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Comparison of ELISA and Rapid Screening Techniques for the Detection of HBsAg among Blood Donors in Usmanu Danfodiyo University Teaching Hospital Sokoto, North Western Nigeria

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Abstract

Hepatitis B Virus (HBV) infection is one of the leading causes of death worldwide. The most important marker for HBV infection is Hepatitis B Surface Antigen (HBSAg). HBSAg rapid screening test methods are the most popular methods used in most developing countries even though the Enzyme-Linked Immunosorbent Assay (ELISA) and Nucleic Acid Testing methods are considered as more reliable methods worldwide. The aim of this study was to compare the analytical sensitivity of a rapid kit and ELISA method in the detection of HBV infection among blood donors. One hundred (100) blood donor samples which tested negative with Rapid screening test kit were re- tested using ELISA method. Out of 100 rapid test negative blood donor samples tested, 9 (9%) tested positive with ELISA method. Thirty blood samples which tested positive with rapid screening test kit were also re-tested using ELISA method. All 30 samples (100%) tested positive with ELISA method. This study shows that the rapid screening test kits are inferior and are associated with more false negatives compared to the ELISA technique. There is need for the Nigerian Government to develop a safe blood donor screening strategy for HBV by combining the use of the less sensitive rapid screening techniques with the more sensitive ELISA and NAT screening method to ensure the safety of blood donation in the country and to limit the risk of transfusion-transmissible hepatitis B infection.

Keywords: HBsAg; ELISA; Rapid screening test; Blood donors; Sokoto; Nigeria

Introduction

Hepatitis B Virus (HBV) infection is a global public health problem. It is estimated that approximately 360 million people are infected worldwide with the virus [1]. Viral hepatitis is a systemic disease primarily involving the liver. Most of the cases of acute viral hepatitis are caused by Hepatitis A (HAV), Hepatitis B Virus (HBV) or Hepatitis C Viruses (HCV). HBV has a double standard DNA encoding for P, X, core and surface proteins. The complex antigen found on the surface of HBV is called Hepatitis B surface antigen (HBAg). Antibodies against HBV proteins are other immunological markers of infection, of which Anti-Hepatitis B core antigen, Hepatitis B envelope antigen and Hepatitis B envelope antibody are also identified shortly after HBSAg, and are important markers of past or present HBV infection. In a typical Hepatitis B infection, Hepatitis B surface antigen (HBSAg) will be detected within 2 to 5 weeks before symptoms or jaundice develop [2]

Presently, both serological and molecular screening tests are employed for the diagnosis and patient monitoring of HBV infection. Nucleic acid test (NAT) is preferable in terms of its simplicity, rapidness and sensitivity but in some cases of occult HBV especially, NAT may miss some positive samples. NAT has the ability to decrease window period. However, it has been shown that NAT is too costly for testing individual samples [3]. Among all HBSAg assays, ELISA techniques are the most frequent used because of their effectiveness. In many developed countries, HBSAg screening is usually done with ELISA techniques [4]. Blood transfusion services are a vital part of modern health care system, with every unit of blood there is 1% chance of transfusion associated problem including transfusion transmittable diseases. Transfusing infected blood to unsuspected patients in need is a crime. It is mandatory to test each and every unit of donor blood for antibodies to HIV-1 and HIV-2, syphilis, HBSAg and HC Virus. ELISA is recommended and preferred screening technique for blood banking [5]. Many blood banks still do not have this facility and rather prefer rapid screening kits because they are easy to perform, cheap and are user friendly kits, and do not require sophisticated equipment and elaborate training. A preliminary study was therefore conducted to evaluate the efficacy of these rapid testing kits for screening of blood donor's [6].

HBSAg rapid test srip is a rapid screening test for the qualitative detection of HBSAg in whole blood, serum or plasma specimen. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBSAg in whole blood, serum or plasma [7]. While Enzyme-linked Immunosorbent Assay (ELISA) is an enzymatic immuno-assay technique of the "sandwich" type for the detection of Hepatitis B virus in human serum or plasma. The test uses monoclonal antibodies selected for their ability to bind themselves to the various sub-types of HBSAg now recognized by the World Health Organization (WHO) and the most part of variant HBV strains [8,9].

Materials and Methods

This prospective study was conducted in the Department of Haematology and Blood Transfusion Services Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto, Nigeria from June to

December 2011. During this period, serum/plasma samples from the blood donors were collected and tested for HBSAg using ACON rapid screening kit. Both positive and negative samples for HBSAg were labeled and marked. The test is a rapid chromatographic immunoassay for the qualitative detection of Hepatitis B surface antigens. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in whole blood, plasma or serum. The sensitivity, specificity, within run precision CV (%) and the between run precision CV(%) of the ACON Hepatitis B surface antigen Rapid test strip was 99.7%, 99.8%, < 7% and < 9% respectively. The samples were then re-tested using ELISA technique (HBSAg ULTRA, Bio-Rad France). HBsAg Ultra ELISA test is a one-step sandwich enzyme immunoassay using a solid phase coated with monoclonal antibodies (three). In the first step, the sample (100 µl) is incubated with a peroxidase conjugate (monoclonal antibody from mouse and polyclonal antibody from goat) in a microtiter plate. After a washing step, a tetramethylbenzidine substrate solution is added. The presence of HBsAg is proportional to binding of the conjugate and peroxidase activity. The colorimetric reaction is stopped, and the optical density is measured by bichromatic reading (450/620 nm). The cutoff value is calculated as the mean negative control value plus 0.050. Samples with a signal/cutoff ratio of ≥1.0 are reactive, and those with a signal/cutoff ratio of <0.9 are negative. Information on the aanalytical sensitivity of the kit (detection limit) was calculated as 0.08 ng/ml.

