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Validity of the Rapid Strip Assay Test for Detecting HBsAg in Patients Admitted to Hospital in Uganda

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Commercially available rapid strip assays (RSAs) for hepatitis B surface antigen (HBsAg) are used for most routine clinical testing in sub-Saharan Africa. This study evaluated the validity of RSA and a more sophisticated enzyme immunoassay (EIA) with confirmation by nucleic acid testing (NAT) in hospitalized patients in Uganda. Sera from 380 consecutive patients collected and tested for HBsAg and anti-HIV in Kampala, Uganda by RSA were sent frozen to Dallas for EIA including HBsAg, total anti-hepatitis B core, hepatitis B e antigen, and anti-HIV. NAT was performed on all HBsAg-positives and on a random sample of 102 patients that were HBsAg-negative by both assays. Overall, 31 (8%) were HBsAg positive by RSA while 50 (13%) were HBsAg-positive by EIA; 26 were concordant between the two assays. Of 55 HBsAg-positive patients, nearly all showed detectable serum hepatitis B virus (HBV) DNA by bDNA (46) or PCR (4) assay. The 26 patients who were HBsAg positive by both EIA and RSA had significantly higher median serum HBV DNA levels than the 24 patients who were HBsAg positive by EIA alone. An additional 12/102 (12%) HBsAg negative patients had very low serum HBV DNA levels by NAT. Several differences in expected results of serologic testing were observed in this large series of African patients. RSA HBsAg testing is less sensitive than EIA; even EIA failed to detect all HBV DNA positive sera. A more complex testing protocol than RSA alone will be needed in Africa to improve patient care. ***J. Med. Virol. 82:1334–1340, 2010.*** © 2010 Wiley-Liss, Inc.

KEY WORDS: rapid strip assay; enzyme immunoassay; nucleic acid testing; HBsAg

INTRODUCTION

Worldwide, there are estimated to be two billion people who have been exposed to hepatitis B virus (HBV) and 370–400 million chronic carriers [Alberti et al., 2005; Ocama et al., 2005]. This highly contagious, easily transmissible virus is endemic in sub-Saharan Africa where 56–98% of the population has been exposed and 9–20% are believed to be chronic carriers [Burnett et al., 2005]. The high prevalence is in part compounded by the low HBV vaccination coverage rates in this region [Emmanuel et al., 1988; Braka et al., 2006] and the high prevalence of HIV infection in Africa. Studies of a large number of HIV positive patients have demonstrated the higher prevalence of chronic HBV infection in this setting [Burnett et al., 2005]. Rapid antigen tests, sometimes called rapid strip assays (RSAs), in the form of cards or test strips are currently used as the principal means of diagnosing HBV infection (by testing for serum hepatitis B surface antigen, HBsAg) in African countries. These tests are inexpensive, easy to perform and have a rapid turnaround time [Sato et al., 1996]. However, their sensitivity and specificity are not clearly known in African patients; neither is the concordance between these tests and other standard measures such as enzyme immunoassay (EIA), the standard test elsewhere. Thus, the true prevalence of chronic HBsAg carriers in Africa is unknown. The aim of this study was to evaluate the validity of RSA tests as compared with standard assays such as EIA and nucleic acid testing (NAT) in determining the prevalence of HBV carriage in a group of 380 consecutive patients from an African urban hospital using a variety of tests.

ES, PO, CKO, MMK, HJY, NA, and DT all have no conflicts of interest.

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PATIENTS AND METHODS

This was a cross-sectional study in which consecutive patients admitted to the emergency medical ward of Mulago Hospital, Kampala, Uganda who met inclusion criteria were recruited in January 2006. The study was approved by the Department of Internal Medicine, Makerere University Medical School, Faculty of Medicine Research and Ethics Committee, Uganda National Council of Science and Technology, and the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas. Consecutive patients admitted to the ward for acute care of general medical problems were approached, and briefed on the study. Informed consent was obtained from those who expressed interest in participating. Minor children (ages 13–17; n = 12) were also included but had to assent before their legal guardians could consent on their behalf. Unconscious patients as well as those who declined any of the tests were excluded; 380 patients were recruited. Data collected along with serum specimens, included socio-demographic factors and risk factors to acquiring hepatitis B infection. Participants also had a physical examination relevant to liver disease.

