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## Comparative analysis of haematological parameters in HIV patients with co-infections of hepatitis B & C, and HIV-negative patients in Rivers State, Nigeria

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**Abstract:**

**Background:** Human immunodeficiency virus (HIV) has continued to be a threat to global health with several deaths recorded despite the introduction of highly active antiretroviral therapy (HAART). Co-infection of hepatitis B and C is now one of the leading causes of death among HIV-infected patients due to some haematological abnormalities and immunological impairment. This study was conducted to compare some haematological parameters of HIV-infected patients with hepatitis B and C co-infections from three hospitals in Rivers State, Nigeria

**Methodology:** This was a comparative cross-sectional study of randomly recruited HIV-patients from antiretroviral therapy (ART) clinic and HIV-negative patients from medical out-patient department (MOPD) of three different hospitals in Rivers State, Nigeria. Socio-demographic information of each participant was obtained with a structured questionnaire. Four millilitres of blood were collected from each participant by venipuncture; 2 ml each were dispensed into ethylene diamine tetra acetic acid (EDTA) and plain bottles for estimation of full blood count (FBC), cluster of differentiation 4 (CD4), HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) serology.

**Results:** A total of 375 participants (M:F ratio 1:1.5, age range 10-69 years) comprising 150 HIV patients on ART, 135 ART-naive HIV patients, and 90 HIV-negative patients (control) were recruited. Comparison of haematological parameters among HIV-negative (control), HIV-infected, and HIV/HBV/HCV, HIV/HCV and HIV/HBV co-infected patients showed significant increase ( $p < 0.05$ ) in mean lymphocyte count (%) of  $36.69 \pm 13.25$ ,  $42.02 \pm 12.75$ ,  $46.53 \pm 8.36$ ,  $47.64 \pm 14.35$ , and  $49.61 \pm 5.81$ , and a significant decrease ( $p < 0.05$ ) in mean neutrophil count (%) of  $54.43 \pm 13.52$ ,  $46.33 \pm 13.04$ ,  $44.23 \pm 9.30$ ,  $41.66 \pm 12.94$  and  $40.86 \pm 7.56$  respectively. The mean platelet count ( $10^9/L$ ) in HIV-negative control, HIV-infected, and HIV/HCV, HIV/HBV and HIV/HBV/HCV co-infected patients showed significant decrease ( $p < 0.05$ ) of  $235.25 \pm 109.52$ ,  $229.26 \pm 104.70$ ,  $152.25 \pm 56.64$ ,  $138.69 \pm 56.25$ , and  $130.33 \pm 79.51$ , as well as a significant decrease in CD4 cell counts (cells/ $\mu$ l) of  $803.40 \pm 211.24$ ,  $619.67 \pm 334.13$ ,  $590.63 \pm 312.20$ ,  $550.15 \pm 311.72$ , and  $406.49 \pm 261.75$  respectively.

**Conclusion:** Alterations in the haematological parameters can lead to serious complications in HIV individuals co-infected with HBV and/or HCV. Therefore, HBV and HCV screening for every HIV-infected patient should be made mandatory in Nigeria

**Keywords:** antiretroviral; co-infection; hepatitis B virus; hepatitis C virus; human immunodeficiency virus

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## Analyse comparative des paramètres hématologiques chez les patients séropositifs co-infectés par les hépatites B et C et chez les patients séronégatifs dans l'État de Rivers, au Nigeria

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## Résumé:

**Contexte:** Le virus de l'immunodéficience humaine (VIH) a continué d'être une menace pour la santé mondiale avec plusieurs décès enregistrés malgré l'introduction de la thérapie antirétrovirale hautement active (HAART). La co-infection par l'hépatite B et l'hépatite C est aujourd'hui l'une des principales causes de décès chez les patients infectés par le VIH en raison de certaines anomalies hématologiques et d'une déficience immunologique. Cette étude a été menée pour comparer certains paramètres hématologiques de patients infectés par le VIH avec des co-infections par l'hépatite B et C dans trois hôpitaux de l'État de Rivers, au Nigéria.

