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## **Seroprevalence of HBsAg among Female Patients Seeking Healthcare in Bo Government Hospital, Southern Sierra Leone: 14-Months Database Study**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author JBK designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author MK managed statistical analyses, literature searches and wrote the study ethics. All authors read and approved the final manuscript.*

**Original Research Article**

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### **ABSTRACT**

**Background:** Chronic hepatitis B virus (HBV) seroprevalence varies globally but is positively correlated with the prevalence of HIV since both have the same route of transmission-contact with infected body fluids. Sierra Leone has a nascent hepatitis B epidemic and there is a paucity of research data and public health awareness about the infection. Bo where this research is done is the second largest city of Sierra Leone and is the regional headquarters of southern Sierra Leone.

**Aim:** To determine the seroprevalence of hepatitis B surface antigen (HBsAg) among female healthcare seekers who used the main government referral hospital in Bo, southern Sierra Leone.

**Methodology:** We retrospectively analysed the serological diagnostic results of HBsAg for female healthcare seekers at the Bo Government Hospital. We later determined the HBsAg seroprevalence for various categories of the study subjects; pregnant women, lactating mothers, girls 5-18years and adults >18years for the period under review.

**Place and Duration of Study:** This study is a multicenter investigation conducted at the

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Bo Government Hospital laboratory, the Departments of Community Health and Clinical Sciences and Environmental Health Sciences at Njala University in Bo southern Sierra Leone. All laboratory diagnostic tests for HBsAg diagnosis were conducted at the Bo Government Hospital laboratories from May 2012 to June 2013. Literature review and data analysis were done at Departments of Community Health and Clinical Sciences and Environmental Health Sciences at Njala University

**Study Subjects:** A total of 2,218 female subjects took part in this investigation; girls 5-18yrs (15.2%), adults females >18yrs (48.9%), pregnant women (17.4%), and lactating women (18.3%). Study subjects were between 5-45yrs; mean age 26yrs; HBsAg+ mean age was 24yrs; HBsAg- mean age was 29yrs.

**Results:** One thousand and fifty four (47.5%) of all females who sought healthcare service at the Bo Government Hospital laboratory during the period under review were tested positive for HBsAg; 159(7.1%) pregnant women, 168 (7.5%) lactating mothers, 154 (6.9%) were girls 5-18yrs and 573 (25.8%) were >18yrs. Forty-five percent (154/339) of all girls between 5-18years who took part in this study were HBV seropositive.

**Discussions:** Few independent investigations in Sierra Leone have estimated the national HBsAg seroprevalence at 2.6%-5.1%. One import of this study is the high 154/2218(6.9%) HBsAg seroprevalence rate among girls 5-18 years which can be attributed to multiple sex partners, unsafe sex practice and other socioeconomic and behavioral factors that might have been prevailing during the period under review. The generally high HBsAg seroprevalence rate in our study can also be as a result of our large cohort size and the low sensitivity and specificity of the Rapid Determine diagnostic test kits used in our study compared to previous similar studies conducted in Sierra Leone.

**Conclusions:** The high seroprevalence rate of HBsAg among girl between 5-18years in this study emphasizes the need for a robust public health preventive and control measures for HBsAg including HBsAg vaccination, routine blood screening, and safe sex practice as a means of reducing the spread and prevalence of HBV infection in Bo.

*Keywords: Hepatitis; chronic; seroprevalence; healthcare.*

## 1. INTRODUCTION

An estimated 350 million people are chronically infected with HBV worldwide [1]. The seroprevalence of hepatitis B surface antigen (HBsAg) varies globally and countries can be defined as having a high, intermediate and low seroprevalence of HBV infection based on the prevalence of HBsAg carriers [1]. About 15%-40% of HBV carriers develop serious sequelae but with no hepatic complications during their lifetime [2]. Most HBV infection is transmitted parenterally among adults through unprotected sexual intercourse. Horizontal spread which is the acquisition of the infection in early life through close personal contact amongst children and infected families is also a common mode of transmission. Vertical transmission which involves the spread of HBV from mother to their fetus also plays an important role in maintaining the transmission cycle of the infection in most parts of Africa where healthcare facilities such as regular blood screening and diagnosis are mostly inadequate. Immigrants from high or intermediate prevalence countries and in those with high risk behaviors are at high risk of HBV infection compared to residents of low risk nations.

