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Full Length Research Paper

Comparative study of the effects of single and dual infections of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) in peripheral blood lymphocytes of infected individuals in Ebonyi State, Nigeria

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A total of 700 blood specimens of patients' attending hematology units of Federal Medical Centre (FMC), Mile Four Hospital, Ebonyi State University Teaching Hospital (EBSUTH) Abakaliki, Mater Misericodia Hospital Afikpo and Private Medical Laboratory Units in Abakaliki town were serologically screened for the presence of human immunodeficiency virus, (HIV), and hepatitis virus and also assayed for absolute lymphocyte count using the improved neubeur chamber heamocytometric method. Fifty two (7.43%), ninety four (13.42%) and eight (1.14%) individuals were positive for HIV, HBV and HIV/ HBV infections respectively. The range of the mean lymphocyte counts for positive individual were 0.6 ± 0.06 - 0.84 ± 0.04 for HIV, 0.51 ± 0.17 - 0.7 ± 0.11 for HBV and 0.35 ± 0.08 - 0.46 ± 0.18 for HIV / HBV x 10^9/L respectively. Individuals who were below 10 years of age were mostly affected by HIV (25.0%), HBV (25.0%) and HIV/ HBV (12.5%). Highest rate of multiple sexual partnerships (63.7%) occurred among the age group of 21 - 30 years. Statistical analysis (χ^2) showed significant relationship between HIV, HBV and HIV/ HBV infections with age. To aid clinical management, the variation of absolute lymphocyte counts may be a guide in monitoring the progression of the diseases.

Key words: HIV, HBV, peripheral blood, lymphocyte count.

INTRODUCTION

Hepatitis B virus infection is highly prevalent among Human Immunodeficiency virus (HIV) infected persons due to shared transmission routes which includes sexual activities, contact with contaminated object, blood transfusion and parenteral routes of transmission (Yan et al., 2007). Sexual activities were reported as the most common means of transmission in Nigeria (Uneke et al., 2005; Forbi et al., 2008) and sero-prevalence of HIV, HBV and co-infection of HIV and HBV were also reported in different regions in Nigeria. (Forbi et al., 2007; FMOH, 2004; Ejele and Ojule, 2004; Uneke et al., 2005). The immune response to HIV and HBV are humoral and cell mediated responses, co-infection or multiple infections of these diseases result in adverse changes to humoral and cell mediated responses (Kuby, 1997). One of such changes is the reduction in the number of circulating lymphocytes in the blood (lymphocytopenia) of infected individuals, lymphocytopenia increases as the severity of these diseases increases. The extent of its increase is estimated among other methods through the

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use of absolute lymphocyte count (Cheesbrough, 2000). In addition HIV exacerbates the clinical disease conditions of HBV in HIV/ HBV co-infected patients, HBV reduces the latent period of HIV and consequently increases the rapid onset of disease conditions (Dore and Cooper, 2001; Kuby, 1997).

The complex nature of the interaction of the agents under study on the immune system poses serious problem to the clinical management of dual or co-infection of these diseases. Consequently this study was designed to aid the clinical management of single or dual infection of these diseases by providing information on disease prevalence and hematologic changes associated with diseases occurrences.

MATERIALS AND METHODS

Study area

The study was conducted from January, 2007 through June 2008 in Abakaliki and Afikpo town in South-Eastern Nigeria of Ebonyi State. These two towns constitute mostly of traders, civil servants, students and farmers.

Study population / sampling techniques

The study was a cross sectional survey of Seven hundred individuals (including adults and children) aged between < 1 - 60 years old that visited the hematology units of Federal Medical centre (FMC), Mile Four Hospital, Ebonyi State University Teaching Hospital (EBSUTH) Abakaliki, Mater Misericodia Hospital Afikpo and Private Medical Laboratory Units in Abakaliki and Afikpo town. Information about the age, age at first sex and multiple sexual partnerships were obtained through a questionnaire given to each patient after ethical clearance was obtained from the ethical committee of Ebonyi State University Teaching Hospital Abakaliki. There were no legal constraints in obtaining information about the sexual activities of adolescents in this part of the globe. Information about the sexual activities of children below the age of 10 years was not obtained. The participants provided informed consent before samples were collected and they were counseled. About 5 mls of blood was aseptically collected by vene-puncture from each patient, two milliliters (2 ml) of venous blood were put into ethyl diaminetetracetic acid (EDTA) bottles and used to assay for the lymphocytes. Sera were separated from the remaining venous blood, stored at -20°C until assayed for the presence of HIV and HBV. Infected patients were referred for post-counseling and treatment.