Testing for anti-HIV was performed with the RSA and EIA: 171 were positive by both assays, 23 positive by EIA only, 9 by RSA only, and 177 were negative by both assays. Thus, 194 (51.1%) patients were considered to be HIV positive (all the EIA positive patients) on admission and these results were used in the remainder of the analysis (Fig. 1). No confirmatory western blot was performed.

Initial Testing for HBsAg Using RSA in Kampala

In Kampala, HBsAg testing was done on fresh serum samples using the Cortez Rapidtest® (Cortez Diagnostics, Calabasas, CA) within 1–2 hr. The one-step RSA HBsAg test is based on the principle of the sandwich immunoassay for determination of HBsAg in serum/plasma. Monoclonal and polyclonal antibodies are employed to identify HBsAg. After dipping the test strip in a cryovial containing serum for about 10 sec, the strip was laid flat on a clean, dry, and non-absorbent surface for 10–20 min and the result read. The presence of HBsAg triggers the chemical reaction leading to a pink color observed in the test region, and a similar pink colored band in the control region. In the absence of HBsAg, only one colored band would appear in the control region and none in the test region. The total absence of color in both regions would constitute an invalid test result.

Hepatitis B Serology Testing by Enzyme Immunoassay (EIA)

Additional aliquots of the 380 sera obtained from this cohort were stored at −80°C and subsequently shipped on dry ice to the University of Texas Southwestern Medical Center at Dallas. Serum HBsAg was measured using the Siemens Centaur® EIA platform (Siemens Diagnostics, Tarrytown, NY), a sandwich immunoassay using direct chemiluminometric technology. Patient sera are mixed with the Lite Reagent which contains biotinylated anti-hepatitis B surface (anti-HBs) mouse monoclonal capture antibody and aridinium-ester labeled anti-HBs mouse monoclonal antibody. If HBsAg is present, antigen–antibody complexes will form. Then streptavidin-coated magnetic latex particles are added to capture the antigen–antibody complex into the solid phase. After washing, the microparticles are flushed by acid/base addition. The relative light units (RLUs) detected by the ADVIA Centaur system are used to calculate the Index Value from the Master Curve. A result of reactive or non-reactive is determined according to a cutoff of 1.0 Index Value. The sensitivity and specificity of EIA for HBsAg on US samples is considered to be 100% (483/483) and 99.94% (5,259/5,262), respectively [van Helden et al., 2004].

Serum anti-hepatitis B core antibody (anti-HBc) was also tested using the Siemens Centaur® EIA platform

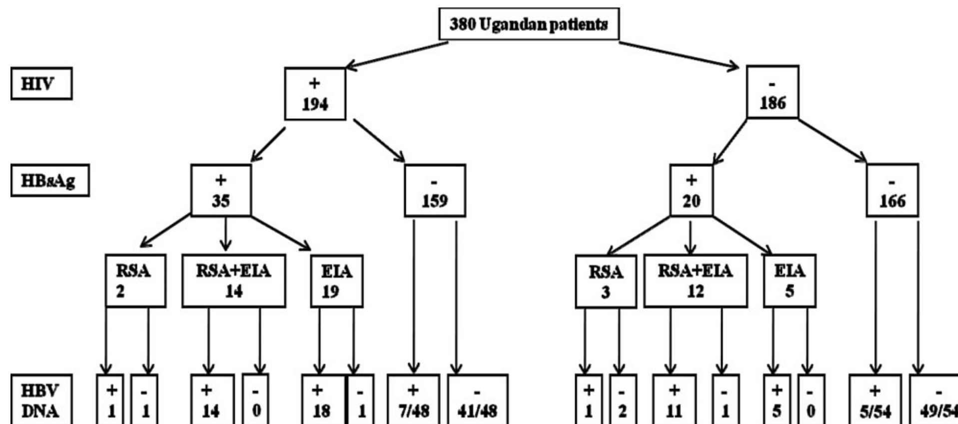

Fig. 1. Flow chart of HBV testing in 380 hospitalized Ugandan patients: 194 had HIV infection, 31 were HBsAg positive by RSA, and 50 patients by EIA. Overall, 55 patients were HBsAg positive: of these, 50 patients were serum HBV DNA positive. An additional 12 were HBV DNA positive but HBsAg negative.

(Siemens Diagnostics) in all samples. Serum anti-HBs, hepatitis B e antigen (HBeAg), and HBe antibody (anti-HBe) were also tested using the Siemens Centaur® EIA platform (Siemens Diagnostics) in 52 HBsAg positive sera.

