

Article

Prevalence of Hepatitis B Virus Seromarkers in Female Sex Workers in Enugu State, Nigeria

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Abstract: Hepatitis B virus (HBV) is a sexually transmitted virus with a wide range of terminal complications. As such, female sex workers (FSWs) are an important group in the epidemiology of the virus. This study was aimed at evaluating the seroprevalence of HBV markers and the exposure rate of the virus among FSWs in Enugu State, Nigeria. A cross-sectional study was carried out among brothel-based FSWs, involving 200 participants recruited using a consecutive sampling method. Blood specimens were collected and tested for HBV markers using chromatographic immunoassay rapid test kits. Additional information was obtained through the administration of a well-structured pre-tested questionnaire. Data were entered into Statistical Package for Social Sciences (SPSS) version 20.0 and analyzed using the Descriptive Statistics and Chi-Square test in SPSS. Out of the 200 sampled individuals, 82(41%) tested positive for at least one seromarker, with 44(22%) showing evidence of natural infection and 38(19%) indicating a vaccine response. Hepatitis B core antibody (total anti-HBc) was present in 42(21%) of the participants, while 8(4%) had hepatitis B surface antigen (HBsAg), which is indicative of current infection. This study revealed intermediate prevalence, a high exposure rate and a low vaccination rate among the study population. There is a need for more effective intervention strategies among FSWs in the study area.

Keywords: hepatitis B virus; seromarkers; female sex workers; acute infection; chronic infection; vaccination; exposure rate



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1. Introduction

Hepatitis B virus (HBV), a hepatotropic virus, constitutes a public health problem affecting millions of people globally, causing illness, disability and death. The prevalence of this virus varies from one geographical region to another. The Western Pacific region and sub-Saharan Africa have the highest prevalence rates, with about 5–10% of their adult populations being chronically infected [1–3]. It is saddening that in spite of the high prevalence and highly infectious nature of HBV, it is poorly diagnosed and under-reported in most African countries [3,4].

HBV is highly infectious, with as few as 1–10 HBV virions sufficient to initiate an infection. The virus is known to be transmitted via contact with infected blood or body fluids [1]. In highly endemic regions such as sub-Saharan Africa, the main route of transmission is perinatal [5,6]. Horizontal transmission of HBV in adulthood is another route that is being played down, especially among high-risk groups, and this can engender perinatal transmission [7].

HBV infection can cause potentially life-threatening inflammation of the liver, which can be seen in the form of acute or chronic liver disease [8]. The virus damages tissues in the liver of patients with HBV infection. The extent of damage is dependent on two factors: how effectively the virus replicates and how vigorously the immune system reacts to the presence of the virus. HBV is not directly cytopathic [9]; the immune system is the main cause of tissue damage. The immune system, in a bid to clear the virus, damages the liver, which, over time, may lead to scarring, cirrhosis and other complications [10].

In the serological diagnosis of hepatitis B, there are diverse seromarkers, and their presence or absence in an individual has different clinical and epidemiological significance. HBV seromarkers include: hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), hepatitis B core antibody (anti-HBc IgG), hepatitis B early antigen (HBeAg), hepatitis B envelope antibody (anti-HBe) and hepatitis B core IgM antibody (anti-HBc IgM) [11–13]. These markers may occur individually or in various combinations, and this is entirely dependent on the natural history of the infection [14]. Early in the course of HBV infection, within 4–6 months, HBsAg is seen in the serum. During an acute HBV infection, HBeAg is seen shortly after the appearance of HBsAg, and this indicates higher HBV replication, infectivity and possibility of chronicity. For persons who recover from HBV infection, HBeAg seroconverts to anti-HBe, followed by HBsAg seroconversion to HBsAb [15]. HBsAb is also the only detectable seromarker in persons whose immunity was acquired from HBV vaccination. On the other hand, hepatitis B core antigen (HBcAg) is not detectable in the bloodstream; however, its antibody (anti-HBc) can be detected in the serum. Anti-HBc can be seen in the serum of persons who have ever been exposed to HBV infection and can suggest acute or chronic infection [11]. Anti-HBc IgM is usually used to differentiate between acute and chronic cases, as it is rarely present in non-acute cases but almost always present in acute cases [16]. However, because it is also present in rare chronic cases, a cutoff value for the determination of acute cases is normally recommended [17].

