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Evaluation of Diagnostic Tests for the Detection of HBsAg in Sample DBS in Children Born to HIV-Positive Mother to HIV-1 in Senegal (Determine AgHBs® vs Microscreen ELISA and HBsAg® Architect HBsAg Qualitative II®)

Abstract

Introduction: According to the World Health Organization, Senegal is the country presenting one of the highest prevalence of HBV (≥ 8%) in the world. However, the diagnosis of this disease remains inaccessible in some areas of the country. We hypothesized that the use of Dried Bloot Spots (DBS) may be potentiated as in the cases of HIV through the use of rapid diagnostic tests to facilitate decentralization of the biology of HBV.

Objective: The aim of our study was to evaluate and to compare the sensibility, specificity of three new diagnostic tests with the gold standard test Architect HBsAg Qualitative II® for the diagnosis of HBsAg from DBS samples. .

Method: This is a retrospective and comparative study of DBS samples collected from 930 patients between July 2007 and November 2012 in the decentralized sites of Mother-Child HIV Transmission Programme in Senegal. These samples were submitted to hepatitis B antigen detection using three independent tests: the test kit Determine HBsAg®, ELISA Kit Microscreen HBsAg® and ELISA kit Architect HBsAg Qualitative II ®.

Results: Patients were predominantly male with 520 (56.0%). Use of HBsAg ® Determine kit showed no reactivity to HBsAg suggesting zero sensitivity. However, we found a sensitivity of 43, 48% [23.22 -63.74], a specificity of 99.30% [98.34 to 100.27], a PPV of 83.33% and a VPN of 95.64% for the ELISA Microscreen AgHBs® overlooked ELISA Qualitative Architect II HBsAg ®. Inversely to the sensitivity of the Architect II Qualitative HBsAg® vis-à-vis Microscreen HBsAg ELISA® is 83.33 % [62.25 to 104.42], specificity was 95.64% [93.32 to 97.96] the positive predictive value was 43.48 (PPV), and predictive negative value (NPV) is 99.30%.

Conclusion: ELISA Microscreen AgHBs® is the only new test that shows interesting results on the detection of HBsAg. These data and could glimpse its usefulness in the diagnosis of this disease on DBS.

Keywords: HBV; DBS; HIV; Rapid diagnostic tests; Senegal

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Abbreviations: HBV: Hepatitis B Virus; MTCT: Mother-Child Transmission Programme; DBS: Dried Bloot Spots; VPN: Negative Predictive Value; PPV: Positive Predictive Value

Introduction

Infection with Hepatitis B virus (HBV) is a major public health problem worldwide. In 2010, the World Health Organization (WHO) estimates that over 2 billion people have been in contact with 350 million of those suffering from chronic HBV infection. More than one million of them die each year from complications related this infection, particularly cirrhosis and hepatocellular carcinoma, making HBV the second major cause of cancer after smoking. The risk of chronicity is 90% in children under one year, 25-30% in children aged 1 to 5 years in the context of family transmission [1-4].

Sub-Saharan Africa is a high endemic area for HBV, with a porting prevalence of HBsAg exceeding 8%. Transmission of HBV from mother to infant occurs mainly during the perinatal period. It is particularly high (70-90%) when the mother carries HBV replication markers. In the absence of active replication, the risk of transmission is only 10 to 40% [5]. The prevalence of HBsAg was 12.7% in a cohort of patients living with HIV in Burkina Faso [6], 15.4% in Niger donors [7] and 13.9% in Mali among blood donors respectively. Diarra et al. [8] in North Africa, the prevalence of HBsAg seem low, with only 4% in pregnant women, in Tunisia [9] and 1.6% among volunteer in the general population in Morocco [10]. In Senegal, according to the national program the fight against hepatitis (PNLH), 85% of the population have at least one marker of HBV and porting of chronic infection in the general population is 17%; mortality from this virus is not assessed but seems high [www.hepatites.sn/le-pnlh However, safe and

effective vaccines against hepatitis B are available [1,11-13]. In many countries, mass immunization campaigns, as recommended by WHO, has led to significant reductions of the incidence of HBV infection in infants, children and adolescents [14].

