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PREVALENCE AND IMMUNE STATUS OF HIV/HBV CO-INFECTED PREGNANT WOMEN

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ABSTRACT
HIV/HBV co-infection places patients at high risk of liver-related morbidity and mortality and the interaction of the two viruses can further complicate treatment. Pregnant women are especially at high risk for increased morbidity and mortality due to infection, and information about HIV/HBV co-infection in pregnant women is scanty. This study examined the occurrence of HBV antibodies in HIV-1 positive pregnant women and the relationship to Ante-retroviral therapy (ART) and other demographic characteristics. Blood samples were collected from 135 HIV pregnant positive women who were either on ART or Not, from May – June, 2008 at the Jos University Teaching Hospital (JUTH) and the Plateau State Specialist Hospital (PSSH). Presence of hepatitis B surface (HBsAg) antigen in serum was determined using Antec strips (Antec diagnostics UK) and their immunologic status were determined by measuring the CD_4^+ counts using SL_3 cyFlow counter (Partec, Germany) . Sixteen 16 (11.8%) of the women examined were seropositive for Hepatitis B virus. Occupation was significantly associated with the prevalence of the hepatitis co-infection in the population examined (8.8% of house wives and 5.5% of business women had co-infection, $p<0.05$). The immunologic status (CD_4^+ of most of the HIV/HBV co-infected pregnant women (81.5%) was low (below 300 cells/mm^3) although all were on Anti retroviral therapy. The 11.8% prevalence rate reported in this study confirms the endemicity of HBV /HIV co-infection in Nigeria, and this supports the calls for screening for Hepatitis B as a routine in antenatal care.

Keywords: HIV, Hepatitis, Co-infection, CD_4, Pregnant women

INTRODUCTION

Of the 33.3 million people currently living with HIV/AIDS, 68% reside in sub-Saharan Africa ([1]). HIV/AIDS weakens a person's ability to fight infections; HIV-infected people are three to six times more likely to develop a chronic or long-term hepatitis B infection because of their weakened immune systems than individuals without HIV (2). Approximately 5%–10% of HIV-infected persons also have chronic HBV infection, defined as testing positive for HBsAg for more than 6 months.

An estimated 400 million people are infected with Hepatitis B virus (HBV) with the majority of cases occurring in regions of Asia and Africa where the virus is endemic. In these continents up to 70% of adults show serologic evidence of current or prior infection and 8 to 15% have chronic HBV infection (2). Hepatitis B virus (HBV) is the most common cause of serious liver infection; it causes transient and chronic infections of the liver. Clinical presentation of Hepatitis B ranges from subclinical to symptomatic hepatitis and, in rare instances, fulminant hepatitis ([3]). HBV and HIV have common characteristics such as transmission routes (vertical, parenteral, and sexual), and the propensity to establish chronic infections which are often difficult to treat with currently available anti-viral agents ([4],5,6). HIV/HBV co- infection is associated with increased liver related morbidity and mortality. The pathogenesis and clinical manifestations of both are due to the interaction of the virus and the host immune system ([7]). The immune system attacks hepatitis B virus and causes liver injury. Activated CD4+ and CD8+ lymphocytes recognize various HBV-derived peptides located on the surface of the hepatocytes, and an immunologic reaction occurs. Impaired immune reactions, (such as cytokine release, antibody production) or relatively tolerant immune status results in chronic hepatitis (8). The immune suppression caused by HIV infection accelerates the course of liver disease caused by chronic HBV infection and results in increased mortality compared to HIV monoinfected patients (9).

