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Research Article

High Rates of Hepatitis B and C Viral Infections in Asymptomatic Pregnant Women and Females of Reproductive Age in Ilesa and Ibadan, Southwestern Nigeria

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Abstract

Clearly, HBeAg-positive mothers are infected with HBV and about 70-90% of such mothers, when pregnant, can vertically infect (i.e. infectious mothers) their non-immune newborns peri-partum; 90% of such infants are likely to develop chronic HB/hepatocellular carcinoma. We aimed to detect evidence of HB and C viral infections/infectiousness in archived sera of asymptomatic pregnant women from Ilesa (PWIL, n=27) and Ibadan (PWIB, n=25) and females of reproductive age from Ilesa (FRAIL, n=40), and determine associated participants' variables. Sera collected in Wesley Guild Hospital, Ilesa, Osun State and Jericho Nursing Home, Ibadan, Oyo State, Nigeria were screened with commercial ELISA kits for HBsAg, HBeAg and HCV antibody, and results obtained were analyzed with t-test, ANOVA and binary logistic regression. The 92 women were 17-42 years old (average: 30.66 yrs). Overall positivity for HBsAg, HBeAg and HCV antibody were 90.70% [n=86]; 60.98% [n=82] and 45.24% [n=84] respectively. Women positive for the 3 tests were 72 of which 94.44% had HBsAg or HBeAg (HBV infection), with 58.70% of all pregnant women and 66.67% of FRAIL having HBeAg (HBV infectiousness). HCV seropositivity (HCV infection) of 55.00%, 28.00% and 47.37% respectively occurred in FRAIL, PWIL and PWIB. Dual (HB-HC) infection rates were 44.44% [n=72] while FRAIL were the only co-variate of HCV antibody positivity (odds ratio [OR]: 3.14, p=0.04, n=84) and dual infection (OR: 3.00, p=0.02, n=88). The high HB, HC and dual infection rates imply high carrier status among these asymptomatic pregnant women, thus increasing the possibility of infecting their newborns/contacts.

Keywords: Pregnant Women; HBV; HCV; Infectiousness; Nigeria

Abbreviations

HBV: Hepatitis B virus;
HB: Hepatitis B;
HBsAg: Hepatitis B Surface Antigen;
HBeAg: Hepatitis B Envelope Antigen;
HCV: Hepatitis C Virus;
HC: Hepatitis C;
HCC: Hepatocellular Carcinoma;
PW: Pregnant Women;
PWIL: Pregnant Women From Ilesa;
PWIB: Pregnant Women From Ibadan;
FRA: Females of Reproductive Age;

FRAIL: Females of Reproductive Age From Ilesa;
DNA: Deoxyribonucleic Acid;
RNA: Ribonucleic Acid;
PCR: Polymerase Chain Reaction;
ELISA: Enzyme-Linked Immunosorbent Assay;
LGA: Local Government Area;
OAUTHC: Obafemi Awolowo University Teaching Hospital Complex;
ANOVA: Analysis Of Variance;
BLR: Binary Logistic Regression;
LAUTECH: Ladoke Akintola University of Technology

Introduction

Hepatitis B and C viruses (HBV, HCV) are DNA and RNA viruses respectively with common routes of acquisition and transmission between infected and susceptible humans [1, 2]. Developing countries are known to have high burden of the two viral hepatitis [3, 4], which are of special concern in females of reproductive age (FRA) and pregnant women (PW) [5, 6, 7]. In spite of availability of vaccines and antiviral therapy, HBV remains a major cause of morbidity and mortality worldwide [8, 9]. In the same vein, HCV, a leading cause of chronic liver disease, is a global public health challenge with the majority of cases occurring in Africa [1].

Although adults are more likely to have acute infection following acquisition of HBV and recover [10], non-immune infants that acquire the virus vertically or in early childhood stand a 90% chance of developing chronic hepatitis B (HB) [11]. Similarly, most individuals (about 75% to 80%) exposed to HCV develop chronic infections [12]. Unlike acute infections, chronic hepatitis B or C can progress to liver cirrhosis, hepatocellular carcinoma (HCC) and death [13, 14]. Consequently, dual infection of an individual with both viruses poses a great health problem [15].