In Senegal, the vaccination is carried out from 6 to 8 weeks with the hexavalent vaccine (*Diphtheria, Tetanus, Pertussis, Polio, Haemophilus inflenzae b* and *hepatitis B* in a complete scheme of 3 injections, the first two a month of Meanwhile, the third at 6 months after the date of the second injection [1,13,15]. The markers of HBV are numerous and their prescription differs depending on the context. Several strategies defined for the screening of this infection exist. In Senegal, HBsAg is the marker used to screen HBV infection. The reference specimen for research of this marker is serum/plasma [4,16,17]. Dried Bloot Spots (DBS), widely used for HIV serology and neonatal diagnosis of HIV infection [18,19] have also been evaluated for the detection of viral and serologic markers of HBV [20,21]. There is quick and accessible testing for HIV DBS [22]. The use of DBS may be potentiated, as in the case of HIV, through the use of rapid diagnostic tests to further facilitate the decentralization of the biology of HBV. This proposal nationwide will have to go through the evaluation of their performance on the media [23]. However, to date, no assessment data such tests have been published in the African context. In this situation, this work has been undertaken and aims to evaluate diagnostic tests for the detection of HBsAg: Determine the AgHBs® (Abbott Diagnostics Japan), the ELISA kit Microscreen HBsAg® (Span Diagnostics Ltd.) and the ELISA kit Architect HBsAg Qualitative II collected in different health centers and hospitals in Senegal. ®(Abbott Diagnostics Japan) from DBS.

Methodology

Population

This is a retrospective and comparative study of DBS samples collected between July 2007 and November 2012 in decentralized sites of Mother- Child Transmission Program (MCTP) of HIV in Senegal. All DBS valid collected from patient's age from 2 weeks to 15 years old and born to HIV-positive mothers were included. DBS invalid, serum child out of PMTCT are excluded.

Confection and DBS management

DBS was made by direct recollection drop blood on filter paper after bite with vaccine style heel for children under 10 kg and in the toes or fingers for children over 10kg. DBS were dried at room temperature in a sealable plastic bag containing desiccant and a humidity indicator are used in many closed envelope. The moisture content is checked every two days. Desiccants and indicator are changed if the indicator in the bag reaches 30% humidity. They are sent to the laboratory of reference along with their profile and their transmission analysis report, avoiding sunlight and moisture during transport. In the laboratory of reference, the sampling of Compliance is checked, then the DBS is retained for immediate or further processing, stored at room temperature or at - 80° C. An analysis report is attached to each DBS and shall contain in addition to the request sampling date, patient ID, sex, age, serological profile of the mother, the treatment regimen and duration of followed if the patient is on ART.

Neonatal HIV diagnosis

HIV status of children was determined by molecular diagnostics using the Cobas Amplicor platforms Cobas TaqMan, NucliSens, Biocentric® PCR or "house" HIV-2. Detection of HBsAg. Two HBV diagnostic tests were evaluated from elution made with PBS at 10%. Each DBS, 3 discs were punched in a saturated zone of blood and a volume of 200µl of PBS 10% was added by DBS disc. The mixture is left overnight at 4° C and the eluate was tested immediately or stored at -20°C until use. ELISA Architect HBsAg Qualitative II ® (Abbott Diagnostics Japan) was the gold standard in our study. This is an automated, closed system, which detects the genotypes A to F and H, with an average analytical sensitivity of between 0.019 and 0.020 U/ml for plasma. It was designed to improve the detection of mutants and sero conversion samples. All samples were tested by this method and the positive cases were retested two times according to the procedure specified by the confirmation test manufacturer.

Was achieved by this technique with the kit HBsAg confirmatory qualitative II®. Determine AgHBs® (Abbott Diagnostics Japan) is an immuno-chromatographique test used for all samples. 100 DBS formed by both positive and negative Architect selected at a pitch of 26, were retested for this control method. Microscreen HBsAg® ELISA (Span Diagnostics Ltd.) is a manual direct ELISA that also detects all subtypes of HBsAg with analytical sensitivity of the kit 0,2PEU / ml in serum / plasma. It was used for the selected positive and 300 negative Architect at a pitch of 3.

Statistical analysis of data

Data were analyzed using EPI INFO 7. The performance tests were determined by calculating the sensitivity, specificity, positive predictive value and negative predictive value. The kappa test was used to measure the degree of agreement between the tests with the interpretation grid Landis and Koch [24].

Results

Population characteristics

The study population consists of 930 patients followed in decentralized sites supported PMTCT. The overall analysis of this population allowed us to highlight different features below. DBS samples came from more regions of Dakar and Ziguinchor, respectively with 34.4% (320/930) and 14.2% (132/930). Patients are male majority with 520(56.0%) and 409 female (44%) with a sex ratio M/F = 1.27 in favor of males. The average age of our population was 26.5 weeks and the median age of 20 weeks. It varies from 2 weeks to over 48 weeks with a standard deviation of 36 weeks. The most represented age groups that are less than 6 weeks and that between 6 and 12 weeks with 23.9% and 17.9% respectively (Table 1).

