Research Article
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Malaria and hepatitis B virus co-infection among pregnant women in Samaru-Zaria, Nigeria

Abstract

Background: Malaria and Hepatitis B virus (HBV) infection are endemic in Nigeria. These infections pose significant public health concerns, putting the well-being of both an expectant mother and the unborn child at risk. This study was aimed at determining the co-infection and risk factors of malaria and HBV among pregnant women on antenatal visits in selected hospitals within Samaru-Zaria, Nigeria.

Methods: A total of 150 pregnant women were included in this study. Structured questionnaires were used to gather data on socio-demographic and risk factors associated with the infections among them. Two millilitres (2mL) of venous blood sample was collected from each of the study participants. Thin and thick blood smears were made from each sample and stained with Giemsa. *Plasmodium* parasites were detected using 100× objective of compound light microscope. Serum was obtained from each blood sample by centrifuging at 1500 rpm for 5 minutes. Hepatitis B surface antigen (HBsAg) was detected from the serum using Swe-Care Rapid One-step Test Strip (Stockholm, Sweden).

Results: The overall prevalence of malaria among the pregnant women was 84.7%, while HBV was 5.3%, and co-infection was 5.3%. Those between 26-35 years had the highest occurrence of both malaria (86.4%), HBV (8.6%) and co-infection (8.6%). Pregnant women that were employed had the highest malaria (86.2%), HBV (6.9%) and co-infection of 6.9%. The most educated women had the highest malarial burden (88.0%), but HBV (10.0%) and co-infection (10.0%) were highest among those with primary education. Women in monogamous marriage had higher occurrence of malaria (86.6%), but those in polygamy had significantly higher HBV (8.2%; P=0.032), as well as co-infection (8.2%; P=0.032). Non-application of insecticides and presence of stagnant water predisposed them to malaria (OR>1). Those who lived with HBV-infected relative(s) were significantly more infected with HBV (100%; P=0.000).

Conclusion: Pregnant women in this study had very high malarial burden, but low HBV infection. The co-infection was also low. Presence of stagnant water and non-application of insecticides were significant risk factors for malaria. Living with infected relative(s) was a risk factor for HBV; while polygamy was associated with the co-infection. These infections endanger maternal and foetal health. Therefore, it is important that every pregnant woman attends antenatal care from early stage of pregnancy, and gets routine screening for malaria and HBV infection. Early and adequate treatments for these infections will ensure good pregnancy outcome.

Keywords: co-infection, malaria, HBV infection, pregnant women, antenatal, risk factors

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Jesse Banas Jemuel, Henry Gabriel Bishop
Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria

Correspondence: Henry Gabriel Bishop, Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria, Tel +2347064608775, Email gabrielhenrybishop@gmail.com

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Introduction

Five species of *Plasmodium* namely, *P. falciparum, P. ovale, P. vivax, P. malariae,* and *P. knowlesi* (a human-adapted species) are known to cause malaria in man.[1] Malaria endangers the outcome of pregnancy, affects mothers, foetuses and newborn babies.[2,3] Warm tropical climate, poor housing, bushes, accumulation of stagnant water and lack of personal protection from mosquito bites promote spread of malaria. *Plasmodium* infection is primarily transmitted via bites of infected female *Anopheles* mosquitoes,[3] and through other minor routes like blood transfusion,[4] tissue/organ transplant or congenitally transmitted to a foetus.[5] Six African countries including Nigeria, Democratic Republic of the Congo (DRC), Uganda, Côte d'Ivoire, Mozambique and Niger are notable for high malarial burden, but Nigeria remains the most endemic country.[1,3] Infection with malaria parasites is common during pregnancy, and is one of the killer diseases worldwide.[6] Pregnancy affects the normal physiological state of women, which increases the risk of infections with consequent alteration of maternal immunity.[2]

Hepatitis B virus (HBV) is an enveloped DNA virus that infects the human liver and causes hepatic necrosis and inflammation. HBV is endemic in sub-Saharan Africa, Asia, the Pacific, the Amazon, parts of Europe.[7,8] Globally, HBV infection has been a public health problem; it affects more people than Human Immuno-deficiency Virus (HIV).[9,10] Both HBV and HIV are world's most important chronic infectious viral diseases.[11] HBV is transmitted congenitally, also via blood transfusion, sexual contact, or contact with body fluids including saliva, sweat, semen, vaginal secretions, breast milk, urine and faeces.[7,8,12–14] Active HBV infection can be detectable via Hepatitis B surface antigen (HBsAg) as a marker for infectivity. This marker is useful for determination of prevalence or endemicity of HBV infection in the general population.[13] Children born to infected mothers are at high risk of developing chronic liver disease.[11,13]

In areas that are endemic for both malaria and hepatitis, there is likelihood for co-infection among individuals, but the interactions between malarial parasites and HBV are still poorly understood.[15] *Plasmodium* species and HBV are among the most common infectious

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diseases that affect underdeveloped countries.[1] Hence, this study aims to determine the prevalence of malaria, HBV infection and their co-infection; as well as some associated risk factors among pregnant women in Samaru-Zaria, Nigeria.