HBV infection is referred to as a silent killer because the majority of carriers are asymptomatic and consequently fail to seek appropriate medical attention [18]. While susceptibility is general, vaccination, early detection and prompt treatment are the keys to breaking transmission [19]. Interestingly, hepatitis B virus is one of the few sexually transmitted infections (STIs) with a vaccine. Vaccination is the most cost-effective strategy for preventing HBV infection and its consequences. In Nigeria, the National Programme on Immunization policy offers infants the opportunity to access the HBV vaccine. The adult population is not known to be sensitized or encouraged to get vaccinated, not even those in high-risk groups, except for health workers.

Female sex workers (FSWs) are one of the key risk groups for HBV infection. There is a high HBV transmission risk for FSWs due to their multiplicity of sexual partners necessitated by their work [20,21]. A study in North-Central Nigeria put the prevalence of HBV among FSWs at 17.1% [22]. The activities of FSWs have a huge impact on the epidemic pool of HBV in the general population, directly or indirectly.

Although there exist few studies on the prevalence of HBV seromarkers in Nigeria, there is also a paucity of evidence regarding the prevalence of seromarkers of hepatitis B infection among FSWs in the study area of Enugu State. Evaluating the seromarkers of HBV among FSWs in the State will not only bridge the knowledge gap of the prevalence of this virus in the target population but also indicate the extent of their exposure and vaccination rate. Therefore, this study aimed to assess the seroprevalence of HBV infection and its seromarkers among female sex workers in the study area.

2. Materials and Methods

2.1. Study Area

The study area is Enugu State with a large number of brothel-based female sex workers. Based on the pre-survey conducted (unpublished data), there are four major locations with such settlements, namely, Obollo-Afor, Ugwuoba, Emene and New Gariki. Obollo-Afor is a semi-urban town and the headquarters of Udenu Local Government Area (LGA) in Enugu North senatorial zone, and Ugwuoba is in Oji River LGA in Enugu West senatorial zone. Emene and New Gariki are located within Enugu metropolis in Enugu East senatorial zone.

2.2. Ethical Approval and Considerations

Ethical approval was sought and obtained from the Ethical Committee of University of Nigeria Teaching Hospital, Ituku-Ozalla. Participants were briefed on the relevance and implication of the study, stressing the need for their participation. The briefing was also summarized in handbills, which were given to each participant. The consent of the participants was sought, and those willing to participate voluntarily thumb printed a consent form. The participants were counseled on the nature of the study and its importance. They were informed of their HBV status after testing, and those positive for HBsAg were referred for treatment. The unvaccinated were also encouraged to get vaccinated, and those who showed interest were referred to hospitals and clinics with vaccination centers.

2.3. Data Collection

Information on demographic data, transfusion and vaccination histories were obtained from the participants with the aid of a well-structured pre-tested questionnaire. This was administered to the participants and translated to the local dialect (Igbo) depending on the literacy level of the individual. For reasons of privacy, all data were kept confidential in accordance with World Medical Association (WMA) Declaration of Helsinki [23].

2.4. Study Design and Selection of Sample Population

This descriptive cross-sectional study involved major brothel sites that were listed, from which 200 FSWs were enrolled in the study using convenience sampling technique. In Enugu State, prominent brothels are located in New Gariki, Emene, Ugwuoba and Obollo-Afor areas. From these locations, a total of 11 brothels, including all in New Gariki, Emene and Ugwuoba and randomly selected ones from Obollo-Afor, were included in the study. All FSWs in the selected brothels were eligible for the study, and those who consented were included.

