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Prevalence of hepatitis B virus and HIV infections among pregnant women visiting healthcare institutions in Ebonyi State

Nwuzo A. C. [1], Emioye A. A.[2], Moses I. B.[1]*, Adiat A. M.[3], Ugbo E. N.[1], Agbom J. N.[4], Ariom T. O.[5], Okata-Nwali O. D.[2], Ugadu I. O.[6], Ayomoh E.[6]

[1]Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, P.M.B. 053, Abakaliki, Ebonyi State, Nigeria.
[2]Department of Biology/Microbiology/Biotechnology, Federal University Ndufu-Alike, Ikwo Ebonyi State, Nigeria.
[3]Department of Obstetrics and Gynecology, University College Hospital, Ibadan, Oyo State.
[4]Department of Science Laboratory Technology, Federal College of Agriculture, Ishiagu, Ivo, P.M.B. 7008, Ebonyi State.
[5]Institute of Food Security, Environmental Resources and Agricultural Research, Federal University Abeokuta, P.M.B. 2240, Ogun State, Nigeria.
[6]National Veterinary Research Institute, P.M.B. 01, Vom, Plateau State, Nigeria.

This study aims to determine the prevalence of hepatitis B virus (HBV) and HIV infections among pregnant women visiting Healthcare Institutions within Ebonyi State, Nigeria. Blood samples were collected from 100 pregnant women who visited Rural Improvement Missionary Hospital (RIMH) and General Hospital (GHE) within Ebonyi State. The presence of hepatitis B surface antigen (HBsAg) in serum was determined using Antec strips (Antec Diagnostics UK) while commercially available Enzyme linked immunosorbent assays (ELISA) based rapid diagnostic test kit (Biotech diagnostic, USA) was used to assay for HIV. Sixteen 16 (11.8%) of the pregnant women examined were sero-positive for HBV, 15(10%) were positive for HIV and 4% were positive for co-infection of HBV and HIV. Occupation was significantly associated with the prevalence of the hepatitis B co-infection in the population examined as 8.8% of housewives and 5.5% of business women had co-infection at $p<0.05$. The prevalence rate of 11.8% for HBV; 10% for HIV; and 4% for HBV and HIV co-infection reported in this study confirms the endemicity of HBV and HIV within the study area. This could cause serious public health problem if not properly tackled. Thus, it is very pertinent to carry out HBV and HIV screening as a routine in ante-natal care.

Key words: Hepatitis B virus (HBV), HIV, pregnant women, co-infection.

INTRODUCTION

Human immunodeficiency virus (HIV) and hepatitis B virus (HBV) are the two most common chronic viral infections in the world today. With an estimated 260,000 children infected with HIV at the end of 2011, Nigeria accounts for more than 10% of the global paediatric HIV burden (Ogboghodo et al., 2006). Hepatitis B infection

*Corresponding author. E-mail: e-mail: ben_iyke70@yahoo.com. Tel: +2348134136233.

affects organs such as the liver, thereby resulting in both acute and chronic disease. The mode of transmission of Hepatitis B viral infection is via blood contact especially with the infected blood or fluids of an infected individual. About 250 million individual were currently prone to viral hepatitis chronically especially HBV (WHO, 2001). The most transmitting routes of HBV/HIV are via contacting by sexual route which may include genital-oral sex (Zhu et al., 1998). Transmission can also be via the blood, body fluids and close personal contact which includes an HBV/HIV carrier mother infecting her child usually during birth. Other transmission routes could be via open wounds, bites, cut, transfusion of contaminated blood or blood products when donor blood is not properly screened; needle stick injuries and other sharp injuries. Transmissions of HIV and HBV from mother to child occur from utero or may occur via blood contact or infected body fluids during birth (Mohammadi et al., 2009). This transmission is accountable for 35-50% of carrier's cases. Vertical transmission of HBV and HIV also occur in almost 10% of infants as soon as the disease take place especially during the early phase of pregnancy and in 65 to 90 percent of infants in the last phase of pregnancy (Ezegbudo et al., 2004). The pathogenesis and clinical manifestations of HIV affects essential cells in the human immune system such as helper T cells (particularly CD^+_4 T cells), macrophages, and dendritic cells (Cunningham et al., 2010). The death rate and the birth rate of HIV reduce as a result of treatment with antiretroviral therapy (Pallela et al., 1998). Though antiretroviral drug is still not available globally, but extension of this treatment as at 2004 has contribute immensely to turn the rate of deaths as a result of AIDS and new disease that may occur in different parts of the world (Ogboghodo et al., 2003). Chronic HBV do not significantly affect the development of HIV and do not affect the HIV suppression or CD^+_4 counts responses through antiretroviral therapy initiation. HBV replication has been inhibited due to lamivudine in 86.4% of HIV/HBV-co infected patients (WHO, 2008).

