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## Impact of HIV and Hepatitis B Virus Coinfection on Selected Haematological Markers of the Patients in Umuahia, Abia State, Nigeria

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### Abstract

The study was done to determine impact of HIV/HBV co infection on selected haematological markers of the patients. The study was done in Umuahia, Abia State, Nigeria. One hundred and eighty six subjects were recruited for the study. Eighty (80) subjects were HIV positive patients and twenty six subjects were HIV/HBV co infected patients. Thirty (30) subjects were HBV subjects, fifty (50) subjects were the control. Two milliliters (2.0ml) of venous blood was collected following aseptic techniques from each subject into EDTA anticoagulated containers for the CD4 count and Full blood count. Two different HIV screening kits were used (determine and Unigold test kits) for the detection of HIV-seropositivity following serial algorithm. One step Hepatitis B surface Antigen (HBsAg) test strip method. The CD4 + T cells were determined using flowcytometry method. The results showed significant increase ( $P < 0.05$ ) in all the parameters studied when compared among the HIV monoinfected patients and HIV-HBV co infected patients. There was significant decrease ( $P < 0.05$ ) in CD4+ T cells of the HIV-HBV co infected subjects compared to HIV monoinfected subjects. The results also showed significant difference ( $P < 0.05$ ) in platelets, WBC, absolute neutrophil, PCV and haemoglobin and no significant difference ( $P > 0.05$ ) in absolute lymphocyte of HBV subjects compared to the controls. The study also showed significant difference ( $P < 0.05$ ) in platelets, absolute lymphocytes, absolute neutrophil and no significant difference ( $P > 0.05$ ) in WBC, PCV and haemoglobin. This shows that HBV leads to thrombocytopenia which can cause a lot of bleeding disorders. The viral infection did not affect WBC, PCV and haemoglobin in HIV subjects compared to HBV subjects. The viral infection affects the leucocytes and platelets more than the erythrocytes line. It shows that HBV infection to HIV positive patients is dangerous and should

be prevented. HIV positive persons should be counseled to avoid illicit and unprotected sexual intercourse and other ways that could expose them to the transmission of HBV because of the adverse effects on their health status.

**Keywords:** HIV; HIV-HBV co-infection; Selected haematological markers; Umuahia

### Introduction

Africa is one of the most impoverished regions in the World and bears the highest level of contagious infections such as HIV [1]. HIV-HBV seroprevalence rate according to Obi et al. [2] is 5.5% to 12.3% in Nigeria.

The rate of progression and complications from viral hepatitis are accelerated in patients with HIV co infection according to Puoti [3] and Thio [4]. HBV infected individuals are 6 times more likely to develop chronic hepatitis B than HIV negative individuals [5]. Accurate assessment of HBV infection in HIV co infected individuals is necessary in order to base therapeutic decisions as opined by Thio [4]. WHO encourages HBsAg testing especially in areas of high HBV prevalence and additional testing for HBV markers such as HBsAg and HBV DNA and to assess stage of liver disease [6]. The course of acute HBV may be modified in the presence of HIV infection with a lower incidence of icteric illness and lower rates of spontaneous clearance of HBV [7]. Persons with HIV/HBV co infection have higher levels of HBV DNA and lower rates of clearance of hepatitis B e antigen (HBeAg) according to Thio [8] and Piroth et al. [9].

It is important to carry out this research on the impact of HIV/HBV co infection on the selected haematological markers to help in predicting the level of derangement to the haematologic system of the patients and the progression of the infection.

## Aim

To determine the impact of HIV-HBV coinfection on the selected haematological markers of the patients.

## Materials and Methods

### Study area

The study was done in the Department of University Health Services, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria and Daughters of Mary Mother of Mercy Hospital Ahieke, Ihe Ndume, Umuahia Abia State, Nigeria.

### Subjects

One hundred and eighty-six subjects were recruited for the study. Eighty (80) subjects were HIV positive patients and twenty-six subjects were HIV/HBV co infected patients. Thirty subjects were HBV subjects, fifty subjects were the control. All the subjects were selected from the two hospitals.

### Ethical consideration

The details of the study were explained to the subject before the blood samples were collected. The subjects were allowed to join in the recruitment voluntarily and withdraw at any stage. Informed consents were obtained from them and confidentiality of the results assured to them.

## Laboratory Investigations

### HIV screening

Two different HIV screening kits were used (determine and Unigold test kits) for the detection of HIV-seropositivity following serial algorithm.