Interferon does not appear to adversely affect the embryo or fetus. However, the data is limited, and the potential benefits of interferon use during pregnancy should clearly outweigh possible hazards (10, 11). Lamivudine used in combination with zidovudine caused pronounced and sustained increases in CD4-cell counts and reductions in HIV-1 viral load in four separate studies, done in both previously antiretroviral untreated and pretreated HIV-1-positive patient Interestingly, lamivudine is also used to treat hepatitis B infections (12, 13). HBV replication has been proven to be inhibited by

lamivudine in 86.4% (95% CI, 75.7–93.6) of HIV-HBV–coinfected patients (14, 15). Chronic HBV does not substantially alter the progression of HIV infection and does not influence HIV suppression or CD4+ cell responses following ART initiation (16,17). Initial data do not suggest that Lamivudine is teratogenic (18). Lamivudine has been used in the latter half of pregnancy in attempt to prevent perinatal transmission of hepatitis B virus infection with mixed success (19). Mother to child transmission of HIV and HBV occurs often either in utero or through exposure to blood or blood contaminated fluids at or around birth. Such perinatal transmission is believed to account for 35-50% of carriers (18). Perinatal childhood infection of HBV is characterised by symptomlessness, but places carriers at a high risk of developing chronic disease (3). It has also been shown that vertical transmission of hepatitis B virus occurs in about 10% of neonates when the infection occur in the first trimester and in 60% to 90% of babies in the third trimester (20). Screening for HBsAg is routine in pregnancy in most developed countries, 122 Colleges of Obstetrics and Gynecology worldwide screen pregnant women for Hepatitis (21, 22). In Nigeria, HIV is routinely tested for pregnant women but not HBV despite their overlapping routes of transmission. Sero-prevalence studies on HBsAg in Nigeria show that the prevalence of the infection in pregnant women range from 2-15% (23, 24, 25).

The prevalence of hepatitis B virus (HBV) infections in Nigeria have been reported as 17.1% among female sex workers in Nigeria and 11.9% in HIV infected persons (26, 27). Imade *et al.*, had done a study in 2004 and reported a prevalence of 11.5% but did not check the immune status of the women in Jos and Oladokun *et al.*, reported a prevalence rate of 8.9% co-infection rate among pregnant women in Ibadan (28, 29). This study was carried out to determine the prevalence of hepatitis B among HIV infected pregnant women in Jos and also to establish the effect of co-infection on their immunological status.

MATERIALS AND METHODS

Study Design

A cross sectional study was carried out among HIV positive pregnant women at the Jos University Teaching Hospital (JUTH) and Plateau State Specialist Hospital (PSSH) between the months of May and June 2008. Blood samples were collected from one hundred and thirty five (135) of the subjects and Ethical clearance for this study was obtained from both the JUTH Institutional Health Research Ethical Committee and PSSH health research ethical committee. Patients were approached with a letter introducing the subject and their consent was obtained by the subject's signature on the questionnaire given to them. Laboratory numbers was indicated on the questionnaires and same number on the sample bottle to protect patients' identity. The outcome of this finding was referred to the clinician for extra attention and care. The samples were then tested for HBV surface antigens and CD_4+ counts.

Sample Collection

A total of 135 blood samples (The sample size was derived using the formula for sample size calculation: $N = z^2pq/d^2$ (where z = Standard normal deviation at 1.96 (which corresponds to 95% confidence interval; p = Prevalence of Hepatitis B surface antigen in ante-natal women in Nigeria from previous studies; q = 1-p; and d = degree of accuracy/ precision expected = 0.05). were collected from HIV positive pregnant women by venipuncture. 5mls of blood was collected aseptically and emptied into sterile vacutainer tubes and labelled. The uncoagulated blood was allowed to separate into serum at room temperature.

Detection of HbsAG Using Antec Strips

The serum was collected into clean containers and used to test for hepatitis B surface antigen HbsAg using the one step disposable Hepatitis B surface antigen test strips (Antec diagnostics; United Kingdom). The test procedure was carried out following manufacturer's instructions and the results interpreted and recorded accordingly.

Determination of Absolute CD4+ count

The blood was placed on a mixer in order to get a homogenous mixture. All the test tubes were labeled accordingly. Monoclonal antibody PE, 20µl was pipetted into a tube; 20µl of the patient's blood sample was added. The mixture was thoroughly mixed and incubated in the dark for 15 minutes. No-lyse phosphate buffered saline (PBS) dilution buffer, 800µl was added and the mixture connected to a cyflow counter (Partec, Germany) for analysis.