There is limited information on the effect of HBV co-infection on baseline laboratory profile of HIV-infected children in sub-Saharan African countries. This study is therefore aimed to determine the prevalence of HBV and HIV infections among pregnant women visiting General Hospital, Ezzamgbo and Rural Improvement Hospital, Ndiagu Achara, Ikwo L.G.A, Ebonyi State.

MATERIALS AND METHODS

Data collection

Letters of introduction were given to the patients in order to get their consent and their subjects' signatures were obtained. Questionnaires were also given and the numbers were indicated on the laboratory forms and the sample bottles in order to defend the identity of the patients. The medical records of all registered patients enrolled for the investigation were also taken. Each patient was prepared for the test by professional counseling. Exactly 3 ml

of blood sample was collected aseptically via vein puncture and emptied into an Ethylene Diamine Tetra-acetic Acid (EDTA) bottle.

HIV screening

Each patient was prepared for the test by professional counseling. Whole blood, serum or plasma was thereafter aseptically obtained from the patient, stored and properly labeled. The test kit was aligned appropriately and labeled with the patient's details. The patient's sample was then applied on the test pad of the test kit and allowed to stand for 15 min. The whole blood was used; and a buffer from the manufacturer was applied to run alongside the blood. The Unigold and Stat-pack strip were also used for the HIV screening (RVST) to confirm the result of the Alere determine strips and the result was interpreted. The National Algorithm for HIV screening was also employed in this test. Procedure was carried out using the manufacturer's instruction and the results were recorded and interpreted accordingly.

HBV screening

The patient was prepared for the test by professional counseling. Whole blood, serum or plasma (3 ml) was aseptically obtained from the patient, stored and properly labeled. The test kit was aligned appropriately and labeled with the patient's details and the test pad of the test kit (Antec Strips Diaspot Diagnostic, United Kingdom) was inserted into the whole blood collected aseptically and was allowed to stand for 15 min. Result was recorded and interpreted. Procedure was carried out using the manufacturer's instruction and the results were recorded and interpreted accordingly.

HIV/HBV confirmation

HIV was confirmed by either Amplicor HIV-1 deoxyribonucleic acid Polymerase Chain Reaction (PCR) (Roche Diagnostics, Branchburg, NJ) for Adult >18 **years** or Western blot (Immunetics, Cambridge, MA) for Adults >18 **years**. The baseline laboratory parameters assessed included HBV surface antigen (HBsAg), CD4+T cell count and CD4+T cell percent, viral load, and alanine transaminase (ALT). HBsAg was used to categorize hepatitis status of the patients. HBsAg was determined using enzyme immunoassay (EIA) (Monolisa HBsAg Ultra3; Bio-Rad). HIV RNA levels were measured using Roche COBAS Amplicor HIV-1 monitor test version 1.5 (Roche Diagnostics, GmbH Mannheim, Germany) with a detection limit of 400 copies/ml. Flow cytometry was used to determine CD4+T cell count (Partec, GmbH Munster, Germany) and the CD4+T percent determined by automated method. ALT levels were measured with Roche COBAS C311 Auto Analyser (Roche Diagnostics, GmbH Mannheim, Germany).

Inclusive criteria

Only pregnant women irrespective of their occupation and tribes, registered in the ante-natal care of General Hospital, Ezzamgbo, Abakaliki and Rural Improvement Missionary Hospital, Ndiagu Achara were required for the investigations.

Exclusive criteria

Breast feeding mothers, non-pregnant women and children were not involved in the investigations.

Questionnaires

The questionnaires were prepared and given to the pregnant

Table 1. Distribution of HBV among pregnant women visiting Rural Improvement Missionary Hospital (RIMH) Indiagu Achara, Ikwo and General Hospital, Ezzamgbo (GHE) with respect to age.