### Hepatitis B assay

**Procedure:** The pouch was brought to room temperature and opened to remove the test strip. The test strip was immersed vertically in the serum with arrows pointing downwards for 10 to 15 seconds, making sure that the maximum line on the test strip is not exceeded. The strip was placed on a non-absorbent flat surface and the result read at 15 minutes.

### CD4+ T cells determination

CD4 + T cells were determined flowcytometry.

### Determination of Haemoglobin Level

**Method:** Haemoglobin cyanide technique [10].

**Procedure:** 20 µL (0.02 mL) of well-mixed venous blood was carefully measured and dispensed into 4 mL Drabkin's neutral diluting fluid. It was stoppered and left at room temperature,

protected from sunlight for 4 to 5 minutes. The colorimeter was set at 540 nm wavelength. The colorimeter was zeroed with Drabkin's fluid and the absorbance of the samples was read. Using the table prepared from the calibration graph, the haemoglobin values were obtained.

### Packed cell volume determination

**Method:** Microhaematocrit [10].

**Procedure:** The plain capillary tubes were filled with EDTA venous blood samples, up to three-quarters of the tube. The unfilled ends of the tubes were sealed with a plasticine sealant. The tubes were balanced in a microhaematocrit centrifuge, taking note of the slot numbers. They were centrifuged for 5 minutes at 12000 RCF for 5mins. The PCV values were obtained by aligning the capillary tubes on the microhaematocrit reader, with the base of the red cell column on the zero line and the top of the plasma column on the 100 line.

### Total white cell count

**Procedure:** 380 µL of Turk's solution was dispensed into a test tube and 20 µL of well mixed EDTA anti-coagulated blood was added and mixed well. The counting chamber was mounted with cover slip and allows charging with test solution. The charged chamber was left undisturbed for 2 to 3 minutes for the cells to settle. The chamber was mounted on a light microscope, the rulings were focused and the cell counted using 10 objectives. The total number of cells counted was subjected to the first principle formula.

$WBC = N \times 20 \times 10^6 / 0.4$ ; Where N=number of cells counted; 20=dilution factor; 0.4=depth of the well

### Platelet count

**Procedure:** With the test tubes labeled accordingly 0.038 mL of filtered ammonium oxalate diluting fluid was dispensed into the tubes and 0.02 mL of well mixed venous blood of the subjects added and allowed for 20 minutes undisturbed on blotting paper and covered with a lid. The chamber was placed on the microscope and the platelets counted.

### Blood film/Differential white cell count

Making, fixing and staining blood films

**Procedure:** Thin blood films were made from well mixed EDTA anticoagulated blood, air dried and covered with Leishman stain for 2 minutes, double diluted with buffered water of pH 6.8 and allowed to stain for 10 minutes. The stain was washed with tap water. The back of the slide was wiped clean and stood in a draining rack for the smear to dry. A drop of immersion oil was placed on the lower third of the blood film and covered with a clean cover glass.

The film was examined microscopically using 10 times objective with condenser iris closed sufficiently to see the cells clearly and changed to 100X for differential count (**Tables 1 to 4**).

## Results

**Table 1** Showing haematological markers of HIV monoinfected patients and HIV-HBV co infected patients.

Markers	HIV patients	HIV-HBV Patients	P-Level
CD4+ ( $\mu\text{L}/\text{cells}$ )	296.83 $\pm$ 24.56	225.75 $\pm$ 18.62	P<0.05
Platelets ( $\text{X}10^9/\text{L}$ )	312.33 $\pm$ 34.21	290.75 $\pm$ 26.42	P<0.05
WBC ( $\text{X}10^9/\text{L}$ )	6.57 $\pm$ 1.20	4.53 $\pm$ 0.86	P<0.05
<b>Absolute</b>			
Lymphocytes ( $\text{X}10^9/\text{L}$ )	3.40 $\pm$ 0.75	2.53 $\pm$ 0.52	P<0.05
<b>Absolute</b>			
Neutrophil ( $\text{X}10^9/\text{L}$ )	3.17 $\pm$ 1.52	2.90 $\pm$ 0.86	P>0.05
PCV (%)	36.07 $\pm$ 10.32	31.27 $\pm$ 8.21	P<0.05
Haemoglobin (g/dl)	12.0 $\pm$ 2.50	10.87 $\pm$ 1.57	P<0.05