Many high risk groups have been identified in the transmission of HBsAg including healthcare emergency personnel, infants born to infected mothers, individuals with multiple

sex partners either past or present, illicit drug users (injecting, inhaling, snorting, popping pills) and homosexuals. HBV carriers are at increased risk of developing cirrhosis, hepatic decompensation, and hepatocellular carcinoma [3].

## **2. METHODOLOGY**

This study is a multicenter investigation that was conducted at the Bo Government Hospital laboratory, Department of Community Health and Clinical Sciences and the Department of Environmental Health Sciences at Njala University in Bo southern Sierra Leone. All laboratory diagnostic tests for HBsAg were conducted at the Bo Government Hospital laboratories from May 2012 to June 2013. Protocol design, literature reviews, and data analysis were done at the Departments of Community Health and Clinical Sciences and Environmental Health Sciences of Njala University in Bo from July 2013 to August 2013. This study analysed HBsAg diagnostic laboratory results for all female patients who visited the Bo Government Hospital laboratory for the period under review. Patient data were retrieved from the laboratory register after a written consent for the use of such medical data was obtained from authorities of the Bo Government Hospital laboratory. Ethical approval for this study was obtained from the Ethical Board for Research at Njala University in Sierra Leone. All subjects for this investigation were recorded as anonymous for the purpose of this research.

In order to determine the population with high HBsAg seroprevalence we categorized all subjects who visited the Bo Government Hospital laboratory during the period under review into; pregnant, lactating mother, girls 5-18years and adult >18years. For each category of subject we determine through laboratory records those subjects who are HBsAg seropositive. Only females who sought healthcare at the Bo Government Hospital laboratory between the periods May 2012 to June 2013 were considered for this investigation. Females were exclusively considered for this study because of their elevated risk for HBV infection as well as their role in the vertical transmission of HBV. Women are documented as very important in the vertical transmission of HBV to their fetus. Females who were diagnosed and confirmed as HBsAg seropositive elsewhere but transferred to Bo Government Hospital laboratory for onward treatment were not included in this study. All subjects had no prior knowledge about their HBsAg status and some of them were only referred to the Bo Government Hospital laboratory for HBV testing based on the suspicion of being infected with either HIV or viral hepatitis or coinfection with both diseases. Reasons for patients been suspected for HIV infection, viral hepatitis infection, or coinfection with both HIV and HBV leading to subsequent referral for HBV laboratory testing are; results obtained from physical diagnosis and case history findings which includes but not limited to passing of frequent stool, weight loss, presence of skin rash, anemic, loss of appetite, persistent and unproductive coughing at night. Other the study subjects however were primarily referred for laboratory diagnostic tests for other infections like malaria, tuberculosis and worm infestation. Others also were referred for diagnostic test for non infection conditions like anemia, diabetes etc but ended up undergoing HBV test as secondary diagnosis. Bo Government Hospital is using ICD 10 for its HBV classification. Although hepatitis B e antigen (HBeAg) seropositivity is an indication of active viral replication and infectivity, this study however did not determine the presence of serum HBeAg for logistic reasons. The presence of serum HBsAg in HBV patient indicates either an inactive carrier state, acute or chronic HBV infection. All subjects who were initially tested seropositive for HBsAg in this study were requested to undergo re-diagnosis after six weeks of their initial positive diagnosis for confirmation test. Subjects who became confirmed cases of chronic hepatitis B

were later placed on antiviral treatment. Chronic HBV patients unlike acute cases have HBsAg present in their serum after six weeks of initial diagnosis.