Age range (Years)	Number examined	Number of HBsAg- positive	%Positive for HBV
RIMH			
18-22	17	2	15.4
23-27	30	6	46.1
28-32	22	5	38.5
33-37	1	0	0.0
38-42	0	0	0.0
Total	**70**	**13**	**100**
GHE			
18-22	03	0	0.0
23-27	11	2	66.7
28-32	12	1	33.3
33-37	03	0	0.0
38-42	01	0	0.0
Total	**30**	**3**	**100**

RIMH $\chi2$ =5.4, P=0.05, df=4; **GHE**$\chi2$ =5.4, P=0.05, df=4

women attending Ante-natal care of General Hospital, Ezzamgbo, Abakaliki and Rural Improvement Missionary Hospital, Ndiagu Achara, Ikwo. The information on the questionnaires includes age, occupation, educational qualification, tribes, trimester and marital status.

Ethical consideration

A written informed consent was obtained from the patients/ guardians for the research. Ethical clearance was obtained from the ethical committee of both hospitals.

Statistical analysis

Statistical analyses such as SPSS and Chi- square (X^2) were used to compare the data of mono-infected pregnant women positive for HBV and HIV to those that were co-infected. The hypothesis was also set up to test whether the result depended on the age group or not. The baseline laboratory parameters of HIV mono-infected patients were compared with those that were co-infected with HBV using nonparametric univariate methods; the Kruskal-Wallis test was used for continuous variables. Linear regression analyses were used to determine whether HBV status was associated with baseline CD4+T cell counts/percent, viral load, or ALT values. *P* value <0.05 was considered significant. Analysis was done with Epi Info version 3.5.4.

RESULTS

Out of 70 blood samples collected from pregnant women attending Rural Improvement Missionary Hospital (RIMH) Ndiagu Achara, the patients within the age range of 23- 27 years presented the highest HBV prevalence with a frequency of 6 (46.1%). This was closely followed by 28- 32 years with a frequency of 5 (38.5%). The least

prevalence frequency was 2 (15.4%). This frequency was recorded within the age group of 18 to 22 years. No prevalence frequency was recorded among the age groups of 33-37 and 38-42 years. Thus, the total prevalence of HBV infection examined among pregnant women in RIMH is 18.6%.

At General Hospital Ezzamgbo, a total number of 30 blood samples were collected from pregnant women. The patients within the age range of 23-27 years presented the highest HBV prevalence with a frequency of 6 (46.1%). The least prevalence frequency was recorded within the age group of 18-22 years with a frequency value of 2 (15.2 %) while no prevalence was recorded among the age groups of 33-37 and 38-42 years (Table 1). There was a statistical significant difference in the prevalence frequency values obtained within the different age groups.

A total of 70 blood samples were collected from pregnant women visiting, Rural Improvement Missionary Hospital (RIMH), Ndiagu Achara, Ikwo for the HIV screening, it was observed that patients within the age range of 23-27 year presented the highest HIV prevalence frequency with a value of 4 (57.1%). The least prevalence frequency value observed was 1 (14.3%) and was recorded within the age group of 18-22 years while the age groups of 33-37 and 38-42 years showed zero prevalence frequency.

At General Hospital Ezzamgbo, 30 blood samples were collected from pregnant women. Statistical results revealed that patients within the age range of 28-32 year had the highest HIV prevalence frequency value of 2 (66.7%). The least prevalence frequency value of 1 (33.3 %) was recorded within the age group of 23-27 years while zero prevalence frequency were recorded among

Table 2. Prevalence of HIV among pregnant women visiting Rural Improvement Missionary Hospital (RIMH), Indiagu Achara, Ikwo and General Hospital, Ezzamgbo (GHE) with respect to age.

Age range (Years)	Number examined	Number of HIV-positive	% Positive for HIV
RIMH			
18-22	17	1	14.3
23-27	30	4	57.1
28-32	22	2	28.6
33-37	01	0	0.0
38-42	0	0	0.0
Total	**70**	**7**	**100**
GHE			
18-22	03	0	0.00
23-27	11	1	33.3
28-32	12	2	66.7
33-37	03	0	0.0
38-42	01	0	0.0
TOTAL	**30**	**3**	**100**
RIMH: χ^2 =8.01, P=0.05, df=4; **GHE:** χ^2 =5.4, P=0.05, df=4			

the age groups of 18-22, 33-37 and 38-42 years (Table 2). There was no statistical significant difference in the prevalence frequency values obtained within the different age groups.

Co-infection rate were also observed for the patient having both HIV and HBV. The total blood samples screened for HIV and HBV in RIMH was 70 samples and patients within the age group of 23-27 year showed the highest rate of co-infection for HBV and HIV with a prevalence frequency value of 2 (66.7%). The least prevalence frequency value of 1 (33.3 %) was observed among the age group of 28-32 years while zero prevalence frequency were recorded among the age groups of 18-22, 33-37 and 38-42 years.