Materials and methods

Study area and design

The study was conducted in two selected hospitals within Samaru-Zaria, Nigeria, including Ahmadu Bello University Medical Center (ABUMC) and Primary Healthcare Samaru (PHCS). This study was hospital-based, cross-sectional and a descriptive research conducted between 19th June to 16th July 2023.

Study population

The study population comprised of pregnant women at different gestational periods who received antenatal care at ABUMC and PHCS. Pregnant women that did not consent to the study were excluded. All information from the study subjects were used only for the sake of this study and kept confidential.

Administration of questionnaires

Structured questionnaires were administered to the consented pregnant women to obtain data on socio-demographic and risk factors that may be associated with malaria and Hepatitis B virus infection among them.

Determination of sample size

Given the reported prevalence of 0.5% for co-infection of malaria and HBV infection among pregnant women,[16] the sample size was determined using the formula by[17]:

$n = [(Z^2pq) /d^2]$

n = sample size

Z = 95% confidence level with class interval (CI) of 1.96

d = allowable error of 5%, which is = 0.05

p = 0.5%, which = is 0.005 [16]

q = (1 - p)

q = 1 - 0.005, which is = 0.995

Hence, $n = [((1.96)^2 \times 0.005 \times 0.995) / (0.05)^2]$

$= 7.644784$

$n \approx 8$

However, for the sake of this study, the sample size was raised to 150 to improve its accuracy.

Collection of blood samples

One hundred and fifty (150) blood samples were collected from the pregnant women on antenatal visits to the selected hospitals. Two (2) mL of venous blood was aseptically obtained into ethylene-diamine-tetra-acetic (EDTA) bottles from each participant by experienced medical laboratory personnel. Each sample was appropriately labelled to match correspondingly filled questionnaire and immediately taken for analysis at a laboratory in the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria.

Thin film preparation and staining

Unto a clean grease-free microscope glass slide, a drop of blood was placed at about the middle and a thin tongue-shaped smear was made by using the edge of another clean grease-free glass slide inclined at an angle of 45°. The smear was allowed to air dry, and then appropriately labelled. Each dry smear was fixed in methanol and allowed to air-dry; followed by flooding with Giemsa stain for 30 minutes. The slide was rinsed gently in clean water, drained and inclined to dry on a rack.[18–20]

Thick film preparation and staining

Unto a clean grease-free microscope glass slide, 2 to 3 drops of blood were placed, and evenly spread to cover an area about 15 × 15 mm using the angle of another clean glass slide. The spreading was carefully done to avoid the red cells forming marked rouleaux that can cause the blood to be easily washed off from the slide during staining. The slide was allowed to air-dry for 12-18 hours. Then the slide was placed on a staining rack and flooded with Giemsa stain for 30 minutes; followed by gently washing in clean water, draining and air-drying at an inclined position on a rack.[18–20]

Microscopic examination of stained blood smears

A drop of immersion oil was placed on each Geimsa-stained smear and examined for *Plasmodiun* parasites using 100× objective lens of a compound light microscope. Coloured atlas of Protozoology was used as guided for identification of the parasites.[18]

Screening assay for hepatitis B surface antigen

Each blood sample was centrifuged at 1500 rpm for 5 minutes to obtain serum, which was used for detection of HBsAg using Swe-Care Rapid One-step Test Strip (Stockholm, Sweden). The centrifuged blood samples and the test strips were allowed to assume room temperature prior to the screening. A dipstick was aseptically immersed in vertical position into the serum (but not exceeding the maximum mark) for 10 to 15 seconds. Then the test strip was placed on a non-adsorbent flat surface and the result was read after 15 minutes.