**Table 2** Showing haematological markers of Hepatitis B Virus and control subjects.

Markers	HBV (30)	Control (50)	P-Level
Platelets ( $\text{X}10^9/\text{L}$ )	170.20 $\pm$ 40.23	252.36 $\pm$ 56.62	P<0.05
WBC ( $\text{X}10^9/\text{L}$ )	6.30 $\pm$ 4.20	4.20 $\pm$ 2.40	P<0.05
<b>Absolute</b>			
Lymphocytes ( $\text{X}10^9/\text{L}$ )	2.45 $\pm$ 0.87	2.68 $\pm$ 0.52	P>0.05
<b>Absolute</b>			
Neutrophil ( $\text{X}10^9/\text{L}$ )	3.72 $\pm$ 0.64	2.26 $\pm$ 0.35	P<0.05
PCV (%)	36.30 $\pm$ 5.2	37.69 $\pm$ 3.50	P<0.05
Haemoglobin (g/dl)	12.10 $\pm$ 0.71	12.56 $\pm$ 1.20	P<0.05

**Table 3** Showing haematological markers of HIV and Hepatitis B Virus subjects.

Markers	HIV (80)	HBV (30)	P-Level
Platelets ( $\text{X}10^9/\text{L}$ )	312.33 $\pm$ 34.21	170.20 $\pm$ 40.23	P<0.05
WBC ( $\text{X}10^9/\text{L}$ )	6.57 $\pm$ 1.20	6.30 $\pm$ 4.20	P>0.05
<b>Absolute</b>			
Lymphocytes ( $\text{X}10^9/\text{L}$ )	3.40 $\pm$ 0.75	2.45 $\pm$ 0.87	P<0.05
<b>Absolute</b>			
Neutrophil ( $\text{X}10^9/\text{L}$ )	3.17 $\pm$ 1.52	3.72 $\pm$ 0.64	P<0.05
PCV (%)	36.07 $\pm$ 10.32	36.30 $\pm$ 5.20	P>0.05
Haemoglobin (g/dl)	12.00 $\pm$ 2.50	12.10 $\pm$ 0.71	P>0.05

## Discussion

The result showed significant increase (P<0.05) in all the parameters studied when compared among the HIV monoinfected patients and HIV/HBV co-infected patients. This shows that the coinfection has more debilitating and suppressive effects to the bone marrow and may increase

chances of cytopenia. The observation could be traceable to increased oxidative stress induced by cytokines and reduced total antioxidant status of the co infected patients before commencement of treatment. In a study done by Obeagu et al. [11], total white blood cell showed significant increase (P<0.05), significant decrease (P>0.05) in PCV, haemoglobin, neutrophil and lymphocyte of the HIV positive patients

compared to the control. It means that HIV suppresses haematopoietic system [12-14]. The significant decrease ( $<0.05$ ) in the haematological markers studied in the co-infected patients could be as a result of the synergistic effects of HIV and HBV on the bone marrow leading to bone marrow failure, peripheral destruction and opportunistic infections. The significant decrease of platelet count in the HIV/HBV co infection may be as a result of accelerated platelet clearance due to immune complex [2,15], glycoprotein antibodies, antiplatelet HIV antibodies that cross react with platelet membrane glycoprotein. If the co infection is not well handled may lead to thrombocytopenia and may cause spontaneous bleeding [2].

There was significant decrease ( $P<0.05$ ) in CD4+ T cells of the HIV/HBV co infected subjects compared to HIV monoinfected subjects. This shows that HBV act as an accelerator of pathogenesis of HIV. It has a suppressive effect on the CD4+ T cells as seen in the HIV/HBV co infection. This increases morbidity and mortality rate of those infected if not well treated immediately. Those infected with HIV must avoid being infected with HBV to avert the debilitating danger to the immune system and haematologic system too.

The result equally showed significant difference ( $P<0.05$ ) in platelets, WBC, absolute neutrophil, PCV and haemoglobin and no significant difference ( $P>0.05$ ) in absolute lymphocyte of HBV subjects compared to the controls. This could be attributable to suppression of haematopoiesis by decreasing bone marrow activity.

The study also showed significant difference ( $P<0.05$ ) in platelets, absolute lymphocytes, absolute neutrophil and no significant difference ( $P>0.05$ ) in WBC, PCV and haemoglobin. This shows that HBV leads to thrombocytopenia which can cause a lot of bleeding disorders. The viral infection did not affect WBC, PCV and haemoglobin in HIV subjects compared to HBV subjects. The viral infection affects the leucocytes and platelets more than the erythrocytes line.

## Conclusion

The study showed significant decrease in the haematological markers and CD4+ T cells of the HIV-HBV co infected subjects. It shows that HBV infection to HIV positive patients is dangerous and should be prevented. HIV positive persons should be counseled to avoid illicit and unprotected sexual intercourse and other ways that could expose them to the transmission of HBV because of the adverse effects on their health status.

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