Total leucocyte count

Dilutions of 1: 20 were made with specimen preserved in EDTA vials using 2% galacial acetic acid tainted with gentian violet (Turks solution). The dilutions were incubated for 5 min at room temperature, the improved Neubauer Chamber was filled with the solution using clean pipette, allowed to settle and counted. The number counted was multiplied by the dilution factor (0.05) (Cheesbrough, 2006).

Leucocyte differential count

Thin films were made on microscopic slide with various blood samples, allowed to dry and stained with Leishman stain. The stained films were viewed under oil immersion objective to enumerate differentially the percentage of white blood cells. The absolute number of each leucocyte per litre was calculated by multiplying the value of the total white blood cell counted with the percentage of each leucocyte found in the differential count.

HIV and HBV serologic tests

The HIV status was determined by abridged Enzyme Linked Immunosorbent Assay (ELISA) method using commercially available abriged ELISA Kits: (ACON HIV ½, ACON Diagnostics' USA REF HH - 401, Bio System, USA N0 098 KE) and was confirmed by a second stage confirmatory tests of two - three rapid test kits with different principles (Capillus HIV ½ Assay, Trinity Biotech Ireland and Determine kit list No 7D 23-43, Abbot Japan Co. Ltd) of antibodies and antigen testing methods as recommended by WHO for resource low country including Nigeria (CDC, 2004). Qualitative HBV screening was done using commercially available abridged ELISA kit (ACON HBV Catalog N0 IHBSg 401, ACON Laboratories INC,) reactive samples were confirmed using a particle agglutination assay procedure (sensitivity 94. 4-10% Seroida - HBsPA Fujirebio, INC). Control group were individuals who had no detectable antigen or antibodies to HIV, HBV infections and HIV /HBV co-infection.

Statistical analysis

Chi- square contingency table was used to determine the relationship between age and disease occurrence (HIV, HBV and HIV/HBV infection). Statistical significance was achieved if $P < 0.05$.

RESULTS

Out of the seven hundred individuals that participated in the study, 52 (7.43%), 94 (13. 42%) and 8 (1.14%) were positive for HIV, HBV, infections and HIV/HBV co-infection respectively. Individuals included in the study were aged between < 1 to 60 years old (Mean age 29.7 years). Individuals of age group 21 - 30 years had higher prevalence of HIV and HBV than others (Table 1). Statistical significant relationship occurred between age of individuals and disease occurrences (HIV, HBV and HIV / HBV infections).

The ranges of the mean lymphocyte counts for the controls were 1.3 ± 0.42 - 4.25 ± 0.82 for HIV, 1.09 ± 0.17 - 4.25 ± 0.82 for HBV and 1.03 ± 0.13 - 4.25 ± 0.82 for HIV/ HBV x 10^9/L, respectively (Table II). Individuals presenting with co-infection of HIV/HBV showed higher values (65.2 and 73.7%) of the percentage decrease in mean lymphocyte counts than those with single infection only.

Figure 1 shows the prevalence of HIV, HBV infections and HIV / HBV co-infection according to age among individuals in Ebonyi State. The number tested and those positive are as follows; < 10 years (2/8) for HIV, 2/8 (HBV), 1/8 (HIV/HBV); 11 - 15 years 23/230 for HIV, 32/230 (HBV) and 4/230 (HIV/ HBV); 16 - 20 years (21/300) for HIV, (49/300) HBV and 3/300 (HIV/HBV);

Table 1. Age distribution of HIV and HBV single and dual infections among individuals in Ebonyi State.

Age range	Number tested (NT)	HIV prevalence number positive	(% +ve)	HBV prevalence. number of positive	(%) +ve	HIV/HBV prevalence percentage positive	% + ve
≤ 10	38	-	-	-	-	-	-
11 - 20	36	10	1.42	8	1.4	4	0.57
21 - 30	408	28	4.0	70	10.0	4	0.57
31 - 40	110	8	1.40	2	0.29		
41 - 50	74	2	0.29	8	1.4		
> 50	34	4	0.57	6	0.86		
Total	700	52	7.43	94	13.42	8	1.14

Table 2. Age distribution of mean lymphocyte counts of individuals infected with single and combined infection of HIV and HBV.