Statistical analysis showed that patients within the age range of 23-27 years had the highest prevalence rate of co-infection for HBV and HIV with prevalence frequency value of 1(100 %), while zero prevalence frequency value were recorded among the age groups of 18-22, 28-32, 33-37 and 38-42 years. When the obtained data were subjected to statistical analysis, it revealed statistical difference (Table 3). The occupational characteristics of the pregnant women attending RIMH was also put into consideration and the studies shows that Civil servant had the highest prevalence frequency of 3 (60.0%) for HIV and 4 (66.7%) for HBV at RIMH while 2 (66.7%) and 4 (60.0%) were recorded at GHE respectively. Business women were also pre-disposed to this infection with a prevalence frequency of 2 (40.0%) for HIV and 2 (33.3%) for HBV at RIMH while 1 (33.3%) and 3 (40.0%) were recorded at GHE for HIV and HBV respectively (Table 4). House wives and self-employed had zero prevalence frequency for HIV and HBV. The educational qualification

of the subjects were also evaluated, and it revealed that pregnant women that had no formal education and primary school living certificate had the highest prevalence rate of HIV and HBV infections with prevalence frequencies of 3(50.0%) and 4(50.0%) respectively at RIMH and 2 (66.7%) and 3 (50.0%) at GHE respectively. The prevalence frequencies were 2 (33.3%) for HIV and 3 (37.5%) for HBV at RIMH while 1 (33.3%) were recorded for HIV and 2 (33.3%) for HBV at GHE for pregnant women with primary school certificate. Secondary School Certificate subjects had the lowest rate of HIV infection with a prevalence frequency of 1 (16.7%) for HIV and 1 (12.5%) for HBV and zero prevalence frequency values were recorded among pregnant women with tertiary certificate (n= 100, χ^2 = 0.545, P=0.05, df = 4). The trimester was also put into consideration, and it was observed that at first trimester, there was a zero prevalence frequency of HIV and HBV at RIMH; 1 (6.7%) for HIV and zero prevalence frequency for HBV at GHE. At second trimester, prevalence frequencies of 3 (30.0%) and 5 (33.3%) were recorded for HIV and HBV respectively at RIMH, followed by 1 (33.3 %) for HIV and 1 (25.0%) for HBV at GHE. Among the pregnant women under investigation at third trimester, 7 (70.0%) had the highest prevalence frequency of HIV at RIMH and 9 (60.0%) for HBV; while 2 (66.7 %) for HIV and 3 (75.0%) for HBV were recorded at GHE.

DISCUSSION

This study provides a comprehensive sentinel

Table 3. Prevalence of HBV/HIV co-infections among pregnant women visiting Rural Improvement Missionary Hospital (RIMH) Indiagu Achara, Ikwo and General Hospital, Ezzamgbo (GHE) with respect to age.

Age range (Years) for HBV/HIV	Number examined	Number of HBV/HIV-positive	% Positive
RIMH			
18-22	17	0	0.00
23-27	30	2	66.7
28-32	22	1	33.3
33-37	01	0	0.0
38-42	0	0	0.0
Total	**70**	**3**	**100**
GHE			
18-22	03	0	0.00
23-27	11	1	100
28-32	12	0	0.00
33-37	03	0	0.00
38-42	01	0	0.00
Total	**30**	**1**	**100**
RIMH $\chi2$ =5.4, P=0.05, df=4; GHE $\chi2$ =4, P=0.05, df=4			

surveillance of HIV and HBV among pregnant women attending Rural Improvement Missionary Hospital (RIMH), Ndiagu AcharaIkwo and General Hospital, Ezzamgbo. The association between HIV and HBV with their socio-demographic parameters such as age, occupational characteristic, educational background and the trimester of the pregnant women were observed. The total prevalence of HBV infection examined among pregnant women in RIMH was 18.6%. This is similar to the work done by Uneke et al. (2007) in southeastern Nigeria, Harry et al. (1993) in Maiduguri, Nigeria, with prevalence frequency of 14.7 and 11.2%, respectively. Nevertheless, the prevalence frequency of 18.6 % in our study is relatively higher than 5.4 and 6.1% reported by Obi et al. (2005) in Abakaliki and Odie (2005) in southeast Nigeria respectively. Prevalence frequency of 8.1 and 6.5 % were reported by Somi et al. (1999) and 2010 respectively, in Tanzania.