2.5. Sample Collection

Blood samples were collected from participants and analyzed between April 2018 and October 2018. Following counseling, 4–5 mL of venous blood was aseptically taken from each participant via venipuncture from the cubital fossa and discharged into a plain vacutainer bottle. The blood was allowed to clot and then centrifuged at $1000 \times g$ for 10 min. Serum was aspirated with Pasteur pipettes and stored in cryovials at -17°C if not used immediately.

2.6. Sample Analysis and Test Procedure

All sera were assayed for five hepatitis B virus markers: hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (HBsAb) (anti-HBs), hepatitis B core antibody (HBcAb) (total anti-HBc), hepatitis B early antigen (HBeAg) and hepatitis B envelope antibody (HBeAb) (anti-HBe).

These markers were screened using HBV Diagnostic kit (Micropoint Bioscience Inc., Santa Clara, CA, USA), an immunochromatographic kit that combines the 5 markers in a panel on one single cassette and can measure all of the markers at the same time but singly. It measures HBsAg, anti-HBs and HBeAg with a sandwich method and anti-HBe and anti-HBc with a neutralization competitive inhibition method. The kit is reported

by the manufacturer to be very specific, with no interference from other viruses such as hepatitis A, C and E viruses, HIV or the etiology of syphilis, another common sexually transmitted infection. It has been evaluated against enzyme-linked immunosorbent assay (ELISA) [24,25] and reported to have a 100% specificity, 81.8% sensitivity, 100% positive predictive value and 100% negative predictive value [25]. During analysis, serum samples were brought to room temperature. The test cassette for HBV seromarkers was carefully laid on a level surface. A Pasteur pipette was used to draw serum from the cryovial tubes, and 2 drops were administered into the sample well(s) of each cassette accordingly. Respective sample buffers were added to each well, and the results were read within 5 min and interpreted as either negative or positive. For HBsAg, anti-HBe and HBeAg, the appearance of 2 red bands on the control and test lines indicated positive, while the appearance of only 1 red band on the control line indicated negative. For HBeAg and anti-HBc, the presence of 2 red bands on the test and control lines indicated negative, while the appearance of only 1 red band on the control line indicated positive. In both cases, the absence of red bands on the test and control lines was indicative of an invalid test.

2.7. Data Analysis

Data generated were entered into Statistical Package for Social Sciences (SPSS) version 20.0 and analyzed using Descriptive Statistics. Differences in proportions among the different subgroups and between test results and participant responses were compared using SPSS Chi-Square (χ^2) test. Probability values (*p* values) less than or equal to 0.05 were considered significant.

3. Results

3.1. Serological Patterns of HBV among FSWs

HBsAg, anti-HBs and anti-HBc are the primary markers of HBV infection. The various combinations of these markers in a sample indicate whether there has ever been an exposure to HBV, the stage of the infection, the presence of protective antibodies and the source of the antibodies. Table 1 shows the serological patterns obtained from the analysis of 200 samples and their interpretation. The analysis showed that 1(0.5%) of the FSWs tested positive for the three markers, 6(3%) FSWs tested positive for HBsAg and anti-HBc but tested negative for anti-HBs, and 1(0.5%) additional FSW tested positive for only HBsAg and negative for the other two markers. Additionally, a total of 118(59%) participants tested negative for the three markers and were considered to have not been exposed to HBV (Table 1).

Table 1. Frequency and interpretation of pattern of occurrence of the three major HBV markers.