During pregnancy, hepatitis B and C viruses not only cause high rates of vertical transmission leading to fetal and neonatal hepatitis, they are also associated with maternal complications like premature contractions and preterm delivery [16, 17]. Mothers who are HBe antigen-positive have 70%–90% risk of transmitting HBV to their infants (i.e. high infectiousness); in addition, presence in serum of HBsAg or HBeAg indicates acute or chronic HBV infection/infectiousness [18]. Females of reproductive age as well as PW having detectable HBsAg with or without HBeAg can transmit HBV to their sexual partners during unprotected sexual intercourse [19]. Also, sexual transmission of HCV, though less common, does occur during unprotected sexual contacts especially among those having more than one sexual partner [20]. Most sera positive for anti-HCV antibodies reportedly are also HCV PCR-positive, indicating that the antibodies are markers of ongoing infection and do not correlate with clearance of infection (http://www.who.int/about/copyright/en/).

There have been previous reports of dual HBV-HCV infections in Nigeria. Using the enzyme-linked immunosorbent assay (ELISA), Esan *et al*. [21] documented 0.15% co-infection of HBV and HCV among 649 apparently healthy PW in Federal Medical Centre, Ido-Ekiti, Ekiti State while Ezechi *et al*. [22] reported that two women (0.08%) in a cohort of PW living with human immunodeficiency virus had dual HBV-HCV infection. There have also been reports of dual HBV-HCV infection outside Nigeria [6, 23]. This retrospective study was therefore conducted using sera of asymptomatic/apparently healthy PW who have been described as a sentinel population whose prevalence data may be extrapolated to the general sexually active heterosexual population [24], as well as sera of FRA. The data obtained will provide information on the proportion of such individuals with HB, HC or HB-HC dual infection, and the possibility of infecting their newborn babies or sexual partners, and also help to determine associated participants' variables.

Materials and Methods

Study area and population

Archived serum samples used for this study were from apparently healthy women visiting two health facilities in southwestern Nigeria. The first group comprised females of reproductive age (FRAIL) and pregnant women (PWIL) attending Wesley Guild Hospital, Ilesa, Osun State for gynecologic and antenatal care, respectively. The second group consisted of pregnant women (PWIB) visiting Jericho Nursing Home, Ibadan, Oyo State for antenatal care. The sera were collected between October, 2011 and May, 2012. Wesley Guild Hospital is located in Ilesa West local government area (LGA) of Osun State and is one of the six health-care units of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), which by virtue of its location provides tertiary, secondary and primary health-care services to Osun, Ekiti and Ondo States, and some parts of Oyo, Kwara, Kogi, Lagos and Edo states in Nigeria. Ilesa, compared to Ibadan, has a relatively low socio-economic status and the people are predominantly Yorubas, with other tribes from various parts of the country and outside Nigeria. Jericho Nursing Home is located in Ido LGA, Oyo State and serves women in the LGA and other parts of Ibadan in Oyo State. Ibadan is the largest city in West Africa and home to Yorubas majorly, other Nigerian indigenes and foreigners. Figures 1 and 2 show demographic data of the women.

Study design

This is a retrospective, health facility-based cross-sectional study approved by the Health Research Ethical Committee, College of Health Sciences, Osun State University, Osogbo, Osun State and conducted between May and November, 2014. The serum samples and interviewer-administered question-

naires used for this study were collected from consenting participants and had previously been used for rubella and human papillomavirus prevalence studies between October, 2011 and May, 2012 [25, 26]. Pertinent data extracted from the questionnaires for the purpose of the present study are shown in Figures 1 and 2.

Serum samples

Archived sera used for this study were retrieved from the storage freezer (-20°C) but some of them were insufficient for the three different tests performed. In all, there were 92 serum samples, but those that were sufficient for the three tests with pertinent demographic data were 72 in number.