One commercially available rapid assay widely used because of its higher sensitivity and excellent specificity compared with other available rapid assays is the Rapid Determine HBsAg immune chromatographic assay (Inverness Medical Japan Co., Ltd, Chiba, Japan). This study used the Rapid Determine HBsAg immunoassay test for HBsAg diagnosis of all study subjects. The analytical sensitivity and specificity of the Rapid Determine HBsAg diagnostic test kit is 95% and 99% respectively. The Rapid Determine HBsAg immunoassay is commonly used in resource-constraint countries like Sierra Leone for HBsAg diagnosis because it provides a technically undemanding, affordable and flexible approach towards HBsAg diagnostic testing. It is simple to perform, don't require extra laboratory equipment and its results easy to interpret. One other important reason for the use of the Rapid Determine HBsAg test in resource-poor setting is the fact that its reagents can be stored at room temperature without the need for continuous refrigeration and thus contribute to the safety of blood collected in blood bank. In most developing countries the Rapid Determine HBsAg immunoassay is used both for diagnosing suspected HBV infection as well as for monitoring HBsAg status. However, one major challenge for HBsAg rapid tests is the detection of low levels of target antigen that are present in a relatively high proportion of asymptomatic blood donors resulting to low clinical sensitivity compared to that of EIAs.

Procedures for HBsAg diagnosis using the Rapid Determine HBsAg immunoassay serological kit involves collecting 50ul of blood sample with a finger stick and an EDTA capillary tube, transfer of blood sample to a sample pad and adding a drop of chase buffer to the blood sample on the sample pad. The blood sample is allowed to wait for 15 minutes before results are read. To ensure the Rapid Determine HBsAg immunoassays' validity, a procedural control is incorporated into the device and is labeled "control".

In interpreting the Rapid Determine HBsAg immunoassays HBV test results, a visible red color (bar) in the patient's window would be interpreted as positive, one red bar in the control window of the strip (labeled C) and no red bar in the patient window would be interpreted as negative, and invalid result implies when there is no red bar in the control window of the strip, and even if a red bar appears in the patient window of the strip, the result is invalid and should be repeated. A Rapid Determine HBsAg test result could also be interpreted positive even if the patient bar appears lighter or darker than the control bar. However, if the control bar does not turn red following the HBsAg test with the Rapid Determine HBsAg immunoassay, the test result is invalid and the blood sample should be retested.

We set to determine the overall HBsAg seroprevalence by taking the proportion of HBsAg positive per the total number of patients screened (No. of HBsAg seropositive/Total No. of patient screenedx100). We also determine the HBsAg seroprevalence per category of female population screened (No. of HBsAg seropositive for each category/Total No. of patient screenedx100). We later compared our HBsAg seroprevalence rate with previous HBsAg seroprevalence survey results conducted in Sierra Leone as well as compared HBsAg seroprevalence rates among the various categories in our study.

### **3. RESULTS AND DISCUSSION**

A total of 2,218 female healthcare seekers visited Bo Government Hospital laboratory from May 2012 to June 2013. Our study recorded 47.5% (1,054/2,218) HBsAg seroprevalence rate for all categories of subjects during the period under review; girls 5-18yrs 6.9%

(154/2218), pregnant women 7.1% (159/2218), lactating mothers 7.5% (168/2218), and adult females >18yrs 25.8% (573/2218). Study subjects were between 5-45yrs; mean age 26yrs; HBsAg<sup>+</sup> mean age was 24yrs; HBsAg<sup>-</sup> mean age was 29yrs Table 1.

**Table 1. Frequency of HBsAg among descriptive variables N=2218**

Variables	No. HBsAg <sup>+</sup>	(%)	No. HBsAg <sup>-</sup>	(%)	Total	No screened per category	(%)
Pregnant	159	7.1	229	10.3	388		17.4
Lactating	168	7.5	238	10.7	406		18.4
5-18yrs	154	6.9	185	8.3	339		15.3
> 18yrs	573	25.8	512	3.0	1,085		48.9
Total	1,054		1,164		2,218		100

**Table 2. Seroprevalence of HBV and HIV in relation to sex in Sierra Leone**

Sex	No Screened	No (%) with HIV-1/2	No(%) with HBsAg	No (%) HIV+HBsAg coinfection
Male	102	17(54.8)	26(60.5)	6(46.2)
Female	96	14(45.2)	17(39.5)	7(53.8)
Total	198	31	43	13