Serologic Patterns	Frequency Occurrence (%)	Interpretation
HBsAg ⁺ , anti-HBs ⁻ , total anti-HBc ⁺	6(3)	Infected and Contagious
HBsAg ⁺ , anti-HBs ⁺ , total anti-HBc ⁺	1(0.5)	Infected and Contagious
HBsAg ⁺ , anti-HBs ⁻ , total anti-HBc ⁻	1(0.5)	Infected and Contagious
HBsAg ⁻ , anti-HBs ⁺ , total anti-HBc ⁻	38(19)	Uninfected with immunity from vaccination
HBsAg ⁻ , anti-HBs ⁺ , total anti-HBc ⁺	19(9.5)	Exposed with immunity from previous HBV infection
HBsAg ⁻ , anti-HBs ⁻ , total anti-HBc ⁺	17(8.5)	Exposed with no evidence of immunity
HBsAg ⁻ , anti-HBs ⁻ , total anti-HBc ⁻	118(59)	Non-exposed and non-immune to HBV
Total	200	
HBsAg ⁺ present	8(4)	HBV carrier status
Anti-HBs ⁺ present	57(28.5)	Immunity status
Total Anti-HBc ⁺ (only) present	17(8.5)	Previous exposure

HBsAg = hepatitis B surface antigen; anti-HBs = hepatitis B surface antibody; total anti-HBc = hepatitis B core antibody.

3.2. Prevalence of HBV among FSWs

HBsAg is the hallmark indicator of hepatitis B infection. Samples that tested positive for HBsAg were considered positive for HBV, which is an indication of current infection. Table 2 shows that out of 200 FSWs examined, 8 tested positive for HBsAg. This indicates

that 4% of the sampled population was presently infected with HBV and could spread the virus.

Table 2. HBsAg prevalence in the population.

HBV Status (HBsAg)	Prevalence %
Positive	8(4) (Intermediate Endemicity)
Negative	192(96)
Total	200(100)

KEY: High Endemicity ≥ 8 ; Intermediate Endemicity: 2–8; Low Endemicity: < 2 .

For the pattern observed from the positive samples, as shown in Table 3, 1(0.5%) of the FSWs indicated an early convalesce stage or HBV virus reactivation stage, and 1(0.5%) of the sampled FSWs was in the early stage of HBV infection, while 6(3%) of the FSWs indicated late acute or early chronic HBV infection with low infectivity. Generally, no sample was HBeAg positive.

Table 3. Frequency and interpretation of patterns of occurrences of all HBV seromarkers for HBsAg-positive samples.

Serological Patterns Observed	Frequency Occurrence (%)	Indication
HBsAg+, anti-HBs ⁺ , HBeAg ⁻ , anti-HBe ⁻ , anti-HBc ⁺ .	1(0.5)	Early convalesce stage
HBsAg+, anti-HBs ⁻ , HBeAg ⁻ , anti-HBe ⁻ , anti-HBc ⁻ .	1(0.5)	Possible early stage of HBV infection
HBsAg+, anti-HBs ⁻ , HBeAg ⁻ , anti-HBe ⁺ , anti-HBc ⁺ .	6(3)	Possible late acute or chronic HBV infection with low infectivity
Total	8(4)	

HBsAg = hepatitis B surface antigen; anti-HBs = hepatitis B surface antibody; HBeAg = hepatitis B early antigen; anti-HBe = hepatitis B early antibody; anti-HBc = hepatitis B core antibody.

3.3. Susceptibility of FSWs to HBV

The susceptibility of FSWs to HBV was investigated. Out of the 200 FSWs sampled, 82(41%) showed evidence of HBV antibodies, 44(22%) from natural infection and 38(19%) from vaccination. The tests also revealed that 118(59%) of the FSWs were susceptible to HBV; i.e., they had neither been infected with nor vaccinated for HBV (Figure 1).