Quantitation and Sequencing of Serum HBV DNA

Serum HBV DNA level was determined in the samples that tested positive for HBsAg by branched DNA (b-DNA) assay (Versant® 3.0, Siemens Diagnostics; lower limit of detection 357 IU/ml). HBsAg positive samples with undetectable serum HBV DNA by bDNA were further tested using a sensitive PCR assay (Amplicor®, Roche Diagnostics, Nutley, NJ; lower limit of detection ~50 copies/ml). An additional group of 102 samples testing negative for HBsAg in both assay systems were tested by the bDNA method to identify any cases of occult hepatitis B infection, defined as negative for HBsAg and positive for HBV DNA, typically at very low titers [Aye et al., 1997]. For genotyping and determination of resistant mutations, the HBV reverse transcriptase region [amino acid 1–344] was amplified by PCR as described [Tenney et al., 2004] using a Roche Amplicor thermocycler (Roche Diagnostics). The resulting products were sequenced to determine both the genotype and presence of known drug resistant mutations, the sequences being compared to the NCBI online genotyping program (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi).

Statistical Analysis

The Statistical Program for Social Sciences (SPSS 17.0 for windows; SPSS Inc. Chicago, IL) was used for all statistical analyses. Non-parametric testing was used to compare the continuous variables. Chi-square test or Fisher exact test was applied for categorical variables. P values of less than 0.05 were used to indicate statistical significance. Receiver operating characteristic curve (ROC) analysis was used to evaluate the RSA and EIA for the detection of HBsAg.

RESULTS

Demographic Data and HIV Results

Of the 380 patients studied, 211 were female with median age of 38 (13–87) years (Table I). Ten patients were from neighboring countries while the majority (232) came from the Central Ugandan Ganda tribe; however, there were 19 other tribes represented. The provisional diagnoses on admission (number) included malaria (59), pulmonary tuberculosis (47), disseminated tuberculosis (19), accelerated hypertension (19), lobar or bronchopneumonia (16), gastroenteritis (15), diabetes mellitus (12), liver failure/cirrhosis (8), hepatitis (6), and hepatocellular carcinoma (2). Forty-seven were jaundiced on admission, 32 had demonstrable ascites.

Detection of HBsAg by Cortez RSA and EIA

Of the 380 patients tested with both Cortez RSA (Kampala) and EIA (Dallas), 31 (8.2%) patients were HBsAg positive by RSA and 50 (13.2%) patients were HBsAg positive by EIA; 26 patients were HBsAg positive by both assays. In toto, 55 (14.5%) patients were HBsAg positive by at least one test (Table II and Fig. 1). HIV positive patients had a higher prevalence of serum HBsAg compared to HIV-negative patients [18.0% (35/194) vs. 10.8% (20/186), $P = 0.04$].

Serum HBV DNA Determination in HBsAg Positive Patients

Of 55 HBsAg positive patients (Table II and Fig. 1), 46 patients had detectable serum HBV DNA level by the bDNA assay; an additional 4 were positive by PCR only. The 26 patients that were HBsAg positive by both RSA and EIA had significantly higher median serum HBV DNA levels than did the 24 patients who were HBsAg positive by EIA only [median (range): 9.12×10^4 ($<3.57 \times 10^2$–1.79×10^7) vs. 1.02×10^3 ($<3.57 \times 10^2$–1.79×10^7) IU/ml, $P = 0.01$; Fig. 2]. The five patients that were HBsAg positive by RSA only included only one patient positive by bDNA who had a very low but detectable HBV DNA level (1,318 IU/ml). While 46/55

TABLE I. Comparison of Baseline Demographic Characteristics and HBsAg Results by EIA and RSA, HBV DNA Status