Serologic Assay

Three different ELISAs to detect HBsAg, HBeAg and anti-HCV antibody were performed on the sera. For HBsAg detection, the AiD® HBsAg ELISA kit (Wantai Biological Pharmacy Enterprise Co., Beijing, China) was used while for detection of HBeAg and anti-HCV antibody, HBeAg and Anti-HCV ELISA kits (INTECO DIAGNOSTICS, London, UK) were used. The ELISA and interpretation of results were done according to the respective manufacturer's instructions.

Data analysis

The results were presented with descriptive statistics using mean and percentages with 95% confidence interval (CI). Independent t-test and ANOVA were used as appropriate to compare mean age of three groups of women. For the purpose of comparability during inferential statistical analyses, participants with incomplete data or insufficient serum sample for, at least, a serologic test were excluded. Binary logistic regression (BLR) was used to establish statistical association or otherwise between the participants' demographic/behavioral variables and seropositivity of HBsAg, HBeAg, anti-HCV antibody, and 2 or 3 combinations of the seropositivity. Data analysis was done with SPSS version 15.0 for Windows (SPSS Inc., Chicago, IL) and p-value ≤ 0.05 was used as indicator of statistical significance in two-tail hypothesis.

Results

Characteristics of study participants

Overall, there were 92 study participants (Figures 1 and 2) comprising 40 females of reproductive age (FRAIL), 27 PWIL and 25 PWIB. The age range of all the women was 17-42 years (yrs) (mean age: 30.66 yrs [95% CI: 29.72-31.60 yrs]). The age range for the FRAIL was 17-40 yrs (mean age: 29.85 yrs [95% CI: 27.04-32.66 yrs]) while the corresponding values for PWIL and PWIB were 19-42 yrs (mean age: 31.30 yrs [95% CI: 29.44-33.16 yrs]) and 27-35 yrs (mean age: 31.28 yrs [95% CI: 30.44-32.12 yrs]) respectively. Comparison of the mean age of the three categories of women showed that they were statistically homogeneous (p = 0.33).

All the FRAIL responded "no" to alcohol consumption while most of them had tertiary education, were married, had one sexual partner and responded "no" to use of condom during sexual intercourse (Figure 1). The PW generally had tertiary education, belonged to the category of "others" (i.e. traders, etc.) regarding occupation, carried first pregnancy, were in the second trimester of pregnancy and had family size ≥ 3 (Figure 2).

Serology

Though there were 92 participants in all, only 86, 82 and 84 sera respectively were tested for HBsAg, HBeAg and anti-HCV antibody due to insufficient serum samples. Therefore, the prevalence rate for the 78 positive out of 86 sera tested for HBsAg was 90.70% [95% CI: 84.56-96.84%]: 37 of 38 FRAIL (97.37% [95% CI: 92.28-102.46%]), 21 of 24 PWIL (87.50% [95% CI: 74.27-100.73%]) and 20 of 24 PWIB (83.33% [95% CI: 68.42-98.24%]) were positive. The three groups were comparable (p = 0.82 and 0.68) in HBsAg positivity.

Regarding HBeAg, 50 of 82 serum samples were positive, giving a 60.98% prevalence rate (95% CI: 50.42-71.53%): 24 of 36 FRAIL (66.67% [95% CI: 51.27-82.07%]), 15 of 22 PWIL (68.18% [95% CI: 48.72-87.64%]) and 12 of 24 PWIB (50.00% [95% CI: 30.00-70.00%]) were positive. The three categories of women had statistically similar (p = 0.21 and 0.20) HBeAg positivity. Twenty seven (58.70% [95% CI: 44.47-72.92%]) of 46 PW with sufficient sera had HBeAg. Seven (63.64%) of 11 PW in the third trimester of pregnancy had evidence of active HBV replication (i.e. HBeAg positivity) with 4 (36.36%) having anti-HCV antibody and 2 (18.18%) with dual infection (i.e. HBsAg or HBeAg and anti-HCV antibody). We also noted that 4 PW of the 82 (4.89% [95% CI: 0.22-9.54%]) that tested positive for HBeAg had no detectable HBsAg. While one of these had no serum sample for anti-HCV antibody test, one was positive for the antibody and the remaining two negative for same.