Findings also showed that the age group between 23 to 27 years had the highest prevalence frequency value of 4 (57.1%) when the distribution of HIV among the pregnant women was assessed. There was a significant difference in the trend (p<0.05). It was also observed that the age group between 23-27 years was infected more than the age group greater than 27 years in RIMH unlike the greater distribution of HIV observed among the pregnant women visiting GHE. This may be as a result of chance occurrence among the group (28-32 years) in which a prevalence frequency value of 2 (66.7%) was recorded. This is in agreement with the prevalence studies conducted in southeastern Nigeria by Uneke et al. (2007) and Yahya-Malim et al. (2006), with prevalence frequency values of 45.2 and 33.1%, respectively. However, the

finding contradicts the findings of HIV prevalence studies conducted in Abakaliki, Nigeria by Obi et al. (2005) with a prevalence frequency of 5.4, 6.1 and 6.5%, respectively in southeast Nigeria. There was a significant difference in HIV prevalence among the different age groups of the pregnant women (p<0.05). Usually, the age group between 23 and 27 years is the age at which women are more actively involved in sexual activities. Also, at this same period, some of them get pregnant for the first time. The findings are in line with the report of Yahya-Malima et al. (2006) in which HIV infection was highly associated with third pregnancies at ages below 20 years compared to the first pregnancies at ages above 20 years (Table 4). The findings also showed that the age group between 23-27 and 28-32 years had HBV prevalence frequency of 6(46.1%) and 5(38.5%) respectively at RIMH. Also, at GHE, the age groups of 23-27 and 28-32 years had prevalence frequency values of 2 (66.7%) and 1 (33.3%) respectively. There was a significant difference in the trend (p<0.05). The finding also revealed that the age group of 23-27 year was infected more than the age group greater than 27 years. This is in agreement with the prevalence studies conducted in Africa by Telatela et al. (2007) and Thio et al. (2006) in Tanzania which revealed the prevalence frequency of 57.3 and 21.7%, respectively. However, the findings are not in consonance with the findings of HBV prevalence studies conducted in Ibadan, Nigeria by Adewole et al. (2006), Ahmed et al, (2005) in western Nigeria which gave prevalence frequencies of 21.2, 24.1 and 23.2%, respectively. There was no significant difference in the age groups among the pregnant women observed (p < 0.05).

Table 4. Occupation, educational background, marital status, and trimester of human immunodeficiency virus/hepatitis B virus co-infection in pregnant women visiting RIMH and GHE all in Ebonyi State.

Occupational characteristics	Total no. screened	No. positive for HIV infection (%)	No. positive for HBV infection (%)
RIMH			
Business women	20	2 (40.0)	2(33.3)
House wives	10	0 (0.0)	0 (0.0)
Self-employed	10	0 (0.0)	0 (0.0)
Civil servants	30	3(60.0)	4(66.7)
Total	**70**	**05(100)**	**06(100)**
GHE			
Business women	10	1 (33.3)	3(40.0)
House wives	03	0 (0.0)	0 (0.0)
Self-employed	02	0 (0.0)	0 (0.0)
Civil servants	15	2(66.7)	4(60.0)
Total	**30**	**03(100)**	**07(100)**
n=7 %= χ2 =0.35 P=0.5 df=4			
Educational characteristics			
RIMH			
Primary	20	2 (33.3)	3 (37.5)
Secondary	15	1 (16.7)	1 (12.5)
Tertiary	05	0 (0.0)	0 (0.0)
No formal education	30	3 (50.0)	4 (50.0)
Total	**70**	**06(100)**	**08(100)**
GHE			
Primary	09	1 (33.3)	2 (33.3)
Secondary	05	0 (0.0)	1 (16.7)
Tertiary	02	0 (0.0)	0 (0.0)
No formal education	14	2 (66.7)	3 (50.0)
Total	**30**	**03(100)**	**06(100)**
n= 15, χ2 =0.545 P=0.05 df=4			
Trimester			
RIMH			
First trimester	15	0 (0.0)	1(6.7)
Second trimester	20	3 (30.0)	5 (33.3)
Third Trimester	35	7 (70.6)	9 (60.0)
Total	**70**	**10(100)**	**15(100)**
GHE			
First trimester	05	0 (0.0)	
Second trimester	10	1 (33.3)	1 (25.0)
Third trimester	15	2 (66.7)	3 (75.0)
Total	**30**	**03(100)**	**04(100)**
n= 15, χ2 =0.545 P=0.05 df=4			