*Solayide. AA et al, 2010<sup>4</sup>*

**Table 3. Prevalence of hepatitis B markers among Sierra Leone pregnant women of middle and high socioeconomic status**

No Screened	HBsAg Positive	%	Anti HBs	%	HBsAg and Anti HBs	%
302	19	6.3	15	5.5	0	0

*Wurie IM et al, 2005<sup>5</sup>*

**Table 4. Hepatitis B markers in peri-urban and rural Sierra Leone pregnant women**

No Screened	No positive HBsAg	%	HBs and HBeAg <sup>+</sup> ve	%	HBsAg and Anti HBe <sup>+</sup> ve	%
179	20	11.2	7	3.9	11	6.1

*Wurie IM et al, 2005<sup>5</sup>*

The first HIV and HBV seroprevalence survey conducted in Freetown , Sierra Leone among combined sexes in 2010 indicated 28.3% (56/198) seroprevalence rate for HIV and HBsAg; 16.1% (32/198) males and 12.1% (24/198) females [4] Table 2-the authors of this small survey however recommended a large population based investigation to confirm their result. Few independent investigations in Sierra Leone have estimated the national HBsAg seroprevalence rate between 2.6%-5.1% [5] but some HBV seroprevalence studies have documented alarming seroprevalence rates for the infection for different population groups in Sierra Leone [6-8]. HBV seroprevalence rate for blood donors in Freetown in 1996/1998 was 15.5% (n=2593) [9]. Another Freetown study of hepatitis B marker among pregnant women of middle and high socioeconomic status in 2005 [5] has comparable favorable results like ours with regards to HBsAg seropositivity among pregnant women in Sierra Leone. Wurie IM et al. 2005 recorded a 6.2% Table 3 and 11.2% Table 4 HBsAg seroprevalence amongst pregnant women of middle and high socioeconomic status and pregnant women living in rural and peri-urban settings respectively while ours is 7.1%. Bo is the second city of Sierra

Leone and its strategic location as the headquarter town makes it to have the second largest population [10] in Sierra Leone after Freetown the capital city. Bo is an urban town with various road networks leading to other peri-urban towns and villages in the south.

Our high (47.5%) HBsAg seroprevalence rate which is significantly higher than the estimated national HBsAg seroprevalence rate [5] can be attributed to our large subject population, poor blood screening, increased bloodletting practices like female genital mutilation (FGM) and tribal and cultural markings, low level of analytical sensitivity of the Rapid Determine HBsAg immune chromatographic assay diagnostic testing kit that we used alongside differences in the socioeconomic and demographic backgrounds of our cohort compared to those of the previous investigations carried out in the capital Freetown. Sierra Leone is recorded as an HBV endemic zone. The screening for HBsAg is not included at the national level in routine antenatal check for pregnant women except through private medical care or in cases of high suspicion of HIV infection, HBV infection or coinfection with both. We surveyed 2,218 female healthcare seekers that used the Bo Government Hospital laboratories for a period of 14 months thereby making our result statistically significant. The Freetown HBsAg seroprevalence study surveyed only 198 cohorts. Also, Bo is not socioeconomically better-off compared to the capital city of Freetown because of its poor infrastructure and high unemployment rate. Our research setting is also surrounded by many rural villages with high incidence of bloodletting practices such as tribal cultural markings and female genital mutilation. The Bo Government Hospital serves all the other peri-urban areas and rural villages in the south. Southern Sierra Leone is also documented to have the highest (91.2%) FGM practice compared to 79.6% [11] for Western area (Freetown) where the previous HBV studies were conducted. For most of this bloodletting practices conducted in Sierra Leone unsterilized pieces of equipment are used which places the participants in elevated risk of HBV infection.