Age range	Mean lymphocyte x 109/L ± S. D (HIV)		% decrease	Mean lymphocyte x 10^9/L ± S. D (HBV)		% decrease	Mean lymphocyte x 10^9/L ± S. D (HIV/HBV)		% decrease
	-ve subgroup	+ve subgroup		-ve subgroup	+ve subgroup		-ve subgroup	+ve subgroup	
< 10	4.25 ± 0.82	-	-	4.25 ± 0.82	-	-	4.25 ± 0.82	-	-
11-20	1.42 ± 0.55	0.73 ±0.08	48.6	1.44 ± 0.51	0.7 ±0.11	51.4	1.32 ± 0.45	0.46 ±0.18	65.2
21-30	1.36 ±0.8	0.66 ±0.10	51.5	1.38 ±0.39	0.64 ±0.10	53.6	1.33 ± 0.36	0.35 ±0.08	73.7-
31-40	1.31 ±0.51	0.7 ±0.12	46.6	1.35 ±0.96	0.62 ±0.08	54.1	1.27 ± 0.03	-	-
41-50	1.32 ±0.65	0.84 ±0.04	36.4	1.34 ±0.75	0.51 ±0.17	61.9	1.31± 0.30	-	-
> 50	1.13 ±0.42	0.6 ±0.06	46.9	1.09 ±0.17	0.56 ±0.06	48.6	1.03 ± 0.13	-	-

21-25 years 6/104 (HIV), 10/104 (HBV) and > 25 years 1/20 (HBV). No statistical significant relationship exists between < 10 years and factors (HIV and HBV infections) except for HIV/HBV coinfection.

Figure 2 shows the percentage of multiple sexual partnerships among different specific age groups of individuals in Ebonyi State. Individuals of 21 - 30 years age group had more multiple sexual partners (63.7%) than others.

DISCUSSION

HIV and HBV infections are usually associated with high case fatality rates and constitutes a major public health problem in sub-Saharan Africa (UNAIDS, 2007; Uneke et al., 2005). In this study, prevalence rates of 7.23, 13.42 and 1.4% were observed for HIV, HBV infections and HIV/ HBV co-infection, respectively (Table 1). Similar prevalence rates of 7.0% for HIV (Federal ministry of Health, Nigeria, 2004) and divergent rates of 4.98% (Ejele and Ojule, 2004) and 27.8% (Forbi et al., 2007) for HBV have previously been reported. The prevalence of 15.49% for HBV among HIV infected individuals recorded in this study reveals a decline when compared with 25.9% previously reported by Uneke et al. (2005) for the same locality. Unprotected sexual intercourse, increased premarital sex (WHO, 2003a), Multiple sex partnership (UNSN, 2001; Forbi et al., 2008) as well as early age of sexual abuse (Kalichman and Simbayi, 2004) are possible contributory factors to the occurrence of these infections. Early -age at - first - sex could suggestively be attributed as was showed in our study (Figure 1). Multiple sexual partnership as a contributory factor is asserted in our study as a result of higher prevalence of both agents among the age group that engaged in it more than others (Figure 2). Although there was no significant relationship between HIV, HBV or HIV/ HBV and specific age groups of individuals in the study ($P > 0.05$), the highest prevalence of both infections