Study variable	Overall study population (N = 380)	HBsAg neg (N = 325)	HBsAg positive RSA/EIA (N = 26)	HBsAg positive EIA only (N = 24)	HBsAg positive RSA only (N = 5)	HBV DNA pos pts HBsAg pos (N = 50)	HBV DNA pos pts HBsAg neg (N = 12)
Mean age (years)	38	39	34	36	40	36	40
Gender n (%)							
Males	169 (45%)	144 (44%)	14 (54%)	7 (29%)	4 (80%)	22 (44%)	5 (41.7%)
Females	211 (55%)	181 (56%)	12 (46%)	17 (71%)	1 (20%)	28 (56%)	7 (58.3%)
Risk factors n (%)							
Blood transfusion	67 (18%)	56 (17%)	5 (19%)	5 (21%)	1 (20%)	10 (20%)	1 (8%)
Surgery	69 (18%)	62 (19%)	2 (8%)	4 (17%)	1 (20%)	5 (10%)	1 (8%)
IV drug use/needle sharing	15 (4%)	13 (4%)	1 (4%)	1 (4%)	0	2 (4%)	0 (0%)
Clinical profile n (%)							
Jaundice	47 (12%)	36 (11%)	9 (31%)	2 (8%)	0 (0%)	10 (20%)	3 (25%)
Splenomegaly	76 (20%)	66 (20%)	7 (27%)	3 (13%)	0 (0%)	10 (20%)	5 (42%)
Presence of HIV	194 (51%)	159 (49%)	14 (54%)	19 (79%)	2 (40%)	33 (66%)	7 (58%)

TABLE II. Serum HBV DNA Determination in HBsAg Positive Patients and HBsAg Negative Patients

RSA (Uganda)	EIA (Dallas)	No. of pts	No. of HBeAg+	No. of anti-HBe+	No. of anti-HBc+	No. of anti-HBs+	HBV DNA		Total no. (%)
							bDNA	PCR[a]	
Pos	Pos	26	12/25	9/18	19/26	0/26	24	1	25 (96.1%)
Neg	Pos	24	2/22	4/13	8/24	5/24	21	2	23 (95.8%)
Pos	Neg	5	0/5	3/5	3/5	3/5	1	1	2 (40%)
Neg	Neg	325	NA	NA	142/325	138/325	12/102[b]	NA	12/102 (11.8%)
Total		380	14/52	16/36	172/380	146/380	58	4	62

These numbers still do not match Figure 1. For example, there are 50 DNA positive plus 12 on the figure, giving 62, rather than 64 total. One is that there should be one less.
[a]Serum HBV DNA positive detected by PCR assay in samples that were negative by b-DNA assay.
[b]Serum HBV DNA levels were measured by bDNA assay in 102 samples that were HBsAg negative by RSA and EIA.

HBsAg positive sera tested positive by bDNA, the nine remaining patients included 4 who were HBV DNA positive by PCR assay (two EIA positive only, one RSA positive only, and one HBsAg positive by both EIA and RSA (Table II, Fig. 1). Five patients were HBsAg positive but no HBV DNA could be found by bDNA or a sensitive PCR. Of 46 HBsAg positive patients with detectable serum HBV DNA level by bDNA assay, the 31 HIV positive and 15 HIV negative patients had similar serum HBV DNA levels [median (range): 3.34×10^3 (4.10×10^2–1.78×10^6) vs. 2.36×10^4 (4.17×10^2–1.78×10^6) IU/ml, $P = 0.47$].

Serum HBV DNA Determination in HBsAg Negative Patients

Of 102 sera that were selected at random from the group that was HBsAg negative by both assays, 12 (11.8%) were determined to be HBV DNA positive by bDNA assay, all with very low serum HBV DNA levels [median (range): 937 (380–1,875) IU/ml]. All 12 were confirmed positive by PCR assay. Patients who were HBsAg negative were similar regardless of their HIV status: serum HBV DNA positive rates were 14.6% (7/48) versus 9.3% (5/54) among the HIV-positive and HIV-negative groups, respectively, $P = 0.41$, while serum HBV DNA levels were similar [median (range): 1.00×10^3 (3.80×10^2–1.87×10^3) vs. 8.69×10^2 (6.10×10^2–1.60×10^3) IU/ml, $P = 0.42$] between the HIV positive and negative groups as well. Five of 12 patients were anti-HBc positive. None of the 12 was positive for hepatitis C antibodies by the Centaur assay (data not shown).