We observed that 38 of the 84 sera tested for anti-HCV antibody were positive, giving seropositivity rate of 45.24% [95% CI: 34.59-5.88%]: for FRAIL, 55.00% (95% CI: 39.58-70.42%) positivity (22 of 40 sera) was obtained, for PWIL, 7 of 25 sera were positive (28.00% [95% CI: 10.40-45.60%]) while for PWIB, 9 of 19 sera (47.37% [95% CI: 24.92-69.82%]) were positive. However, comparison of anti-HCV antibody positivity among the three groups gave odds ratio (OR) of 3.14 (95% CI: 1.08-9.19, p = 0.04) for FRAIL compared to PWIL while PWIB had OR of 2.31 (95% CI; 0.66-8.11, p = 0.19) compared to PWIL.

After removing a total of 20 study participants for reasons stated earlier (see data analysis section above), there were

72 women with complete data and test results for the three serologic tests. Our estimation showed that 70 (97.22% [95% CI: 93.43-101.02]) of these tested positive to, at least, one serologic test, leaving two women (both from Ibadan) negative for all the tests (Figure 3). Of the 72 women, 18 were positive for the three tests (Figure 3), giving triple positivity (HBsAg+, HBeAg+, anti-HCV antibody+) rate of 25.00% [95% CI: 15.00-35.00%]. Statistical analysis revealed OR of 4.91 (95% CI: 0.97-24.84, p = 0.05) for FRAIL compared to PWIL and OR of 2.57 (95% CI: 0.41-16.12, p = 0.31) for PWIB compared to PWIL. It is noteworthy that an unmarried 30 year old woman belonging to FRAIL, with secondary school education reported having no sexual partner yet had triple positivity.

Of these 72 women, those positive for HBsAg or HBeAg were 68 (Figure 3), giving HBV infection rate of 94.44% (95% CI: 89.15-99.74%) while 41 (56.94% [95% CI: 45.51-68.38%]) had detectable HBeAg. However, 32 of the 72 women (44.44% [95% CI: 32.97-55.92%]) had a combination of HBsAg or HBeAg and anti-HCV antibody, representing those with dual (HBV and HCV) infection (Figure 3).

Among the 40 FRAIL, 32 (80.0%) responded "no" to use of condom during sexual intercourse. Of these, 18 (56.52% [95% CI: 39.06-73.44%]) had dual infection (HBsAg or HBeAg and anti-HCV antibody), 17 (53.12% [35.84-70.41%]) had evidence of HBV infection (HBsAg or HBeAg) while 22 (68.75% [95% CI: 52.69-84.81%]) had anti-HCV antibody alone. Among the 7 FRAIL that responded "yes" to use of condom during sexual intercourse, there was no dual infection but 4 (57.14%) had HBsAg or HBeAg. There were 5 FRAIL that reported having 2 sexual partners and they all (100.00%) had HBsAg or HBeAg, while 4 (80.00%) had dual infection. The only FRAIL with 3 sexual partners was single and positive only for HBeAg. The married FRAIL were 31 in number with 16 (51.61% [95% CI: 34.02-69.20%]) of them having dual infection while 17 (54.84% [95% CI: 37.32-72.36%]) were HBsAg or HBeAg positive. Of the 9 FRAIL that were single, 5 (55.56%) had dual infection and 7 (77.78%) had HBsAg or HBeAg.

Among the PW, 33 had family size ≥ 3 (Figure 2) of which 6 (18.18%) had dual infection, 29 (87.88% [95% CI: 76.74-99.01%]) had HBsAg or HBeAg and 8 (24.24%) had anti-HCV antibody.