Molecular diagnosis of HIV was positive in 24 cases or 2.6% of the population (Table 1). The techniques used were Cobas Amplicor (563 tests or 60.5%), Cobas TaqMan (181 tests or 19.4%), NucliSens® (163 tests or 17.5%), PCR "house" HIV-2 (16 tests or 1.7%) or Biocentric® (8 tests or 0.9%). The overall analysis of the mothers of the status shows a majority prophylactic use of AZT + 3TC + NVP combination Search HBsAg and performance tests. All DBS of our population were tested ELISA Qualitative Architect II HBsAg® Determine HBsAg and the search for HBsAg. Staffs of 28 DBS was responsive but none were reactive Determine HBsAg®. DBS 310 having positive ELISA Qualitative Architect HBsAg® II, 12 were reactive in ELISA Microscreen HBsAg® (Table 2). HBsAg

was found more in the most represented age group (less than 6 weeks) with 11 positive 5.05% (11/218). Slices of 18-24 weeks and 12-18 weeks followed with 5/109 respectively (i.e. 4.59%) and 3/74 (i.e. 4.05%). HBsAg was found slightly among female children than male children respectively girls 15/409, 13/521 3.67% and 2.5% boys. In the study population, 24 cases of HIV and 28 cases of HBsAg were found, but no cases of co-infection (Table 3) Using Determine HBsAg®, we found no reactivity to HBsAg, resulting in zero sensitivity.

The sensitivity of the ELISA Microscreen AgHBs® vis-à-vis of ELISA Qualitative Architect HBsAg® II is 43 48% [23.22 -63.74], the specificity of 99.30% [98.34 to 100, 27]; the positive predictive value of 83.33% (PPV), and negative predictive value (NPV) of 95.64%. The value of kappa 0.548 watches an average degree of agreement between the two tests (Table 4) .Inversely, the sensitivity of the Architect II Qualitative HBsAg® vis-à-vis of Microscreen HBsAg ELISA® is 83.33 % [62.25 to 104.42], specificity was 95.64% [93.32 to 97.96] the positive predictive value was 43.48 (PPV), and negative predictive value (NPV) is 99.30%.The kappa value of 0.548 shows an average level of agreement between the two tests (Table 5).

Table 1: Characteristic of the study population.

Characteristic	Effective	Percentage
Areas		
Dakar	320	34,4
Diourbel	45	4,8
Fatick	36	3,9
Kaffrine	2	0,2
Kaolack	57	6,1
Kédougou	15	1,6
Kolda	50	5,4
Louga	40	4,3
Matam	14	1,5
Saint-Louis	58	6,2
Sédhiou	63	6,8
Tambacounda	44	4,7
Thiès	46	4,9
Ziguinchor	132	14,2
Non précisée	8	0,9
Sex (ratio M/F=1,27)		
Masculine	520	56,0
Féminine	409	43,9
Non precise	1	0,1
Age brackets (Age median 20 weeks)		
< 6 weeks	218	23,4
6 - 12 weeks	163	17,5
12 - 18 weeks	74	7,9
18 - 24 weeks	109	11,7
24 - 30 weeks	64	6,9
Table 2: T 30 - 36 weeks	101	10,8
36 - 42 weeks	35	3,8
42 - 48 weeks	53	5,7
> 48 weeks	95	10,2
Non précisée	18	2,0
HIV status		
Negative	905	97,3
Positive	24	2,6
Doubtful	1	0,1
Total	930	100,0

Table 2: Distribution of population according to HBsAg tests Research.

Research HBsAg test	Effective	Percentage
ELISA Architect HBsAg		
Reactive	28	3,0
Non reactive	902	97,0
Total1	930	100,0
ELISA Microscreen HBsAg®		
Reactive	12	3,9
Non reactive	298	96,1
Total2	310	100,0
Determine Ag HBs		
Reactive	00	00,0
Non reactive	930	100,0
Total3	930	100,0

Table 3: Distribution ded the results of HBsAg testing based on Characteristic of the population.