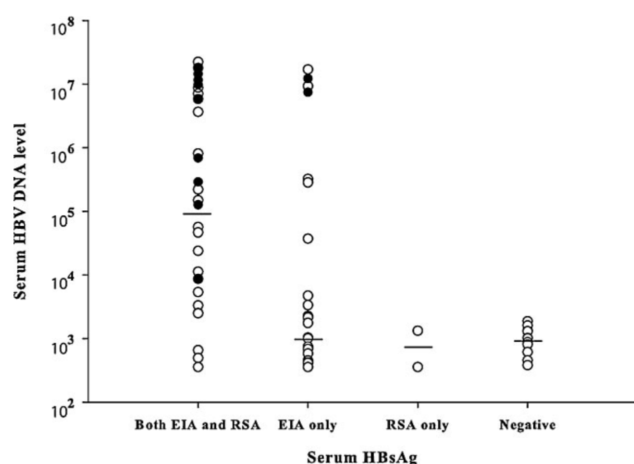

Fig. 2. Serum HBV DNA levels in 50 HBsAg positive and 12 HBsAg negative patients. "●" represents 14 HBeAg positive patients, "○" represents HBeAg negative or not tested patients. Those who were HBsAg positive by RSA and EIA had significantly higher median serum HBV DNA values than those who were positive by EIA or RSA only. Two of five patients who were HBsAg positive by RSA only were serum HBV DNA positive (one by bDNA and another by PCR). Twelve of 102 patients who were HBsAg negative were found to have low serum HBV DNA levels by bDNA assay as well.

Sensitivity and Specificity of HBsAg Assays

As mentioned above, 157 patients were tested for HBV DNA and 62 patients were HBV DNA positive. Using the serum HBV DNA result as the gold standard, the sensitivity and specificity of the HBsAg tests in detecting active HBV infection were 43.5% and 95.8% for RSA and 77.4% and 97.9% for EIA. The positive predictive value (PPV) and negative predictive value (NPV) were 87.1% and 72.2% for RSA, 96.0% and 86.9% for EIA, respectively. Thus, the EIA and RSA have similar specificity but the RSA has much lower sensitivity than does EIA. The areas under the receiver operator curve (AUROC) were 0.69 and 0.86 for RSA and EIA, respectively (Fig. 3).

Frequency of Serum Anti-HBc and Anti-HBs Antibody

In toto, 172 (45.5%) patients were serum positive for anti-HBc antibody (total) (Table II). The prevalence of anti-HBc positivity was 54.5% (30/55) in HBsAg positive patients and 43.7% (142/325) HBsAg negative patients ($P = 0.14$). The prevalence of sera that were anti-HBc positive was significantly higher in patients who were HBsAg positive by RSA compared with those who were HBsAg negative by RSA [71.0% (22/31) vs. 43.0% (150/349), $P = 0.003$]. However, more than 25% of patients positive for HBsAg by RSA had undetectable total anti-HBc levels. Patients who were HBsAg positive by EIA had similar anti-HBc positive rates compared with those who were HBsAg negative by EIA [54.0% (27/50) vs.

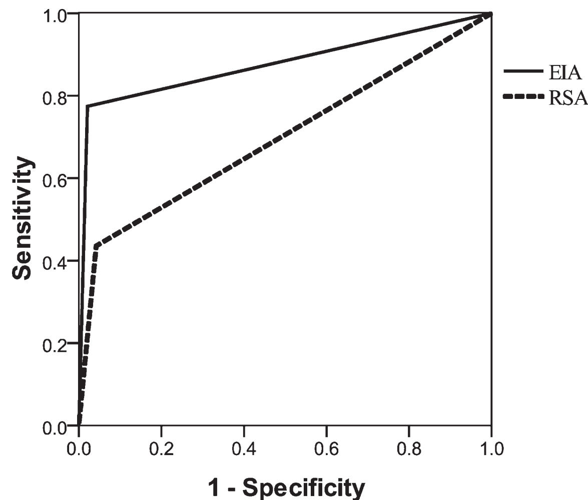

Fig. 3. Receiver operating characteristic curve (ROC) analysis of RSA and EIA for detection of HBsAg in 157 patients who were tested for serum HBV DNA. The solid line represents EIA. The dash line represents RSA. The area under ROC (AUROC) were 0.86 and 0.69 for EIA and RSA, respectively.

43.9% (145/330), $P = 0.18$]. Of 62 HBV DNA positive patients, 33 (53.2%) patients were anti-HBc positive; 40 were co-infected with HIV. The prevalence of anti-HBc total positivity in HBV DNA positive patients with and without HIV co-infection was 57.5% (23/40) and 45.5% (10/22), respectively ($P = 0.36$). Among 95 patients negative for serum HBV DNA, 45 (47.4%) patients were anti-HBc positive; 43 (45.3%) were HIV co-infected. The prevalence of anti-HBc positivity in HBV DNA negative patients with and without HIV co-infection were 41.9% (18/43) and 51.9% (27/52), respectively ($P = 0.33$).