Comparison of dual infection among the FRAIL (n=40) and PW (n=48, 4 had no sera for testing) showed that 50.00% (95% CI: 34.51-65.49%) of the former had dual infection compared to 25.00% (95% CI: 12.75-37.25%) of the latter. The FRAIL had significantly higher (OR: 3.00 [95% CI: 1.22-7.38], p = 0.02) rate of dual infection. 39 of the FRAIL (97.50% [92.66-102.34%]) and 45 (93.75% [95% CI: 86.90-100.60%]) of the PW had HBsAg or HBeAg, and these rates were statistically comparable (p = 0.42).

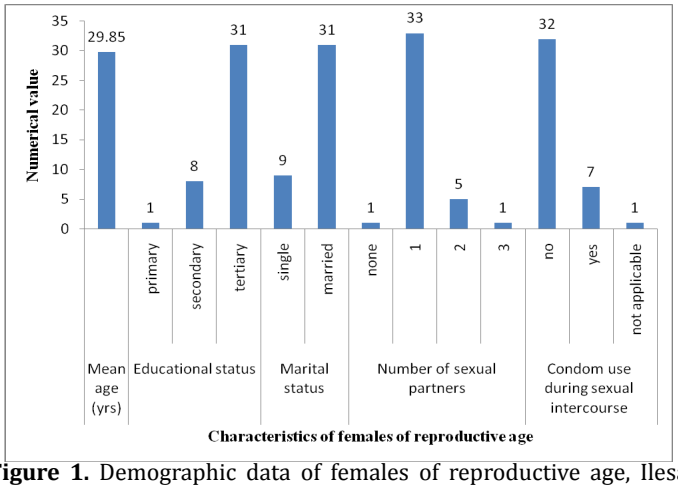

Figure 1. Demographic data of females of reproductive age, Ilesa, Osun State, Nigeria.

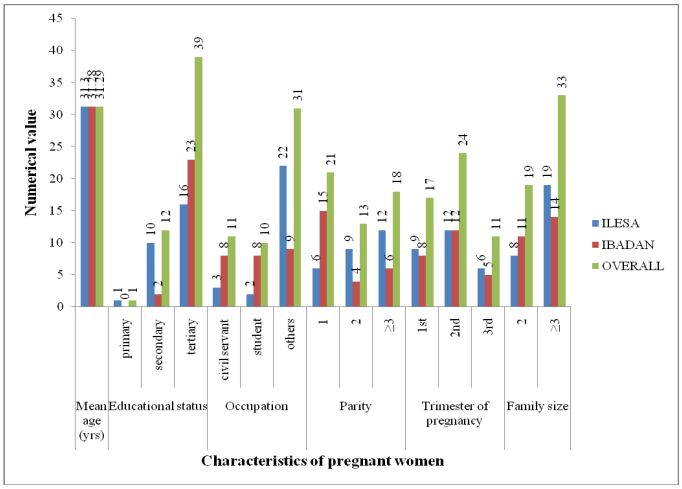

Figure 2. Demographic data of pregnant women from Ilesa, Osun State and Ibadan, Oyo State, southwestern Nigeria.

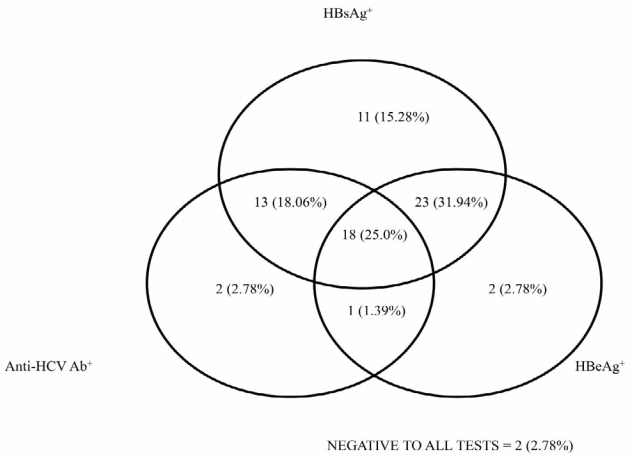

Figure 3. Venn diagram showing relationship among the three sero-

logic tests performed on sera of PW and FRA, southwestern Nigeria (prevalence rates are in parenthesis).