Interpretation of HBsAg test results

The result was interpreted according to the manufacturer's instruction. Positive result was indicated by two distinct red lines (one line on the control region (C) and another line on the test region (T). Negative result was indicated by one red line on the control region (C) with no apparent red line appearing on the test region (T). Invalid result was indicated where control lines failed to appear but test line appeared, or where both control and test lines failed to appear completely.

Data analysis

The data collected from the questionnaires were analyzed using Chi square (χ^2) and Odds Ratio (OR) on IBM SPSS version 23. Statistical significance was set at P-value ≤ 0.05. Any risk factor with OR>1 was considered significant.

Results

Out of 150 pregnant women examined, the overall prevalence of malaria parasites was 127(84.7%). Overall prevalence of HBV was 8(5.3%); while the co-infection of malaria parasites and HBV was also 8(5.3%) as shown in Table 1.

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Table 1 Prevalence and Co-infection of malaria and Hepatitis B virus infections among pregnant women in Samaru, Zaria

Infection (n=150)	Number infected	Prevalence (%)
Malaria	127	84.7
HBV	8	5.3
Co-infection	8	5.3

Table 2 presents some socio-demographic factors of the pregnant women in relation to malaria and HBV infection among them. Those between 26-35 years old had the highest occurrence of malaria (86.4%), followed by 84.6% among those between 36-45 years old; while the youngest women (of 16-25 years old) had the least occurrence of malaria (82.1%). Pregnant women between 26-35 years old also had the highest occurrence of HBV infection of 8.6% and co-infection of 8.6%; but the youngest women between 16-25% years old had neither HBV infection nor co-infection. There were no statistical significant differences in the age distributions of malaria, HBV infection and their co-infection among the pregnant women. Higher occurrences of malaria (86.2%), HBV infection (6.9%) and their co-infection (6.9%) were recorded among pregnant women that were employed, than those that were unemployed who recorded 83.7% and 4.3% of malaria and HBV infections respecitively, with co-infection of 4.3%. Occupational status of the women did not significantly influence the distribution of the infections (P>0.05), but higher risks were found among those that were employed (OR>1). Those with tertiary education recorded the highest occurrence of malaria (88.0%), compared to those with informal education who had the least (75.0%). However, HBV infection and co-infection occurred highest among those with primary education (10.0%). Level of education of the women did not significantly affect the distributions of the infections (P>0.05). Married women had higher occurrence of malaria (85.1%) than divorced women (50.0%). All the case of HBV infection (5.2%) and co-infection (5.2%) were recorded among married women, but absent among the divorced women. Marital status of the women did not significantly affect the occurrence of the infections (P>0.05); however, the married women were more at risks of the infections (OR>1) than the divorcees.

Table 2 Socio-demographic factors of malaria and Hepatitis B virus infections among pregnant women in Samaru-Zaria

Socio-demographic factors	Number examined	Malaria Number positive (%)	HBV Number positive (%)	Co-infection Number positive (%)
Age				
16-25	56	46(82.1)	0(0.0)	0(0.0)
26-35	81	70(86.4)	7(8.6)	7(8.6)
36-45	13	11(84.6)	1(7.7)	1(7.7)
	P-value	0.792	0.08	0.08
Employment status				
Unemployed	92	77(83.7)	4(4.3)	4(4.3)
Employed	58	50(86.2)	4(6.9)	4(6.9)
	P-value	0.678	0.499	0.499
	OR	1.21	1.63	1.63
Education status				
Informal	4	3(75.0)	0(0.0)	0(0.0)
Primary	30	24(80.0)	3(10.0)	3(10.0)
Secondary	66	56(84.8)	3(4.5)	3(4.5)
Tertiary	50	44(88.0)	2(4.0)	2(4.0)
	P-value	0.748	0.62	0.62
Marital status				
Married	148	126(85.1)	8(5.2)	8(5.2)
Divorced	2	1(50)	0(0.0)	0(0.0)
	P-value	0.171	0.735	0.735
	OR	5.727	1.057	1.057
Type of marriage				
Polygamy	53	43(81.1)	8(8.2)	8(8.2)
Monogamy	97	84(86.6)	0(0.0)	0(0.0)
	P-value	0.374	0.032	0.032
	OR	0.665	0.918	0.918

Table 3 presents the risk factors of malaria among the pregnant women. Those that did not use insecticide-treated nets (ITNs) had higher occurrence of malaria (91.7%), but it was not significantly different from 81.4% occurrence among those that utilised ITNs (P=0.145). Presence of stagnant water around houses was a significant risk for the occurrence of malaria (OR>1).