The occupational characteristics of the pregnant women attending RIMH and GHE was also considered and the result obtained showed that Civil servants had the highest prevalence frequency of 3 (60.0%) for HIV and 4 (66.7%) for HBV at RIMH while 2 (66.7%) and 4 (60.0%) were recorded at GHE. Business women were also

pre-disposed to this infection with a percentage frequency of 2 (40.0%) for HIV and 2 (33.3%) for HBV at RIMH while 1 (33.3%) and 3 (40.0%) were recorded at GHE for HIV and HBV respectively (Table 4). This result is in accordance with the study carried out by Imade et al. (2004) in Southern Nigeria Ibadan, with prevalence percentage of 36.0 % for HBV and Oladokun et al. (2004), report that 38.9% of co-infection of HBV and HIV among pregnant women in Ibadan ($\chi2=0.35$ P=0.05 df=4). This may be as a result of their various occupations, or as result of improper orientation, poor awareness or ignorance.

The educational qualification of the subjects were also evaluated, and it revealed that pregnant women that had no formal education and primary school living certificate had the highest prevalence rate of HIV and HBV infections with prevalence frequencies of 3 (50.0%) and 4 (50.0%), respectively at RIMH and 2 (66.7%) and 3 (50.0%) at GHE respectively. The prevalence frequencies were 2 (33.3%) for HIV and 3 (37.5%) for HBV at RIMH while 1 (33.3%) were recorded for HIV and 2 (33.3%) for HBV at GHE for pregnant women with primary school certificate. Secondary School Certificate subjects had the lowest rate of HIV infection with a prevalence frequency of 1 (16.7%) for HIV and 1 (12.5%) for HBV and zero prevalence frequency values were recorded among pregnant women with tertiary certificate (n = 100, $\chi2$ = 0.545, P = 0.05, df = 4). This study shows that educational level of the pregnant women was not related to co-infection rates in this work (p > 0.05), but it was in consonance with the study made by Eke et al. (2008) that recorded no relationship among HBV disease and the level of education between people of a lower income earning in Nigeria. Nevertheless, a relationship was found among pregnant women privileged to have education amongst HIV/HBV infection in Anambra State, Nigeria (Ezegbudo et al., 2004).

The trimester was also put into consideration, and it was observed that at first trimester, there was a zero prevalence frequency of HIV and HBV at RIMH; 1 (6.7%) for HIV and zero prevalence frequency for HBV at GHE. At second trimester, prevalence frequencies of 3 (30.0%) and 5 (33.3%) were recorded for HIV and HBV respectively at RIMH, followed by 1 (33.3%) for HIV and 1 (25.0%) for HBV at GHE. Among the pregnant women under investigation at third trimester, 7 (70.0%) had the highest prevalence frequency of HIV at RIMH and 9 (60.0%) for HBV; while 2 (66.7 %) for HIV and 3 (75.0%) for HBV were recorded at GHE. According to the report of Yahya- Malima et al. (2006), HIV and HBV were highly associated with the third pregnancies usually below 20 years of age compared to the first pregnancies at age above 20 years. This study is in line with the report of Yahya- Malima et al. (2006).

The co-infection of HBV/HIV also showed that the age group between 23 and 27 years had the highest prevalence rate of co-infection with prevalence frequency value of 2 (66.7%) and 1(100%) at RIMH and GHE,

respectively. There was a significant difference in this trend (p < 0.05). It was also noticed that the age group of 23-27 years was infected more than the age group greater than 27 years. This is in agreement with the prevalence studies conducted in Africa by Telatela et al. (2007), and Ajuwon et al. (2005) in Tanzania which revealed the prevalence frequencies of 57.3 and 73.2%, respectively.

Conclusion

This survey indicates a high prevalence of human immunodeficiency virus (HIV) and Hepatitis B virus (HBV) in asymptomatic pregnant women visiting Rural Improvement Missionary Hospital (RIMH) and General Hospital (GHE) within Ebonyi State. The high prevalence of HIV and HBV in our young adults reflects either the absence of health policy fighting against this diseases or low progress in understanding the natural history of HIV and HBV infections. It is therefore of paramount importance to frequently carry out longitudinal studies in order to determine the impact of HBV co-infection on HIV treatment response and hepatotoxicity to anti-retroviral drugs in adult's especially pregnant women.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

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