Discussion

This study tested archived sera collected between October 2011 and May 2012 from pregnant women (PW) and females of reproductive age (FRAIL) for markers or evidence of Hepatitis B and C viral infections to estimate proportions of participants infected or infectious, and determine associated variables. A study of this sort among asymptomatic pregnant women is especially necessary to prevent vertical transmission of Hepatitis B and C viruses and manage infected infants because infants that acquire these viruses from their mothers are more prone to developing chronic infections with possibility of liver cirrhosis, HCC [27] and death later in life.

It has been reported that 8% - 10% of people in the general population in the developing world become chronically infected, most of which acquire HBV at childhood. Among these, the risk of death from HBV-related liver cancer or cirrhosis is approximately 25% [28, 29, 30]. In a similar study that focused on females, overall HBsAg prevalence rate of 90.70% among adult women was high and further reflects potential infectiousness [18, 31] and high endemicity/carrier rate of hepatitis B viral infection in southwestern Nigeria [4, 32, 33]. In addition, 60.98% positivity rate in the study for HBeAg was high and explicitly indicates active HBV replication and infectiousness of the HBeAg-positive women to their infants and/or susceptible spouses during unprotected sexual intercourse.

We observed that 27 PW were HBeAg-positive; based on the report that 70%-90% of non-immune infants born to HBeAg-positive mothers acquire HBV [18], it can hence be estimated that between 19 and 25 surviving children of the study PW stood the risk of acquiring HBV from their mothers. About 90% of which, in turn, may develop chronic HB with high propensity of progressing to HCC [11]. In the same vein, HBeAg-positive PW could infect their susceptible husbands (or partners) during unprotected sexual contacts and family members via non-sexual person-to-person contacts. HBeAg presence in women of childbearing-age is a major determinant of perinatal HBV transmission [34]; Moreover, the FRAIL positive to HBeAg were 24 in this study, most of these were therefore more likely to transmit HBV to their unprotected susceptible sexual contacts [35].

Occult HBV infection is characterized by presence of HBV infection with undetectable HBsAg [36]; in this study, 4 pregnant women without detectable HBsAg tested positive to HBeAg, thereby simulating occult HB. This implied that, in a setting like ours where antenatal screening for HBV infection is based on HBsAg detection, such pregnant women would be regarded as non-HBV infected but unsuspectedly infectious. We suggest therefore, the inclusion of HBeAg assay in screening of pregnant women for HBV infection in southwestern Nigeria.

Presence in serum of anti-HCV antibody does not differentiate between acute, chronic and resolved infection; it however, indicates exposure to HCV and infection with same. Moreover, most sera positive for anti-HCV antibodies reportedly are also HCV PCR-positive, indicating that these antibodies are markers of ongoing infection (http://www.who.int/about/copyright/en/). In this study, 45.24% HCV antibody prevalence rate indicates women with HCV infection and, probable infectiousness. There were 16 pregnant women positive for HCV antibody; 2-12% of these could vertically transmit HCV to their infants [18]. Report has it that the chance of spontaneously clearing HCV infection by infants that acquire HCV perinatally is lower than for those via parenteral routes [18]. This partly explains the reason 75%-80% of such infants might develop persistent/chronic HC later in life [12, 37]. Although, the prevalence rate of anti-HCV antibody was the lowest of the three tests in this study, it should be noted that HCV infection has higher propensity for progression to chronicity compared to HBV infection [38, 39].

Comparison of HBsAg and HBeAg positivity rates among the FRAIL, PWIL and PWIB showed the three groups were statistically comparable in prevalence rates; possible reason for these could be the endemicity of Nigeria for HB viral infection implying comparable exposure to the virus. However, comparison of HCV antibody among the three groups showed significantly higher odds of FRAIL being seropositive and this might be due to the larger sample size of FRAIL compared to PWIL and PWIB.