Statistical Analysis

Data was collected and entered into excel spread sheet and analyzed using the statistical software (SPSS version 18). Statistical analysis included descriptive statistics of percentages, mean and bivariate analysis of t- test and chi- square. Differences were considered significant when $p \leq 0.05$ (Table 1).

Number of samples	Results based on Rapid Kit (ACON)	Percentages (%)	ELISA Positive Result	Percentages (%)
100	All Negatives	100	9	9
30	All positives	100	30	100

Table 1: HBsAg screening using both ELISA and Rapid Kits

Results

During this study, a total of one hundred (100) HBsAg negatives blood donor samples and thirty (30) HBSAg positives blood donor samples screened using the (Acon Laboratories Inc. San Diego.CA) were segregated and re-tested using ELISA technique. Of the 100 samples that initially tested negative with rapid screening kit, 9(9%) tested positive with ELISA technique. All the thirty (30) initially positive samples with the rapid kit also tested positives with ELISA technique.

Discussion

Transfusion-transmissible hepatitis B virus (HBV) is one of the greatest threats to blood safety for transfusion recipients particularly in developing countries and pose a serious public health problem. Improvement of immunoassay for the detection of viral infections, particularly HBSAg has always been predicted to be due to emergence of mutant isolates and the need for early diagnosis of the disease. In this present study, 9 (9%) of samples that initially tested negative with the rapid kits, tested positive with ELISA technique (HBSAg ULTRA ELISA kit). Similarly 30 samples that tested positive with HBsAg Rapid screening kit also tested positive (100%) with the ELISA technique. This indicates that ELISA test is more sensitive and superior for the testing of blood donors for HBsAg. Our finding is consistent with previous report which indicated that ELISA technique is superior to rapid kits in the diagnosis of hepatitis B virus infection among blood donors [9]. Failure of the rapid kits to detect the presence of markers of infectious viral diseases may be due; inadequate coating of the antigen, nature of the antigen used and genetic heterogeneity of the virus [8-10].

In this study we have observed high false negative results with the rapid diagnostic kit compared to ELISA. Our finding is in agreement with previous report [11] which indicated that there is risk of donor blood containing HBV being transfused to patients due to suboptimal testing using HBsAg using rapid kits only. Previous report has shown that hepatitis B is endemic in Nigeria in both the general population and among blood donors [12-15] Testing of blood donors for hepatitis B virus infection in most blood banks in Nigeria utilize only rapid diagnostic kits. Our finding in this study brings to light the fact that blood transfusion may not be safe in Nigeria. Previous report has questioned the continued diagnostic utility of rapid test as sole assay for the diagnosis of HBV in the laboratory [16,17].

This present study shows that the rapid tests are inferior compared to ELISA. Our finding is consistent with a previous report which indicated that rapid test have not shown any promising results compared to ELISA and hence should not be recommended in transfusion centre solely for screening blood donors for HBsAg [18].

Prevention of transfusion-transmitted hepatitis B virus (HBV) in Nigeria continues to rely on serological screening of blood donors using rapid test kits rather than the progressively more sensitive ELISA and NAT technique employed in most developed countries to detect chronic carriers including those with low-level viremia who lack detectable HBsAg[19]. There is need for the combination of HBsAg rapid test along with ELISA and NAT. Cost effectiveness has erroneously been a reason put forward why developing countries cannot incorporate ELISA and HBV DNA detection into HBV screening of donors. It must however be noted that the cost of long-term treatment of a patient who contracts HBV from blood transfusion far outweigh the cost of testing donor units for HBV using ELISA and NAT. An unsafe blood transfusion is very costly from both human and economic points of view. Morbidity and mortality resulting from the transfusion of infected blood have far-reaching consequences, not only for the recipients themselves, but also for their families, their communities and the wider society [20].

We observed discordant results (negative with the rapid kit but positive with ELISA) between the rapid and ELISA kit involving 9 donor's samples. We observed 9% false negative cases with the rapid test. Discordant results between 2 assays for the diagnosis of an infectious disease can cause a huge challenge and have serious

consequences among patients [21]. It can cause undue mental stress and tension. Kit evaluation is vital in determining the diagnostic kit of better performance. The ELISA test in this study showed a better performance (higher positive predictive value, higher negative predictive value and fewer cases of false negatives) value. Positive Predictive Value (PPV) is the ability of an assay to identify actual infected individuals among all persons giving a positive result with the kit being used. Negative predictive value is the ability of an assay to identify correctly the real non-infected individuals among persons giving a negative result with the kit being used. A good assay for an infectious agent like HBV from a diagnostic point of view is one with a high positive predictive value and less cases of false negatives [22].

Serologic testing for hepatitis B surface antigen (HBsAg) has historically been the foundation blood donors screening in Nigeria. However ELISA and most recently HBV nucleic acid testing (NAT) was recently developed to detect HBsAg-negative blood units donated during early acute phase of infection [23]. The major challenges with ELISA and NAT is that they are more expensive, require use of instrumentation, time consuming and requires trained personnel. Nucleic acid amplification testing (NAT) is becoming the gold standard for blood donor screening in the developed world particularly because of its ability to reduce the risk of rapid kit and enzyme immunoassay (EIA)-negative, NAT-reactive donations from entering the blood donor pool [24]. Previous report indicates that implementation of NAT will provide a significant increment in safety relative to serological screening alone [25]. There is increasing need for the Nigerian Government to develop a safe blood donor screening strategy for HBV by combining the use of less sensitive rapid screening techniques with more sensitive and sophisticated evidenced- based ELISA and NAT screening to ensure the safety of blood donation in the country.

Competing interest

The authors declare that they have no competing interests.

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