Rapid Determine HBsAg diagnostic test was widely accepted for use in the diagnosis and screening of infectious diseases in both developing and developed countries in past years. However, questions relating to their sensitivity and specificity have raised concerns for their universal usability. The different rapid tests currently available for HBsAg detection range in clinical sensitivity from 50 to 94% relative to the various EIA tests. Rapid test clinical sensitivity and specificity depends on the technology on which they are manufactured [12]. Even the most sensitive rapid tests like the current high-performance HBsAg rapid (DRW-HBsAg) test developed by Diagnostics for the Real World (Europe) Ltd. that operates on lateral-flow technology do not achieve an assay sensitivity for HBsAg of 1IU/ml [13,14]. The mean detection limits of the DRW-HBsAg and the Rapid Determine HBsAg tests are 0.34 IU/ml (95% CI, 0.22 to 0.46IU/ml) and 3.56 IU/ml (95% CI, 2.27 to 4.85 IU/ml), respectively ( $P=0.002$ ) [12]. None of the thirty-three HBsAg rapid tests that are commercially available have met the performance requirement for the US Food and Drug Administration approval or for CE marking in Europe [15,16]. Assay sensitivity is the main criterion considered during HBsAg testing of blood donors or donations in developing countries with high HBV seroprevalence. Assay test specificity is also important. Assay test specificity picks out the proportion of patients without the disease and who are really negative for the disease. It thus determines the need for diagnostic result confirmation and for resource-constraint countries like Sierra Leone where medical logistics are limited in supply such result confirmation is very important. The Rapid Determine HBsAg test has low sensitivity (95%) compared to its high specificity (99%). An assay specificity of 99% however is also insufficient. One study in Malawi in 2008 raised a salient concern about the specificity of the Rapid Determine HBsAg diagnostic test [17]. Our study may have suffered from the lack of HBsAg test result confirmation since the Rapid Determine HBsAg test used lacks 100% analytical specificity

and sensitivity and this may have been responsible for the generally high (47.5%) HBsAg seroprevalence in our study population. The Rapid HBsAg Determine diagnostic test used in this study has low sensitivity but high specificity. A rapid test with a high specificity is useful for 'ruling in' a disease if a person tests positive. A Rapid HBsAg Determine test with low sensitivity means that the test is unable to detect as positive cases persons who actually have HBsAg; while a low specificity rapid test implies that such test is unable to pick out as negative cases persons who are HBsAg negative. Our high HBsAg seropositivity may also have included a high proportion of false positive cases because of the following reasons; the presence of other acute viral and bacterial infections, prior vaccination pregnancy and drug addition [18]. It thus implies that the sensitivity and specificity of a test though very useful in diagnosing an infection may not be accurate on their part alone if used to estimate the probability of the presence of HBsAg. The two parameters could however be combined into the likelihood ratio to enhance their application and importance.

Further large studies like ours using the most recent rapid test like DRW- HBsAg test which uses signal amplification system (SAS) alongside EIA as a gold standard test would be needed to confirm our study result. DRW- HBsAg test employs SAS technology which improves its analytical sensitivity and specificity.