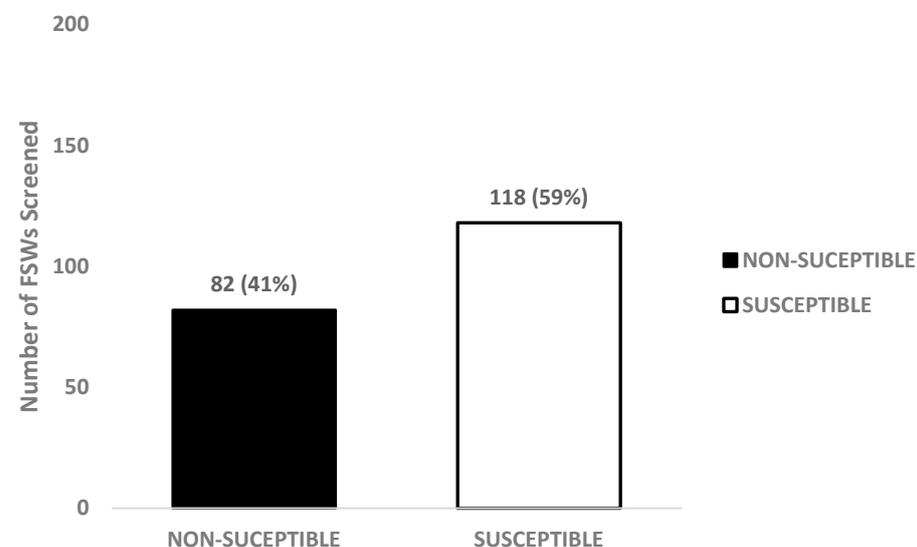


Figure 1. Proportion of Sampled FSWs Susceptible and Non-Susceptible to HBV.

3.4. HBV Vaccination among FSWs

The vaccination rate of the FSWs was also evaluated, and 38(19%) of the study population had been vaccinated against HBV, while 162(81%) of the FSWs were unvaccinated (Figure 2).

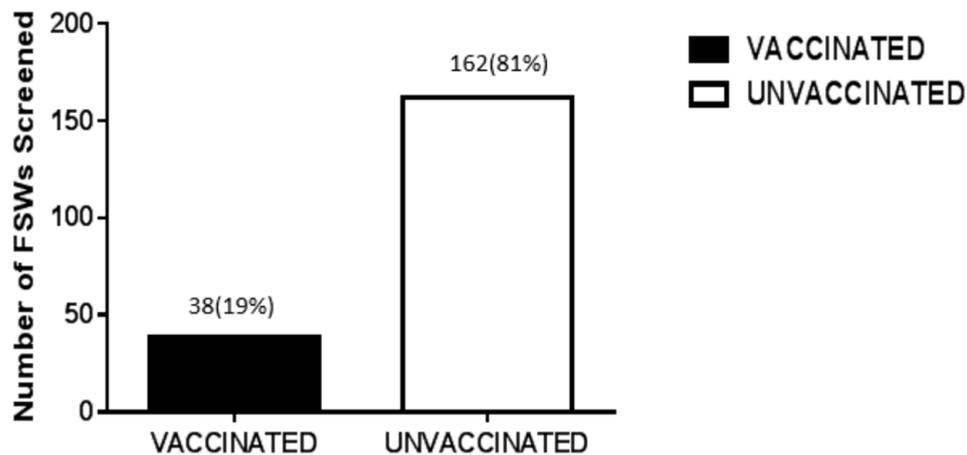


Figure 2. Total Number FSWs Vaccinated and Unvaccinated.

The vaccination status of the FSWs was considered with respect to their response regarding vaccination in the administered questionnaire. Out of the 16(8%) who claimed to have been vaccinated against HBV, only 4(2%) actually tested positive for HBV exposure by vaccination, 2(1%) had an ongoing infection, and 10(5%) were not vaccinated. Among the female sex workers who responded that they had not been vaccinated against HBV, 30(15%) showed evidence of immunity from vaccination, as shown in Table 4.

Table 4. Distribution of HBV infection status among female sex workers in Enugu State versus participants’ responses regarding vaccination.