Anti-HBs was tested on all patients and the results shown in Table II; 146 of 380 were anti-HBs positive, 138 of these were negative for both HBsAg tests. No patient who was HBsAg positive by both RSA and EIA was also positive for anti-HBs.

Frequency of HBeAg and Anti-HBe Among HBsAg Positive Samples

Of the 55 patients who were HBsAg positive, 52 had additional available serum testing for HBeAg: 14 (27%) patients were HBeAg positive. Those who were HBsAg positive by both EIA and RSA were significantly more likely to be HBeAg positive than those who were HBsAg positive by EIA only or by RSA only [48% (12/25) vs. 9.1% (2/22) vs. 0% (0/5), $P = 0.004$]. Of the 34 patients who had serum available for testing for anti-HBe, 15 patients were anti-HBe positive (Table II).

Genotyping and Resistance Mutations

In 31 HBsAg positive patients who had serum available, 29 patients were HBV DNA positive by PCR: 26 samples were genotype A and one, genotype D. The genotypes were indeterminate in the remaining two samples. Three patients had a mutation in the YMDD region at codon 204, from methionine to isoleucine (M204I), indicative of resistance to lamivudine. Additional sera were available on 10 of 12 patients who were *HBsAg negative* and had detectable serum HBV DNA level by b-DNA assay. Nine samples tested were genotype A, while one sample demonstrated genotype D. One patient in this cohort was shown to have the M204I mutation.

DISCUSSION

In this cross-sectional study of patients admitted to a medical ward in a large city hospital in Kampala, Uganda, a number of surprising serologic findings were observed: a low apparent prevalence of HBV (8%) if RSA alone is used, but a much higher apparent prevalence (13.2%, 50/380) observed using EIA. The true prevalence of ongoing HBV infection appears to be even higher if HBsAg negative, HBV DNA positive patients are included. The overall prevalence is within the expected range for HBV in a highly endemic area such as sub-Saharan Africa, but seems low for an in-hospital population, given the very high prevalence of HIV (51%) observed in this study in a region where the reported community HIV prevalence is 6–7% [Tswana et al., 1996; Abebe et al., 2003; Ocama et al., 2005; Uneke et al., 2005]. The relatively low prevalence of chronic HBV infection in the face of high HIV carriage would seem to imply that HBV exposure occurred early and prior to HIV, so that patients were immune to HBV at the time of HIV infection. This is consistent with the high prevalence of anti-HBs in this population (38% overall) and with infection with hepatitis B acquired frequently early in life in African countries. A recent, large population-based seroprevalence study across Uganda showed a variable HBsAg carriage rate, with Kampala having only 5.3% HBsAg seroprevalence, with other regions as high as 20.7% [Bwogi et al., 2009]. It is not possible to extrapolate the incidence figures to the population as a whole; however, these data underline the relative high burden of HIV and comparatively low burden of active HBV infections in this African in-hospital setting.

Overall, the viral loads observed in this study were similar to those that might be expected in a Caucasian US population but lower than those that might be observed in Asia or in Asian Americans (Fig. 2) [Lok and McMahon, 2001]. It is important to note that 35/39 sera tested were positive for genotype A, similar to a US Caucasian population. Likewise, the median viral load in the RSA and EIA positive group was approximately 9.12×10^4 IU/ml while those positive in the EIA only had viral loads of 1.02×10^3 IU/ml. Similarly, the overall number of patients who were HBeAg positive was relatively low at 27%. Among those positive in both assays, only 48% were HBeAg positive, while only 9% of the EIA only group were HBeAg positive. Among the remaining RSA positive only and HBsAg negative groups, no viral load exceeded 10^4 IU/ml and none was HBeAg positive. These results suggest that RSA, as

expected, perform best in patients with high viral loads but do not identify reliably those who have seroconverted to HBeAg negative and have low viral loads, the majority of cases. The use of color development on a strip followed by visual inspection of the results is much less sensitive compared to the automated detection of a fluorescent signal via EIA. Two of the five patients who were HBsAg positive by RSA only had very low viral loads, the remaining three with undetectable HBV DNA, suggesting that these three might even represent false HBsAg positivity by RSA, resulting either from: (1) hemolysis during sample processing, (2) operator's misinterpretation of results, or (3) non-specific binding because both monoclonal antibody and polyclonal antibodies are coated on the strip [Dufour, 2006].