Analyses of the 72 sera of women with complete data and serologic results for the three tests showed that 97.22% were positive to, at least, a serologic test (Figure 3). Twenty-five per cent of the 72 sera had triple seropositivity ($HBsAg^+$, $HBeAg^+$, anti-HCV $antibody^+$). The FRAIL subset of the study participants was about 5 times more likely to be triple positive and this could be due to involvement of majority of the FRAIL in unprotected sexual intercourse as shown in Figure 1. That one unmarried FRAIL of 30 years with no sexual partner had triple positivity pointed to other modes of acquiring HB and C viruses. It is noteworthy that, regarding HBV, 68 (94.44%) of the 72 women were potentially infectious (HBsAg or HBeAg positivity, Figure 3).

Dual HB-HC viral infection has the tendency of causing more aggressive liver disease and rapid progression to chronicity and HCC [15]; 44.44% of the 72 women had dual infection with the stated possibilities among some of them later in life. Analysis showed that 50.00% of FRAIL and 25.00% of all the pregnant women had dual HB-HC viral infection; the prevalence rate of dual infection among the FRAIL was however, significantly higher.

Not many studies in southwestern Nigeria report HB and HC viral infections among asymptomatic pregnant women and females of reproductive age in hospital settings. Among 200 pregnant women attending antenatal clinic of LAUTECH Teaching Hospital, Osogbo, Nigeria, 16.5%, lower than ours, had HBsAg [40]; in the same Teaching Hospital, Opaleye *et al*. [41] documented 7.1% HBsAg positivity among 183 pregnant women. Among 2,392 HIV-positive pregnant women in Lagos, HBV and HCV infection rates of 4.2% and 1.5% respectively were reported by Ezechi *et al*. [22]. Among 649 healthy pregnant women attending antenatal clinic, Federal Medical Centre Ido-Ekiti, Ekiti State, Nigeria, seroprevalence rates of HBV and HCV were 6.78% and 1.39% respectively; with co-infection of one person (0.15%) in age group 31-35 [21]. Regarding HBeAg, Mbaawuaga *et al*. [32] reported that 3.3% of 300 pregnant women in Markurdi, Nigeria tested positive to HBeAg and reported they had high viral replication as well as high risk of transmitting HBV to their neonates.

In women of child-bearing age, a prevalence rate of 8.9% HBV infection was reported in Lagos, Nigeria [42]; while Nwankiti *et al*. [43] documented 27 (9.0%) of 300 such women in Plateau State, Nigeria as HCV antibody positive. Low dual HB-HC viral infection was found in 33 (0.57%) of 5,760 pregnant women in Benin City, Edo state, Nigeria [44]; ditto in young apparently healthy females of Karachi-Pakistan where only one female (0.025%, n=4000) had dual infection [45]. Co-infection of HBV and HCV was however, not observed in 267 pregnant women in rural Okada Community of Edo State, Nigeria [46] and in 200 pregnant women in Gwagwalada, Abuja, Nigeria [47].

We therefore conclude that HB, HC and dual infection with both viruses had high prevalence rates, implying high carrier status for both viruses, among the asymptomatic pregnant women and females of reproductive age attending the two healthcare facilities in Ilesa and Ibadan, southwestern Nigeria. Many of the women involved in this study were most likely infectious to either their newborn babies or susceptible sexual contacts at the time of sample collection with a high possibility of the former developing chronic HBV and or HCV infection(s). Unlike the pregnant women, the females of reproductive age had significantly higher association with either single or dual infection. Continued screening of pregnant women and females of reproductive age for hepatitis B and C viruses is advocated in order to identify those that need monitoring and therapy, and also identify and monitor neonates at risk of acquiring these viruses [21]. HBV-specific IgG is also recommended for those with confirmed exposure to HBV as well as appropriate HB vaccination of exposed newborn infants in 97% of whom the vaccine is effective and lasts for 10–15 years [18].

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