**Méthodologie:** Il s'agissait d'une étude transversale comparative de patients séropositifs recrutés au hasard dans une clinique de traitement antirétroviral (TAR) et de patients séronégatifs du service médical ambulatoire (MOPD) de trois hôpitaux différents dans l'État de Rivers, au Nigéria. Les informations socio-démographiques de chaque participant ont été obtenues à l'aide d'un questionnaire structuré. Quatre millilitres de sang ont été prélevés sur chaque participant par ponction veineuse; 2 ml chacun ont été distribués dans des flacons d'acide éthylène diamine tétra acétique (EDTA) et simples pour l'estimation de la formule sanguine complète (FBC), du groupe de différenciation 4 (CD4), du VIH, du virus de l'hépatite B (VHB) et du virus de l'hépatite C (VHC) sérologie.

**Résultats:** Un total de 375 participants (rapport M: F 1: 1,5; tranche d'âge de 10 à 69 ans) comprenant 150 patients séropositifs sous TAR, 135 patients séropositifs naïfs de TAR et 90 séronégatifs (contrôle) ont été recrutés. La comparaison des paramètres hématologiques chez les patients séronégatifs pour le VIH (contrôle), infectés par le VIH et co-infectés par le VIH/VHB/VHC, le VIH/VHC et le VIH/VHB a montré une augmentation significative ( $p < 0,05$ ) du nombre moyen de lymphocytes (%) des  $36,69 \pm 13,25$ ,  $42,02 \pm 12,75$ ,  $46,53 \pm 8,36$ ,  $47,64 \pm 14,35$  et  $49,61 \pm 5,81$ , et une diminution significative ( $p < 0,05$ ) du nombre moyen de neutrophiles (%) de  $54,43 \pm 13,52$ ,  $46,33 \pm 13,04$ ,  $44,23 \pm 9,30$ ,  $41,66 \pm 12,94$  et  $40,86 \pm 7,56$  respectivement. La numération plaquettaire moyenne ( $10^9/L$ ) chez les patients témoins séronégatifs, infectés par le VIH et co-infectés par le VIH/VHC, le VIH/VHB et le VIH/VHB/VHC a montré une diminution significative ( $p < 0,05$ ) de  $235,25 \pm 109,52$ ,  $229,26 \pm 104,70$ ,  $152,25 \pm 56,64$ ,  $138,69 \pm 56,25$  et  $130,33 \pm 79,51$ , ainsi qu'une diminution significative du nombre de cellules CD4 (cellules/ $\mu l$ ) de  $803,40 \pm 211,24$ ,  $619,67 \pm 334,13$ ,  $590,63 \pm 312,55$ ,  $550,15 \pm 311,72$  et  $406,49 \pm 261,75$  respectivement.

**Conclusion:** Des altérations des paramètres hématologiques peuvent entraîner de graves complications chez les personnes VIH co-infectées par le VHB et/ou le VHC. Par conséquent, le dépistage du VHB et du VHC pour chaque patient infecté par le VIH devrait être rendu obligatoire au Nigeria

**Mots clés:** antirétroviral; co-infection; Virus de l'hépatite B; virus de l'hépatite C; virus de l'immunodéficience humaine

## Introduction:

Human immunodeficiency virus (HIV) and acquired immune deficiency syndrome (AIDS) emerged some decades ago to become a global pandemic especially in the sub-Saharan Africa where it has brought so much pain and hardship to many people. Non-adherence to antiretroviral therapy (ART) and delay in administering ART has resulted in reduction of T-cells in patients with HIV infection. The immunosuppression experienced by HIV infected individuals renders them very susceptible to opportunistic infections which could be viral, bacterial, parasitic or fungal. Co-infection with HBV and/or HCV is now known to be caused by drug resistance among individuals with related haematological abnormalities (1,2).

Globally, HBV and HCV infections remain major causes of public health problems and are responsible for high morbidity and mortality rate. They continue to pose very serious clinical issues in developing countries because majority of the populace are not even aware of these infections and the task of planning for the

screening of the general populace is not an easy one. In most cases, detection is done very late and most of those infected are not able to pay for the high cost of effective management (3,4). The World Health Organisation (WHO) report reports that 2 - 4% and 4 - 5% of the global population of HIV positive patients infected with HBV and HCV respectively (2). In developing countries, the routes of transmission of these viruses and risk factors are similar and include; transfusion of infected blood and blood products, sexual contact, sharing of injection needles by drug addicts, using unsterilized sharp objects during surgical operation and vertical transmission from mother to child (5,6,7).

HIV-infected patients are known to have different haematological challenges including, anaemia, thrombocytopenia, lymphopenia and neutropenia (8). HIV disease causes alteration in these parameters as the disease progresses and can occur among those patients on antiretroviral therapy (ART) and those who are ART-naïve (9). An early examination of these parameters will help the clinicians in better management of HIV patients.