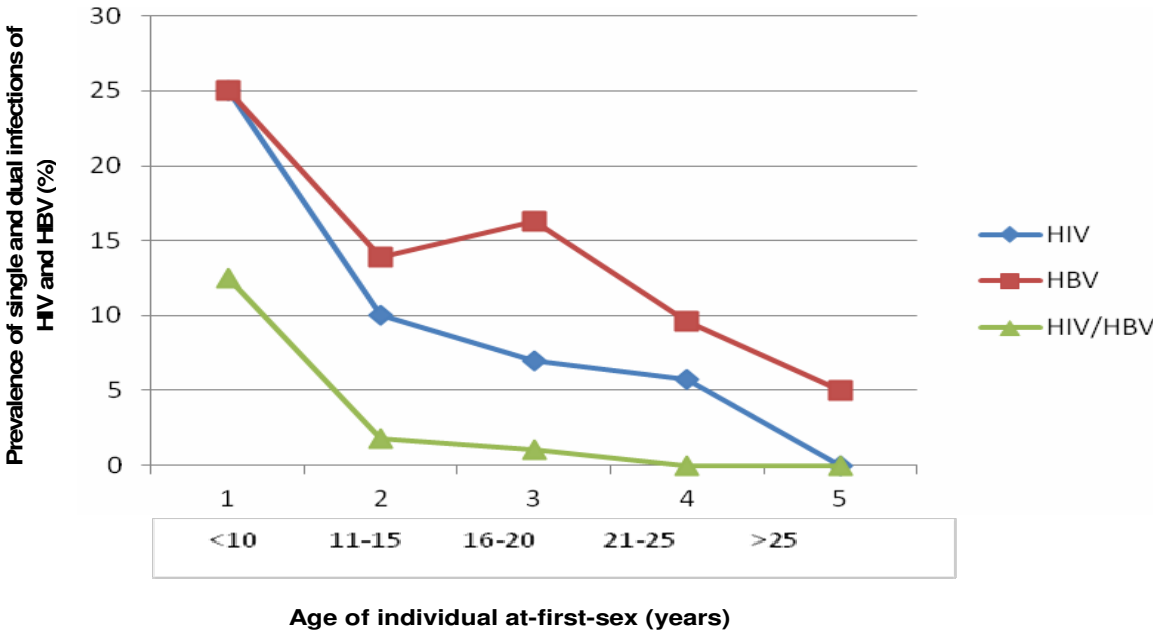

Figure 1. The relationship between age-at-first sex and HIV and HBV single and dual infections.

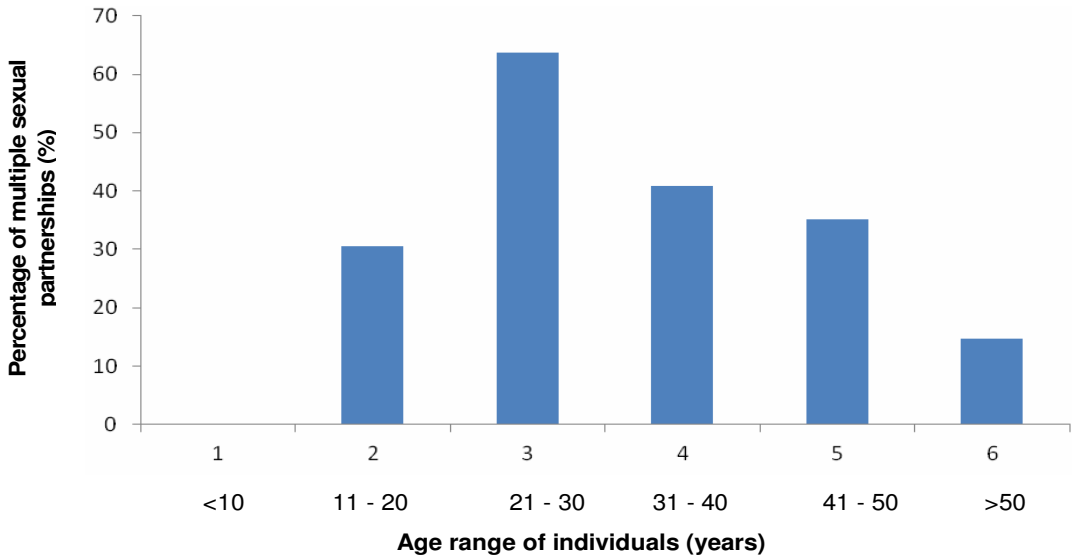

Figure 2. The relationship between age and multiple sexual partnerships among individuals presenting with single and dual infections of HIV and HBV.

were recorded in the 21 - 30 years age group. Sagay et al. (2006) reported higher prevalence of HIV in 25 - 29 year groups whereas Forbi et al. (2008) and Obi et al. (2006) reported higher prevalence of HBV in 31 - 35 and 41 - 45 years age groups respectively.

Comparative evaluation of the absolute mean lymphocyte counts in HIV, HBV infected and HIV/HBV co-infected (Table II) and non-infected individuals showed a decrease in infected individuals. Lymphocytopenia has been reported in single and combined infection with both viruses (Erhabor et al., 2005; Iwalokun et al., 2006 Forbi et al., 2007). This results from massive destruction of CD4 - T lymphocytes by HIV (Singh et al., 2007) and weak T- Cell response with concomitant poor activation of B–cell by HBV. Higher levels of lymphocytopenia were observed in individuals with coinfeciton than those with single infection (Table II) probably as a result of the additive effect of both viruses.

Decrease in CD4 lymphocytes across various age groups has been used as a basis for establishing response to treatment against HIV (WHO, 2003b). In resource poor areas like Ebonyi State of Nigeria where facilities for CD4 count evaluation may be lacking, percentage decrease in absolute lymphocyte count as observed across all age group in HIV, HBV and HIV/HBV infections in this study could be employed for the same purpose.

In considering the high case fatality rate associated with these infections and the need for effective management, the use of percentage decrease in absolute lymphocyte count as an index of monitoring host response to treatment is highly advocated.

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