HBV Infection Status	Vaccination History			Total (%)
	Yes (%)	No (%)	Don’t Know	
Current Infection	2(1.0)	5(2.5)	1(0.5)	8(4)
Vaccine response	4(2.0)	30(15.0)	4(2.0)	38(19.0)
Exposed (immune) from past infection	0(0)	11(5.5)	8(4.0)	19(9.5)
Exposed with no evidence of immunity	0(0)	14(7.0)	3(1.5)	17(8.5)
Unexposed	10(5.0)	89(44.5)	19(9.5)	118(59.0)
Total	16(8.0)	149(74.5)	35(17.5)	200(100)

$\chi^2 = 19.38$; $df = 10$; $p = 0.036$.

3.5. HBV Status of FSWs According to Age

A high rate of HBV was seen among FSWs aged 10–19 years (9.1%) and 40–49 years (8.3%). There was no case of current infection among FSWs aged 50–59 years. The highest rate of past exposure occurred among those aged 50–59 years (100%), followed by those aged 30–39 years (48.5%) and 40–49 years (33.3%). Those aged 10–19 years had the highest unexposed rate of 72.7%. HBV distribution among the age groups was significant ($\chi^2 = 35.81$; $df = 20$; $p = 0.016$) (Table 5).

Table 5. Distribution of HBV infection status by age among female sex workers in Enugu State.

Age Group	Number Tested	Infected and Contagious	Past Exposure	Unexposed
10–19	11(5.5)	1(9.1)	2(18.2)	8(72.7)
20–29	109(54.5)	6(5.5)	34(31.2)	67(61.5)
30–39	66(33)	0(0)	32(48.5)	34(51.5)
40–49	12(6)	1(8.3)	4(33.3)	8(66.7)
50–59	2(1)	0(0)	2(100)	0(0)
Total	200	8(4)	74(37)	118(59.0)

($\chi^2 = 35.81$; $df = 20$; $p = 0.016$).

4. Discussion

The prevalence of HBV infection of 4% obtained in this study is similar to the studies conducted by Zermiani et al. [26] and Silverman et al. [27], who recorded 3.5% and 3.8% among FSWs in Northeast Italy and Nepal, respectively. The HBV rate in this study was higher than the 0% reported by Eledo et al. [28] and 0.2% reported by Figueroa et al. [29]. These lower rates may suggest high levels of safe sex and hygienic practices among the FSWs in those studies. Higher rates of 17.1% [22], 8% [30], 14% [31] and 16.9% [32] of HBsAg among FSWs have been reported in Nasarawa State, Jalingo, Benin City and Benue State, all in Nigeria, respectively. The higher prevalence reported in these studies can be attributed to the predisposing sociocultural practices prevalent in the northern part of the country. These may include poor utilization of immunization services in Muslim communities, which may be pinned to their mistrust of vaccinations [33], communal living, which may promote close contact with the infected in the community, polygamy, which encourages sexual transmission of HBV [34], low literacy level and high poverty [35]. The high HBV prevalence in the reports of Halima et al. [31] can be attributed to the fact that the sampled individuals had sexual disease symptoms and may have contracted HBV alongside other sexually transmitted infections (STIs) since HBV shares a similar transmission route with other STIs [36]. Although the prevalence of HBV infection in this study is low when compared to the 12% prevalence in the general population [37], the average exposure rate is high, which implies that HBV is endemic in the population. Moreover, the prevalence of HBV has been shown to vary from one region of Nigeria to another, with a recent study showing it to be lowest in the southeast of the country [38], the region to which Enugu State, the study area, belongs.

From the results, 9.5% of the participants had anti-HBs and anti-HBc seromarkers, which indicates that a good number of the participants who were previously infected had their infection resolved by developing antibodies against it. This further suggests that the anti-HBs produced due to previous HBV infection can protect against reinfection [39]. It is a well-known fact that infections acquired in adulthood have higher chances of being resolved naturally [40], showing that these FSWs may have acquired their infections in the course of their work.