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Table 3 Risk factors of malaria among pregnant women in Samaru-Zaria

Risk factor	Category	Number examined	Number positive (%)	χ2	Df	P	OR
Use of insecticide treated net	No Yes	48 102	44(91.7) 83(81.4)	2.664	1	0.145	0.397 2.518
Stagnant water around house	No Yes	92 58	78(84.8) 49(84.5)	0.002	1	0.96	0.977 1.023
Application of insecticides	No Yes	63 87	50(79.4) 77(88.5)	2.352	1	0.125	2.002 0.499

Table 4 presents the likely risk factors of HBV infection among the women. Those that shared sharp objects had higher occurrence of HBV (5.9%) than those that did not (5.2%), but it was not a significant difference (P>0.05). Living with infected relative(s) was significantly associated with HBV infection (P=0.000).

Table 4 Risk factors of Hepatitis B virus among pregnant women in Samaru-Zaria

Risk factors	Category	Number examined	Number positive (%)	χ2	df	P	OR
Sharp object sharing	No Yes	116 34	6(5.2) 2(5.9)	0.026	1	0.871	1.146 0.95
Blood transfusion	No Yes	146 4	8(5.5) 0(0.0)	0.232	1	0.63	0.945
Infected relative	No Yes	147 3	5(3.4) 3(100)	54.337	1	0	0.034

Discussion

Changes in physiological condition of a woman due to pregnancy present great challenges to her. Microbial infections like malaria and HBV adversely affect a woman's health and that of the developing foetus. Pregnant women often have decreased immunity, which makes them most vulnerable to malaria.[6] Hence, it is paramount for pregnant women to seek early and adequate health care. Prevalence of 84.7% of malaria among the pregnant women in this study is very high, but not surprising given the endemic nature of the disease in tropical and subtropical areas. Individuals that live in endemic areas (especially women) are particularly prone to frequent mosquito bites during early- and late-hour chores around the house or during daily petty trades, often beside stagnant water channels along major roads. Many women engage in such trading to fend for themselves and their children, or to augment the efforts of their husbands. Several studies had reported different malaria prevalence including 47.2%,[20] 60.0%,[21] 40.2%[22] and 78.4%[23] within Nigeria. Lower prevalence of 4.3%,[24] 8.7%,[25] 21.09%,[3] and 18.7%[26] were also reported from different parts of Nigeria. However, much lower prevalence of malaria had been reported from other African countries including 16.1% in Burkina Faso,[27] 2.2% in Kassala, Eastern Sudan,[28] and from Ethiopia prevalence of 2.8%[29] and 7.2%[30] were reported. However, in India, the prevalence of malaria is about 11.4%.[31] Large difference in reported prevalence of malaria between this study and other locations in Nigeria suggests that Samaru-Zaria may be a focal area for malaria transmission; meanwhile Nigeria is more malaria-endemic than other African and Asian countries. This study is a wake-up call for adequate efforts to be made in order to identify and eradicate local factors promoting malaria endemicity in the Nigeria.

Pregnant women in this study were also infected with HBV with a prevalence of 5.3%. This was much higher than 1.0% reported from Enugu,[32] and 1.51% reported from Gombe.[8] HBV infection is endemic in sub-Saharan Africa.[10] During pregnancy, infection with HBV increases the chances of complications such as preterm delivery.[33] This should raise health concerns for the mother and the foetus. Previous studies within Nigeria had reported higher prevalence of HBV including 7.9% in Kano,[34] 8.3% reported in Abjuja,[35] 6.7% from Bauchi,[36] 10.2% from Benue state,[37] 7.4% reported from Jos,[11] but a very high prevalence of 19.5% was recorded in Keffi.[38]

Prevalence studies on HBV among pregnant women had been reported from different countries across the globe. This indicates that maternal health faces severe challenges from this infection worldwide. From Alexandria in Egypt, HBV prevalence of 3.39% had been reported,[13] but study from Ghana revealed 6.0%.[10] In addition, 5.44% was reported from Vientiane, Laos.[39] Within Ethiopia there had been several reported HBV prevalence including 4.99%,[40] 4.6%,[41] 8%[42] and 8.3%.[43] A study from DRC reported 4.4%.[44] There was also a reported 9.2% HBV prevalence among pregnant women from Gambia,[45] and 6.5% from Burkina Faso.[46] From the Asian continent, particularly southern China, reported HBV infection was 11.74%.[14]