CD4+ % Enumeration

Monoclonal antibody CD_4+ (CD4 MAb PE) and CD_{45}+ (CD4 MAb PE), 10µl was pipette into rhoren tubes, 20µl of the patient's blood samples was added. The mixture was thoroughly mixed and incubated in the dark for 15 minutes at room temperature. No-lyse dilution buffer 1 and 2, 400µl was added just before reading and connected to SL_3 cyflow (Partec, Germany) and analysed.

Interpretation of Immunologic Status Result

The CD4+count of normal patients (healthy immune competent, HIV negative persons in Nigeria) lie between 547-1327 cells/ mm^3. Any CD_4+ Count within the range 200 – 500 cells/mm^3 suggests that the person is immune compromised (some damage has been done to the immune system) (30).

RESULTS

Prevalence Rates

Out of the 135 sera collected from HIV infected pregnant women examined, 16 of them were seropositive for Hepatitis B representing 11.8% of the total (Table 1). Women between the age ranges 36-40 had the highest rate of co-infection, this is not statistically significant (χ^2= 0.074, P=0.05 df=40.05), while women between the age range 16-20 had no co-infection (Table 1).

Association of HIV/HBV co-infection in Pregnant Women and Treatment, Occupational and Educational Characteristics

The occupation of the women examined showed that housewives and business women had higher rates of HIV/HBV co-infection (8.8% and 5.5% respectively), occupation is significantly associated with co-infection (χ^2=0.035 P=0.05 df=4) With respect to educational level, pregnant women who were educated only to secondary level had the highest prevalence of co-infection (4.4%), those with no formal education had the lowest prevalence rates (0.7%) but the difference in the prevalence rates was not statistically significant in women with secondary education compared to the rest (χ^2 = 0.545 P= 0.05 df=3) (Table 2). Among the 135 women examined 18.5% of those who had HIV alone were on antiretroviral therapy (ART) while 6.7% of those with HIV/HBV co-infection were on ART.

TABLE 1; AGE RANGE AND PREVALENCE DISTRIBUTION OF HIV/HBV CO-INFECTED IN HIV POSITIVE PREGNANT WOMEN

AGE RANGE	NUMBER EXAMINED*	NUMBER HBsAg Positiveβ	% POSITIVE FOR HIV/HBV
16-20	10	0	0.0
21-25	31	1	0.7
26-30	48	5	3.7
31-35	28	4	3.0
36-40	18	6	4.4
TOTAL	135	16	11.8

%= Percentage ; *= Number of pregnant women examined; β= Number of pregnant women positive for HBsAg; χ^2 =0.074 P=0.05 df=4

Immunologic Status

The CD4+ counts of the women with HIV/HBV co-infection were lower than those with HIV alone but there was no association between co-infection and the immunologic status of the pregnant women (χ^2= 0.297 P =0.05 df=5). But 81.2% of the women with HIV/HBV co-infection had CD4+ values below 300 cells/mm^3 (Table 3).

TABLE. 2; TREATMENT, OCCUPATIONAL AND EDUCATIONAL CHARACTERISTICS OF HIV/HBV CO-INFECTED PREGNANT WOMEN IN JOS

CHARACTERISTICS	HIV (%)	HIV/HBV (%)
Treatment Characteristics		
*ART	25 (18.5)	9 (6.7)
NON-ART	94 (69.6)	7 (5.2)
n=135		
Occupational Characteristics		
BUSINESS	7 (7.52)	5 (5.5)
HOUSE WIFE	30 (33.0)	8 (8.8)
SELF EMPLOYMENT	17 (18.7)	1 (1.1)
CIVIL SERVANT	12 (13.1)	1 (1.1)
PRIVATE ESTABLISHMENT	10 (11.0)	0 (0.0)
n=91 χ^2 =0.035 P=0.05 df=4		
Educational Characteristics		
PRIMARY	24 (24.0)	5 (5.0)
SECONDARY	30 (30.0)	6 (6.0)
TERTIARY	29 (29.0)	4 (4.0)
NO FORMAL EDUCATION	1 (1.0)	1 (1.0)
n= 100 χ^2 =0.545 P=0.05 df=3		