In this study, 19% of the study population acquired anti-HBs from vaccination. This rate is higher than the 9% reported by Danjuma and Gidado [30] among FSWs in Jalingo and the 10.3% reported by Dan Nwafor et al. [41] among prison inmates in Abuja. In comparison to other high-risk groups, Adelekan et al. [42] reported 54% vaccination among healthcare workers, Ochu and Beyno [43] reported 60.9% among high-risk public safety workers in Kaduna, and Nsirimobu and Peterside [44] reported 85.4% among medical students in Nigeria. Variations in the vaccination rates reported in different studies may be attributed to levels of awareness of HBV infection, vaccination and its accessibility. Therefore, there is a need to educate FSWs and other high-risk groups on HBV infection and the importance of HBV vaccination. It is imperative to make vaccines available in many healthcare outlets for easy accessibility.

The frequency of FSWs who were positive for only the anti-HBc marker in this study is 8.5%. The presence of anti-HBc as the only positive seromarker in this study has many

possible interpretations. It could indicate resolved infection with the loss of anti-HBs, and in this case, the individual may respond to a single booster of the HBV vaccine [45]. It could also represent a false-positive anti-HBc, a condition that has been reported in individuals who failed to respond to a single booster of the HBV vaccine and are thus susceptible to HBV infection. It could mean the resolution of acute infection. It could also possibly mean a low level of chronic HBV infection or occult HBV [45].

Occult HBV can be described as the presence of HBV DNA in the absence of HBsAg and the presence of anti-HBc and/or anti-HBs, which can be detected by molecular assays [46]. All forms of occult HBV can be infectious in immunocompromised individuals. Conversely, the detection of HBV DNA in an individual with only anti-HBc is associated with increased infectivity in both immune-compromised and -competent individuals [47]. This makes the 9.5% anti-HBc prevalence observed in the study, in addition to the unavoidable contact of this group with the general population, a serious public health issue.

The law that excludes FSWs from blood/organ donation should be upheld, and in the same vein, the routine screening of blood for only HBsAg, HIV and HCV is not enough, as anti-HBc screening should be included too.

Furthermore, the rate of 8.5% positive for only the anti-HBc marker in this study is close to the 8.3% reported in the study conducted by Anonedobe et al. [48]. Higher rates of 40.6% and 38.2% anti-HBc markers were seen in the findings of Zermiani et al. [26] and Mbaawuaga et al. [49], respectively. The higher prevalence in these studies can be explained by the fact that the participants in the studies were recruited from health centers, where there are sick people seeking medical care, and their chances of being plagued by HBV are higher.

In this study, the percentage of unexposed people in the population is 59%; this proportion is susceptible to HBV infection in the future. This may imply a high prevalence of susceptible female sex workers in the State. Thus, the targeted vaccination of such individuals will ultimately reduce the rate of HBV transmission. This finding is higher than the 40.2% unexposed rate in the report of Mzingwane and Mamvura [50] and 47.4% in the reports of Ifeora et al. [51]. The lower percentage of the unexposed population in the report of Mzingwane and Mamvura [50] could be attributed to the study population and to the similar transmission routes of HBV and HIV. In the same vein, the lower percentage of the unexposed population in [51] could probably be attributed to exposure from a sex partner, contaminated surgical equipment used during previous childbirth, blood transfusion and other pregnancy-related medical interventions.

This study is limited by the test kits not measuring anti-HBc IgM separately. This made it impossible to make some critical conclusions. A future study should address this and possibly also include nucleic acid measurements.

5. Conclusions

Given the prevalence of HBV infection in this population and the concern about HBV transmission among FSWs and their partners, a national control plan for HBV should be instituted, and attention should be given to FSWs. The low coverage and/or absence of vaccination against hepatitis B among female sex workers is of serious concern and contributes to their increased exposure to HBV infection. Therefore, HBV awareness should be created, and its testing and vaccination should be made easily accessible to this particular population.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of University of Nigeria Teaching Hospital, Ituku-Ozalla (NHREC/05/01/2008B-FWA00002458-1RB000023, 6 May 2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the participants to publish this paper.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

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