Characteristic	AgHBs	Total	
Age brackets (n=912)p=0,415	**Reactive Age brackets**	**Non reactive**	
< 6weeks	11	207	218
6 - 12 weeks	2	161	163
12 - 18 weeks	3	71	74
18 - 24 weeks	5	104	109
24 - 30 weeks	1	63	64
30 - 36 weeks	2	99	101
36 - 42 weeks	0	35	35
42 - 48 weeks	2	51	53
> 48 weeks	2	93	95
Sex (n=929) p=0,302			
Masculine	13	507	520
Feminine	15	394	409
Statut VIH (n=930) p=0,671			
Negative	28	877	905
Positive	0	24	24
Doubtful	0	1	1

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Table 4: Microscreen ELISA HBsAg® performance vis-à-vis de ELISA Architect Qualitative II HBsAg ®.

Distribution of samples tested by Microscreen ELISA HBsAg® et ELISA Architect Qualitative II HBsAg® (P=0,000 et Kappa=0,548)				
		Architect HBsAg		Total
		Reactive	Non reactive	
Microscreen	Reactive	10	2	12
	Non reactive	13	285	298
		23	287	310
Microscreen ELISA HBsAg® performance vis-à-vis de ELISA Architect Qualitative II HBsAg®				
Sensibility		43,48% [23,22 – 63,74]		
Specificity		99,30% [98,34 – 100,27]		
VPP		83,33		
VPN		95,64		

Table 5: Architect Qualitative II HBsAg® performance vis-à-vis de Microscreen ELISA HBsAg ®.

Distribution of samples tested by ELISA Architect Qualitative II HBsAg® et Microscreen ELISA HBsAg® (P=0,000 et Kappa=0,548)				
		Microscreen		Total
		Reactive	Non reactive	
Architect HBsAg	Reactive	10	13	23
	Non reactive	2	285	287
Total		12	298	310
Performance de ELISA Architect Qualitative II HBsAg ® vis-à-vis de Microscreen ELISA HBsAg®				
Sensibility		83,33% [62,25 – 104,42]		
Specificity		95,64% [93,32 – 97,96]		
VPP		43,48		
VPN		99,30		

Discussion

We performed the evaluation of two diagnostic tests: ELISA kits Architect HBsAg Qualitative II ® and Microscreen HBsAg®, in order to implement them as new tools for the diagnosis of HBV. This evaluation was based on the comparison of the sensitivity and the specificity of these two tests by those of the HBsAg Determine® which is the gold standard for this disease. The sample used for this purpose was the DBS. The detection of HBsAg for the diagnosis of HBV-related disease is based on the use of serological tests from serum or plasma [4,25]. DBS, which is widely used for molecular diagnosis and neo-natal diagnosis of HIV infection [11], have been evaluated for the detection of viral and serologic markers of HBV [15,21]. In our study, we aim to transpose the use of DBS for the detection of HBV by rapid diagnostic tests, which in turn may be potentiated as in the case of HIV, to further facilitate the decentralization of the biology of HBV.

In 1981, Villa et al. [24] in order to identify a new epidemiological tool on markers of HBV, have made a comparison study using DBS / serum storage conditions and DBS different elution. They showed allow sensitivity of antibodies with radio immunoassay method and a better detection of HBsAg DBS [25]. More recently, the DBS has been used on enzyme immunoassay techniques to study the prevalence of HBV infection in endemic areas and risk groups such as drug users in Spain [26,27]. The same method was use in a study of Komas et al. [28] in which they determined the prevalence of HBV markers in a cohort of students in Bangui, Central African Republic [28]. Mahfoud et al. [29] in the same year reported the prevalence of HBsAg among Lebanon prisoners as well [29]. Architect HBsAg Qualitative II® as gold standard. Architect HBsAg is an automated, closed system, which detect genotypes A to F and H, with an average analytical sensitivity of between 0.019 and 0.020 U / ml for plasma.

It was designed to improve the detection of mutants and sero-conversion samples. Microscreen HBsAg ® ELISA is a direct manual technique also allowing the detection of all AgHBs subtypes with an analytical sensitivity of the kit 0,2PEU / ml in

serum / plasma. The comparative study serum/DBS by Ross et al. [30] conducted in Germany in 2013 showed a good sensitivity Architect for the use of DBS. Among 1762 on paired samples of serum/DBS they got a specificity of 100% and an analytical sensitivity of 98.6% DBS vis-à-vis of the serum. These data which comforts our position in the use of Architect as the gold standard in our series of evaluation. In fact, their results indicate that it is possible to reliably detect the HBs antigen by analytical systems as the Abbott Architect with DBS samples.