*ART = Patients on Antiretroviral Therapy; n= Number of respondents; χ^2 = calculated chi square value; % =Percentage

TABLE 3: IMMUNOLOGIC CHARACTERISTICS OF HIV/HBV CO-INFECTED PREGNANT WOMEN

CD_4 Count Range (cells/mm^3)	Number Subjects Examined	HIV Positive	HIV/HBV Co-infected
0-100	16	11	5
101-200	9	5	4
201-300	13	9	4
301-400	13	12	1
401-500	4	3	1
501-600	0	0	0
601-700	1	0	1
TOTAL	56*	40	16

χ^2 =0.297 P=0.05 df=0.05; n= 56; *=only 56 out of the total subjects sampled had their CD_4^+ counts determined

DISCUSSION

HBV infection in pregnancy is emerging as an increasingly important issue, although more attention has been given to HCV co-infection because of the higher possibility of developing chronic disease, HBV prevalence rates have been reported to be higher than HCV rates in Nigeria (31,32). In this study, 11.8% of the pregnant women population examined was found positive for HIV/HBV co-infection. In 2004 the rates of HIV/HBV co-infection among pregnant women in Jos was reported as 11.5%, this rate is almost similar to that reported by this study, suggesting that the situation with HIV/HBV co-infection in our environment has not changed (33).

Other Nigerian studies have reported different prevalence rates. In a study in the Federal Capital Teritory (FCT), Abuja, Nigeria the prevalence rate of HIV/HBV co-infection was reported as 7.1% , in Yola North East Nigeria, a prevalence rate of 8.2% was reported among pregnant women. Adesina et al., reported a prevalence rate of 8.9% among HIV infected pregnant women (34, 35, 36). Similarly, in other parts of Africa, varying prevalence rates have been reported; Chasela reported a prevalence rate of 10.4% among pregnant women in Malawi, in Uganda 4.1% was reported among HIV positive pregnant women and a rate of 2.4% in Rwanda also among HIV infected pregnant women (37,38). In Europe, a prevalence rate of 4.9% has been reported among HIV infected pregnant women and a low co-infection rate of 1.5% was reported among pregnant women in America (39,40). Jos is thus hyper-endemic area for Hepatitis B virus infection, according to WHO classification for Hepatitis B endemicity (41,42).

Age is not an important factor related to the prevalence of hepatitis B infection in HIV infected women as revealed in this study. The difference in HIV/HBV co-infection among the various age groups shows that women within the age range 36-40 had higher prevalence rates of HIV/HBV co-infection than women within other age brackets. Eke et al., and Habiba et al., however, reported higher prevalence rates in pregnant women within the age brackets of 20-24 and 25-35 years respectively (43,44). This deviation may have been due to the size of the population studied, a higher population size could change the dynamics.

Occupation was found to be associated with the prevalence of co-infection, among house wives and business women. This is similar to the report of Mohammadi et al., who reported significant association between HIV/HBV co-infection among house wives compared to other occupations in Iran (45).

Educational background was not associated with co-infection rates in our study , (P>0.05), and this was the observation of Eke et al. also, who reported no association between HBV infection and educational level among individuals in low resource settings in Nigeria (43). Ezegbudo et al., however found an association between educational background and HIV/HBV infection in pregnant women in Anambra state Nigeria (46).

With respect to the immune status of the study subjects, although the CD_4+ counts of the women with co-infection were below 300 cells /mm3 there was no statistically significant association between HIV/HBV co-infection and the immune status of the pregnant women. Most studies have reported reduced CD4+ count in HIV/HBV co-infected women when compared with monoinfected women. Otegbayo et al., observed lower CD4+ counts in HIV/HBV co-infected pregnant women in Ibadan, Nigeria (27). Forbi et al., also reported a lower CD4+ count in patients with HIV/HBV co-infection as compared with patients with HIV monoinfection, another study by Idoko et al., reported same in Jos (47,48). However Hoffman et al., (49) reported that HBV does not affect CD4+ cell count in people infected with HIV.

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