Anaemia is a common feature in HIV patients with a rate of 13- 95%, the commonest form being, normocytic normochromic anaemia followed by microcytic anaemia (10,11). Thrombocytopenia is another haematological abnormality observed among about 3-40% of HIV patients and tends to appear in all stages of the infection. Major causes could be platelet reduction due to auto immunity by cytokines, cross-reacting cytokines which are set against HIV proteins, mostly the gp120 and p24. This type of alteration is known as immune thrombocytopenic purpura (ITP) which involves depletion in values of platelet with regular values of haematocrit and white blood cell counts (9).

Another leucopenia normally observed among these subjects is neutropenia which affects about 10-30% of them at the final stage of the infection. Leucopenia could also be caused by co-infections such as, tuberculosis, hepatitis, histoplasmosis, leishmaniasis and others. Co-infection of HBV and/or HCV with HIV affects the bone marrow in a way that the granulocyte macrophage colony-stimulating factor (GM-CSF) which is responsible for haematopoiesis of the white blood cells in stromal with a resultant effect on the granulocyte-macrophage pedigree, are depleted, resulting in leukopenia and neutropenia. Also, HIV may cause lymphopenia with as the disease progresses, with resultant depletion of CD4<sup>+</sup> T-lymphocytes (9,10). Thus, the objective of this comparative study is to describe the haematological changes that could occur in HIV patients co-infected with HBV/HCV and HIV-negative controls, in order to assist in preparing better treatment plan for these patients.

## Materials and method:

### Study area

This study was carried out in three different hospitals within the geopolitical zones in Rivers State, Nigeria; Zonal Hospital Ahoada (Rivers West), Zonal Hospital Bori (Rivers South East) and Rivers State University Teaching Hospital (Rivers East).

### Study design and participants

This was a cross-sectional study of 375 participants consisting of 285 HIV-infected patients (150 on antiretroviral therapy and 135 ART-naive) and 90 HIV-negative patients. The HIV-patients (of both gender and different age groups) were systematically recruited from the ART clinics of the three hospitals (n=95 from each hospital) and the HIV-negative patients from medical out-patient department (MOPD) of the three hospitals (n=30 from each hospital).

### Ethical approval and consent

Ethical approval for the study was obtained from the office of the Permanent Secretary of the Rivers State Ministry of Health and the Rivers State Hospital Management Board. The consent of each participant was obtained through the filling of the consent form.

### Calculation of sample size

The minimum sample size required for the study was calculated using the formula;  $N = Z^2pq/d^2$  where, N=sample size, Z=statistic corresponding to level of 95% confidence level (1.96), p=expected prevalence of 3.8% or 0.038 (12), d=level of significance (allowable error) which is 5% (0.05) and q=1-p. This gives a minimum sample size of 56, which was increased to 375.

### Sample collection and preparation

The socio-demographic information of each participant was obtained using a structured questionnaire. Four millilitres of venous blood were collected from each participant and 2 ml each dispensed into ethylene diamine tetra acetic acid (EDTA) and plain bottles for haematological and serological analysis. Separation of samples in plain bottles was in a centrifuge at 1500 rev/min for 5 minutes. Storage of serum and whole blood was done at -20°C and 2-8°C respectively for samples that were not analysed the same day.

### Determination of full blood count

Full blood count (FBC) was determined using Sysmex XP-300 machine for total white blood count (tWBC), red blood cells (RBC), haemoglobin concentrations (HB), packed cell volume (PCV), neutrophils (NEUT), lymphocytes (LYM), monocytes, eosinophils and basophils (MEB), and platelets (PLT).

Briefly, whole blood was allowed to mix for 10 minutes on the blood mixer. The power switch was turned on, self-check, auto rinse and background check were automatically performed and the "ready" (ready for analysis) appeared. The test and control samples were introduced into the instrument through the probe. Quality control check was done on the sample with the control blood provided (Eight check-3WP) using X control or L-J control program from the manual. Whole blood mode was selected (as the case may be) and samples numbers imputed. The plug was removed from the vacutainer tube while preventing the scattering of blood. Sample or control was introduced through the sample probe and the start button pressed for the sample or control to be aspirated. The sample tube was removed when

the buzzer made a "beep" sound and when the LCD screen displayed "Analyzing". The instrument automatically executed the analysis and displayed the result on the LCD screen. After this, the unit turns to the "Ready" status becoming ready for analysis of the next sample. On the ready status the shut-down key was pressed after each day's work. Shut down screen appeared. The machine prompted for the rinse (cell clean) solution, the cell clean solution was set to the sample probe and the start button was pressed holding the cell clean in the same state. The buzzer made the "beep" sound which indicated the completion of aspiration. The cell clean solution was removed from the sample probe and shutdown executed automatically.