Performance testing

The fact that no positive HBsAg was detected by Determine ® gives zero sensitivity vis-à-vis of the technique Architect HBsAg Qualitative II ®. Among 23 positive samples detected in triplicate by Architect HBsAg, only 10 were processed by Microscreen AgHBs® ELISA (Table 4) Compared to Architect Qualitative ELISA II HBsAg®, to low sensitivity of 43.48% [23.22 -63.74], a specificity of 99.30% [98.34 to 100.27], to positive predictive value of 83.33% (PPV) and negative predictive value (NPV) of 95.64% were found with this test.

The kappa value (0, 548) shows a moderate degree of agreement between the two tests. These results seem to corroborate those found by Maity et al. [31] stating that this test has a low performance in the serum; According to their study which compares diagnostic kits available on trade to assess their effectiveness in detecting HIV infection, HBV, HCV serum HBsAg Microscreen (Span Diagnostics Ltd.,) Microscreen AgHBs® had not revealed the expected results [31] this is an argument against the use and explains its low vis-à-vis sensitivity Architect®. Furthermore, Forbi et al. [32] showed that another ELISA micro plate (Shan test TM HBsAg ELISA) showed low performance DBS vis-à-vis the serum with a sensitivity of 78.6% and a specificity of 88.6% nevertheless higher than ours; the poor performance of the ELISA micro plate would thus resulting in a reduced ability of these techniques to detect HBsAg DBS [32]. Given the results of the tests for determining performance in our study, we find that Determine® HBsAg has zero sensitivity vis-à-vis the Architect HBsAg Qualitative and Microscreen® HBsAg ELISA on DBS. Which is not the case in the work performed on serum Rajaonatahina et al. [33] in Madagascar who evaluated Bioline and Determine with as gold standard Genscreen HBsAg manual ELISA test (BioRad France).

The evaluation tests with 76 positive and 74 will be negative for HBsAg Determine® and 40 positive sera and 49 negative sera for SD Bioline® HBsAg showed characteristics Determine® HBsAg (sensitivity = 96.1%, specificity = 93.2%, PPV = 93.6%, NPV = 95.8%) higher than SD Bioline® HBsAg but close. The results obtained show that the optimal performance of the rapid tests on serum is reached for the detection of HBsAg in their study [33].Their results raise several research questions: HBsAg is it therefore not detectable by the DBS Determine® HBsAg? Or common intrinsic characteristics of our study population - such as HIV-positive mothers, all children under 15 years prophylactic treatment, storage conditions DBS - have they had an impact on the result of this test?

Conclusion

Our study focused on the evaluation of three new diagnostic tests, Determine® HBsAg, ELISA and ELISA Microscreen HBsAg® Architect Qualitative II HBsAg® for detection of HBsAg on Dried Bloot Spots (DBS), the Laboratory of Bacteriology -Virology the Aristide Le Dantec hospital in Dakar. The sample was collected from the DBS decentralized PMTCT sites in Senegal in children born to HIV-positive mothers. The performance tests were determined by calculating the sensitivity, specificity, positive predictive value and negative predictive value, and the degree of agreement between the tests by measuring kappa. The study population was comprised of 930 children, predominantly male with an average age of 26.5 weeks and a median age of 20 weeks. We used ELISA Qualitative Architect II HBsAg® as gold standard and ranked ELISA Microscreen HBsAg® and Determine® AgHBs. Determine® HBsAg was reactive with any sample DBS, which gives it a zero sensitivity. By contrast, among 23 DBS reagents ELISA Qualitative Architect HBsAg® II, only 10 were detected by ELISA Microscreen HBsAg® with a low sensitivity (43, 48% [23.22 -63.74]) Microscreen ELISA AgHBs® vis-à-vis Architect Qualitative ELISA II HBsAg® significant and moderate degree of agreement between the two tests (kappa=0,548.). The Determine® HBsAg cannot be used with DBS to date to facilitate decentralization of the biology of HBV like HIV. But would be on Architect HBsAg Qualitative II in triplicate and Microscreen AgHBs® gave us the results suggesting its usefulness in the diagnosis of HBV. The Following recommendations are based on our study: - HBV screening by Determine HBsAg from DBS samples would not be appropriate for now but would be on Architect HBsAg Qualitative II in triplicate. - Have samples of the mother-child couples to better appreciate the rate of transmission of HBV infection.

Outlook

In order to achieve more substantial results, further work is required and must include samples of the mother-child couples to better appreciate the rate of transmission of HBV infection, the revaluation of DBS storage conditions for search for HBsAg and HBsAg on Determine® conducting research with paired samples DBS / Serum.

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