It was also surprising to find a relatively low sensitivity for EIA in Ugandan patients. About 11% of patients negative for HBsAg had low but detectable serum HBV DNA levels, considered to represent occult infection [Allain et al., 2009]. All had relatively low serum HBV DNA levels ($<$1,875 IU/ml) but were confirmed by PCR. This observation further underlines the need for sensitive nucleic acid assays in this population for transfusion screening purposes. The high prevalence of occult HBV infection might be related to high prevalence of HIV infection in Uganda [Baumert et al., 2005]. Due to shared transmission routes, it is likely that HIV infection increases the risk of HBV co-infection, and enhances HBV replication and disease progression [Baumert et al., 2005]. Of note, none of the 12 testing positive for HBV DNA was anti-HCV positive, a feature said to be associated with occult HBV in other settings [Cacciola et al., 1999]. On the other hand, with the availability of highly active antiretroviral treatment (HAART) in Africa in the recent years, the prevalence of lamivudine resistant mutants is increasing [Selabe et al., 2007]. Lamivudine resistant mutations have been reported to cause the amino acid substitutions in both the pol and envelope genes, thus affecting HBsAg and its detection [Ono-Nita et al., 1999; Yeh et al., 2000]. In this study, only 4 of 39 patients tested had a YMDD mutation (M204I). Currently, negative HBsAg results should be interpreted with caution; a recent study has suggested that the Bayer/Siemens Centaur HBsAg may fail to detect certain HBsAg mutants [Ly et al., 2006].

About 45–47% of serum HBV DNA negative patients had positive anti-HBc antibody, suggesting a high rate of past HBV infection in Uganda. Anti-HBc antibody should be detectable lifelong once infected with HBV [Lee, 1997]. It is surprising that more than 40% of HBsAg positive patients were shown to have no detectable anti-HBc antibody in this cohort of patients. This cannot be explained as immune system dysfunction caused by HIV infection since patients infected with and without HIV had comparable anti-HBc positivity rates. Further studies will be necessary utilizing other EIA; virologic study might also be required to analyze HBV sequence in surface and core region. Low titer HBV DNA positivity is less likely to be associated with active clinical disease [Lok and McMahon, 2001]; however, even low levels of viremia may be associated with transmission of infection at least via blood transfusion.

By contrast, the anti-HBs results appear to follow our expectations more readily: 138 of 325 HBsAg negative patients had anti-HBs as evidence of prior exposure and immunity, while all patients positive for HBsAg by both EIA and RSA were negative for anti-HBs. Those positive for HBsAg by only one of the two assays showed intermediate results—some were positive for anti-HBs in each group (Table II).

HBeAg and anti-HBe testing was performed only on sera that had been shown to be HBsAg positive by at least one assay. Results confirmed that half those found positive by both HBsAg assays were HBeAg positive, while only two additional HBeAg positives were found among the EIA only sera. Also consistent with expectations, of the 16 anti-HBe positive samples identified among 36 samples tested, 12 were negative for HBeAg, and four were positive for both HBeAg and anti-HBe although values were near the cutoff value for both assays in each of these four sera.

This study has several limitations. Serum HBV DNA levels were not screened in all of the 380 sera; only those positive for HBsAg were tested, plus 102 randomly selected additional samples. Thus, the validity of RSA and EIA was based on the subset of 157 samples that were subjected to all three testing methods (RSA, EIA, and NAT). It is also important to note that since this was an in-hospital rather than a population-based prevalence study, the HBV prevalence rates were likely higher than would be observed in out-of-hospital populations. Finally, any of the serologic tests could have failed to detect infection if the patient presented for hospitalization during the "window" period; this is relatively unlikely.

In conclusion, this study has shown that RSA testing needs to be supplemented at least by EIA in order to better identify chronic HBsAg carriers. Neither HBsAg assay effectively identified the significant number of "low viremia" HBV infections that seem particularly prevalent in this population. With such a high number of false negative results associated with the commonly used RSA tests, more widespread use of EIA and possible supplementation by NAT may be necessary to fully screen for HBV infection in Africa. While the cost of such measures appears prohibitive, implementation should be considered as one disease control strategy, particularly for blood bank screening.

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