not included in the calculations. The study showed that out of 180 samples with history of viral Hepatitis B, SD HBsAg ELISA produced 180 positives (100% Sensitivity), Determine presented 177 positives (98.333% sensitivity); Vikia produced 178 positives (99.44%) and Acumen produced 176 positives (97.778%) (Table I). There was no significant difference in the sensitivity values of the rapid tests (P=0.65).

Table I: Percentage sensitivity of diagnostic tests in patients with history of HBsAg (N= 180)

Diagnostic tests	Number of positive samples examined	Number positive produced by tests	Number negative produced by tests	Relative sensitivity of test (%)
VIKIA HBsAg	180	179	1	99.44%
ACUMEN HBsAg	180	176	4	97.78%
DETERMINE HBsAg	180	177	3	98.33%
SD ELISA HBsAg	180	180	0	100%

Specificity of Rapid Tests:

Out of 180 regular blood donors, SD HBsAg ELISA produced 180 negatives (100% Specificity), Determine showed 178 negatives (99.44% Specificity), Vikia and Acumen produced 178 negatives (99.44% Specificity) and 177 negatives (99.448% specificity) respectively (Table II). There was no significant difference in the specificity values of the rapid tests (p=0.65).

The positive predictive value (PPV) and Negative Predictive Value (NPV) of Rapid Diagnostic Tests with respect to SD HBsAg ELISA showed that out of the three rapid tests, Determine had the highest PPV (99.47%) and Acumen showed the least (99.41%). Vikia had the highest NPV (99.44%) while Acumen showed the least NPV (97.79). There was no significant difference in the PPV and NPV between the tests (P=0.32).

DISCUSSION:

There are many problems associated with current methods of screening that may account for lack of public awareness leading to increase risk of transmission of Hepatitis B infection. Commercially available kits for HBsAg vary in their ability to detect all the various mutant forms [9]. In district and peripheral hospitals, diagnostic methods like ELISA are expensive and time consuming. Therefore it seems there is a great demand for a reliable screening method, with high efficiency and rapid diagnostic ability. Since there are many trade names that claim they have these abilities, it was necessary to compare them and determine the most suitable for use especially in District and peripheral hospitals and blood transfusion practice.

The results of the study confirmed that the SDHBsAg ELISA 3.0 was the most sensitive and most specific (100% sensitivity and specificity) test, as reported previously [20, 22], hence it was used as gold standard to compare the three rapid tests used in Yaoundé.

The rapid tests showed relative sensitivity between 97.77% and 99.44%, and relative specificity between 99.44% and 99.44%, when compared to SD HBsAg ELISA 3.0. The values of Positive Pedictive values were greater than 98% and that of Negative Predictive Values were greater than 97%. There was no significant difference in the predictive values between the tests (P=0.32). In a study carried out by Marcillat and colleagues in Brazil in 2007 [21], to evaluate Vikia and Determine, relative sensitivities of 99.0% and 97.80% respectively, were reported. This

is in agreement with our study, which showed that Vikia has a higher relative sensitivity (relative sensitivity 99.44%) than Determine (98.33%). Where research facilities exist, rapid HBsAg test results have to be confirmed by either an EIA or PCR molecular technique. Rapid tests alone may be used only in a small hospital setting where the facilities for EIAs do not exist [23]. The difference in the sensitivity values of the test could be due to the fact that virus variants yield sequences that are not recognized by the antibodies employed in the assays [8]. Vaccine-escape mutants within the 'a' determinant of the S genes are not recognized as effectively by conventional diagnostic tests as the wild type particle [10].

Table II: Percentage specificity of diagnostic tests amongst regular blood donors.

Diagnostic tests	Number of negative samples examined	Number negative produced by tests	Number positive produced by tests	Relative specificity of test (%)
VIKIA HBsAg	180	179	1	99.44%
ACUMEN HBsAg	180	179	1	99.44%
DETERMINE HBsAg	180	179	1	99.44%
SD HBsAg ELISA	180	180	0	100%

CONCLUSION:

From this study, Vikia HBsAg seems to be the most sensitive test among the three rapid tests in Yaoundé-Cameroon. The clinician when developing an HBV screening algorithm should consider antiviral resistance mutations against Lamivudine and Adefovir. Further developments of serological assays should include monoclonal antibodies that recognize epidemiologically relevant surface antigen mutants and further optimization of sensitivity

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