The high 154/2218 (6.9%) seroprevalence rate of HBsAg for girls between 5-18 years may also be attributed alongside the low sensitivity and specificity of the Rapid Determine HBsAg test used in this study to the poor public health behavioral activities and sexual activeness of girls in that age group, high rate of teenage pregnancy, age of first sexual intercourse, early marriage and the socioeconomic factors confronting girls within this age bracket living in Bo during the period under review. Most women including girls in southern Sierra Leone where Bo is located become sexually active at an early age. These women have their first sexual intercourse before 18 years and usually marry when they are between 20-24 yrs. The median age for first married and first sexual intercourse for women in Bo for the age group 20-24 yrs were 16.9 yrs and 16 yrs respectively [11]. Southern Sierra Leone is also recorded to have the highest number of teenage pregnancy with first child compared to other regions in Sierra Leone. The teenage pregnancy with first child for southern Sierra Leone is 7.8% compared to 5.7% (eastern) and 4.2% (western); the median age at first birth for women in southern Sierra Leone is 18 yrs [11]. This unusually high HBV infection of girls between 5-18 years unlike pregnant women who also documented high (7.1%) HBsAg seroprevalence rate in this study is also explainable by the fact that majority of girls 5-18 years have multiple sex partners and rarely practice safe sex-two behavioral health practices that are mostly determined by the prevailing socioeconomic conditions in a community. Most of these girls have little or no saying in deciding when to practice safe sex since most times the right to make that decision is the sole prerogative of the male sexual partner. Also, according to the 2008 Sierra Leone Demographic Health Survey (SLDHS 2008), southern Sierra Leone has the least number of men and women who practice safe sex through the use of condoms compared to other regions in the country [11]. Southern Sierra Leone registered the least number of males who limit their sexual intercourse to one HIV negative partner, use condoms and limit sexual intercourse to one HIV negative partner, and practice sexual abstinence [11]. Most girls in Sierra Leone have several sex partners which they coupled with the act of selling sex (prostitution) as a means for sustenance and survival. Southern Sierra Leone including Bo is documented to have the highest number of male transactional sex compared to other regions in the country [11]. Transactional sex as defined in the SLDHS 2008 report as the exchange of sex for money, favors, or gifts. The act is associated with high risk of contracting HIV, HBV and other sexually transmitted infections such as syphilis and gonorrhoea because of its ability to compromise power and its tendency towards

establishing multiple sex partnerships. Girls 5-18yrs because of their inexperience, prevailing socioeconomic condition and low level of education, can be easily manipulated into having transactional sex with men in a bid to meet their financial and educational needs.

#### **4. CONCLUSION**

The elevated HBsAg seroprevalence among the female population in Bo and especially the high HBsAg seroprevalence among girls 5-18years should not be treated lightly given the important role played by pregnant women in the vertical transmission of HBV to their fetus as well as the fact that the youthful population to which girls 5-18years belongs are very important in the transmission cycle of STIs such as HBV. Youths in Sierra Leone like in most developing countries have the largest population size and this has some implication in the amplification of the future HBV seroprevalence rate in the country. This high seroprevalence of HBsAg seropositivity among pregnant women & lactating mother is alarming and needs special attention by the health authorities in Sierra Leone. The study is suggesting that as a way of reducing the high HBsAg seroprevalence among women especially pregnant women and lactating mothers in Bo there is an urgent need for the implementation of HBV vaccination within the Extended Program in Immunization (EPI) program. This study also recommends a gold standard diagnosis of all HBsAg seropositive patients by other specific diagnostic kits other than the Rapid Determine HBsAg immunoassay alone. The study believes that using the Rapid Determine HBsAg immunoassay test only and alone could lead to over-diagnosis of HBsAg among suspect patients. Also, considering the alarmingly high (47.5%) HBsAg seroprevalence in our study compared to the 2012 WHO HBsAg seroprevalence estimates which placed South Africa in the intermediate 5-7% zone [19], this study can be viewed as a baseline study calling for the production of accurate public health statistical data in the future. As part of its recommendations, this study is also suggesting that, sexually active females and commercial sex workers be encouraged to be practicing safe sex including the use of female condom in order to minimize the transmission of HBsAg. As another safety precaution method, private and public laboratories should be advised to be routinely screening blood products and donors for HBsAg before transfusion to needy patient and that instruments and pieces of equipment used in bloodletting practices such as tribal cultural mark incisions, female genital mutilation and circumcision should be thoroughly sterilized before being used. This study is suggesting that the Ministry of Health and Sanitation through the Education Ministry should increase HBV public health education and awareness raising campaigns especially in schools and tertiary institutions, and calls for further research to investigate the correlation of marital status, age, sex and HBsAg seroprevalence in Bo, southern Sierra Leone.

#### **CONSENT**

All authors declared that a written informed consent letter was obtained from authorities of the Bo Government Hospital laboratory in Sierra Leone prior to the use of medical linked data of HBV seropositive patient for this study.

#### **ETHICAL APPROVAL**

All authors hereby declared that all procedures relating to this study was approved by the appropriate ethics committee of the Department of Community and Clinical Sciences, Njala University and the Bo Government Hospital in Sierra Leone.



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## **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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