As *Plasmodium* infection causes changes in haematological parameters, HBV infection causes changes in liver function. Therefore, co-infection of malaria and HBV will exacerbate the patient's condition because both infections share intra-hepatic stages in their synergistic life cycles. This increases liver injuries and consequently increases morbidity and mortality.[15] In this current study, the occurrence of 5.3% co-infection of malaria and HBV is of significant medical concern. Malaria leads to maternal anaemia, which also affects the developing foetus.[19,20] Tendencies for premature delivery, low birth weight or foetal death are common during infection with either *Plasmodium* or HBV, but these clinical outcomes become aggravated during their co-occurrence.[2,16,47] Multiple infections during pregnancy exacerbate the health of an expectant mother and her unborn child.[2] Therefore, antenatal care should include multiple screening and treatment against bacterial, parasitic and viral infections that may adversely affect women or may be congenital in nature. Several studies conducted had reported malaria and HBV co-infection of 0.5% from Ejule in Kogi State[16] and 3.33% from Owerri.[2] Also, co-infection of 0.7% was reported from Ghana;[47] while systematic review and meta-analysis revealed global co-infection of 6.0%.[1] Co-infection

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is common in areas where the individual infections are co-edmemic.[33] Adapted-immunity and metabolism during pregnancy increase the risks of infections with *P. falciparum* and HBV, but severity of co-infection is higher than in single infection.[33,48] Although malaria-HBV co-infection occurs worldwide, especially in areas where the two infections are both endemic, it seems to be a particular problem in African and Latin-America, as only sporadic cases are reported in industrialized nations.[15]

Women between 26-35 and 36-45 years old had higher malaria burden and HBV infection, as well as the co-infection. These age groups are more engaged in petty socio-economic activities that make them more prone to mosquito bites than the other younger women. This agrees with findings from other studies in which women between 26-30 years old[26] and 31-35 years old[25] had the highest malarial burdens. Another study had shown contrasting highest occurrence of malaria among younger women between 17-19 years old.[49] However, this study found out that age was an insignificant factor in the distribution of malaria, as mosquitoes can transmit the infection to any individual provided there is exposure.

Employed women recorded higher burden of malaria, HBV and the co-infection. However, higher risks of the infections were among those that were unemployed. This is similar with finding of a study in which professionals in medical and engineering fields were significantly more infected.[25] Higher infections among these employed women might have been work-related. However, another study[26] had recorded higher malarial burden among farmers and unemployed women.

Women with tertiary education recorded the highest malaria burden; but HBV and co-infection occurred mostly among women with primary education. Education and awareness increase protection against infection, but other factors like work-related hazards can prone highly educated individuals to various infections. Married women were more infected, and at higher risks of malaria, HBV and the co-infection compared to the divorced women. Women in monogamy had higher burden of malaria, but HBV and co-infection were significantly more occurring among women in polygamy. However, many studies had shown that level of education and occupation are significantly associated with malarial burden among pregnant women in Nigeria. Younger pregnant women with low levels of education and low-income occupations are more likely to be affected by malaria than women that are older, more educated and earn higher income.[25] Women that lived in areas with stagnant water and did not apply insecticide to control mosquitoes in their homes were more at risk of malaria. Presence of stagnant water around the house serves as breeding site for mosquitoes. In areas where mosquito population is not environmentally controlled, malarial burden will be higher.

Higher occurrence of HBV infection was found among women that shared sharps objects; while those with infected relatives were significantly more at risk. These exposures bring the women in contact with the virus. Higher risks of HBV among pregnant women with multiple sexual partners, body tattoos, consumed alcohol, with history of abortion or contact with infected relatives had been reported.[40] Other identified risk factors of HBV reported included blood transfusion, previous surgeries, gestational age and contact with patients with hepatic viral infections.[13]

Conclusion

This study revealed that maternal health faces great health challenge resulting from very high malarial burden (84.7%), but low HBV (5.3%) and low co-infection (5.3%). Pregnant women that were older, married and employed had higher burdens of the individual infections as well as their co-infection. Those that did not use ITNs were more infected with malarial parasites; while presence of stagnant water and the non-application of insecticides increased the risks of malaria transmission among them. Other women that shared sharp objects were more infected with HBV, but living with infected relative(s) was significantly associated with HBV infection. Where an expectant mother battles with these infections, the health of the foetus will be congenitally affected. This study is a wake-up call for adequate protection of maternal health, and the need to increase concerted efforts in reducing endemic malaria and HBV infection in Nigeria.

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Conflict of interest

The authors declare that there is no conflict of interest.

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