#### **Determination of CD4 cell count**

CD4 cells count was determined using the BD FACS Count machine (BD Biosciences, USA) which uses the principle of flowcytometry (13,14). Reagent tubes were labeled, vortexed for 5 seconds and opened using the coring station. Test samples were properly mixed and 50 µl of whole blood was pipetted and added to the reagent tube. The tubes were capped, mixed and incubated for 60 mins in the dark at 20-25°C. The tube was later uncapped and 50 µl of fixative was added to the tubes and recapped. The 'on button' was switched on, daily cleaning carried out, control code verified and control samples were run. The 'sample button' on the machine was pressed, verification of the reagent code was done and sample ID entered. The CD4 tubes were vortexed again, uncapped, placed in the sample holder and the 'run button' pressed. After each analysis, the result was displayed automatically on the screen and the tube was removed.

#### **HIV screening**

The National algorithm for HIV screening in Nigeria which involves the use of three test kits was employed, with two for parallel testing and one for a tie-breaker. The three kits were Determine-HIV 1/2 (Abbott Japan Co., Ltd., Germany), Uni-Gold-HIV 1/2 (Trinity Biotech, France) and Stat-Pak Dipstick (Chembio Diagnostic System Inc). The manufacturers' standard operating procedure (SOP) was followed in performance of the test. HIV seropositivity was defined as a reactive result on two of the test kits. Non-reactive subjects were considered sero-negative.

#### **Determine HIV 1/2 test principle and procedure**

The Abbott Determine HIV 1/2 is an *in vitro* visually read, rapid immuno-chromatographic test for the qualitative detection of anti-

bodies to HIV-1 and HIV-2 in human serum, plasma or whole blood. It involves removing the test device from the pouch and applying 50µl of the test sample to the test pad (marked by arrow symbol) and then waiting for the sample to migrate through the conjugate pad. To ensure assay validity, a procedural control is incorporated in the device and labeled "control". If the control bar does not turn red at the completion of the assay, the test result is invalid and the sample must be retested. The result was read within 15 minutes

#### **HBV and HCV screening**

The screening involved testing serum sample of each patient for the presence of hepatitis B surface antigen (HBsAg) and antibody to hepatitis C virus using 'DiaSpot', a commercially available test kits for the detection of HBsAg or anti-HCV antibody based on principle of sandwich immunoassay in which recombinant antigens are employed sufficiently to identify anti-HCV or HBsAg with high sensitivity and specificity.

Chromogen embedded moves by diffusion to develop colour at test band region where Ag-Ab-Ag complex is formed. Simultaneously, human immunoglobulins present in the serum will be captured by anti-human globulin antibodies at the control region on which the chromogen also impact colour to give a control band. The test device was removed from pouch and dipped into specimen for 3 seconds with the arrow pointing downwards. The device was later laid on a clean, dry and non-absorbent surface. The result was read within 15 minutes.

#### **Quality control**

The samples were tested on the machines after running the controls and the standard operational procedures (SOPs) were strictly followed to ensure accuracy of the results.

#### **Statistical analysis**

Data generated were analysed using the Statistical Package for Social Sciences (SPSS) and Excel (version 22.0) package. Data presented as mean±SD were compared using the Students' *t*-test while demographic data were compared using Chi square test, and *p* value < 0.05 was considered statistically significant.

#### **Results:**

A total of 375 participants from three hospitals; Rivers State University Teaching Hospital (RSUTH), Zonal Hospital Bori (ZHB) and Zonal Hospital Ahoada (ZHA) representing the

three senatorial districts in Rivers State, were recruited for the study (Table 1). One hundred and fifty (40.0%) were HIV-infected patients on ART, 135 (36.0%) were ART-naïve HIV-infected patients while 90 (24.0%) were HIV-negative controls. Male constituted 40.3% (151/375) while females constituted 59.7% (224/375) with a male to female ratio of 1: 1.5. The age groups most represented were 20-29 years (26.7%) and 30-39 years (23.7%), while the least represented age groups were 60-69 years (7.2%) and 10-19 years (8.3%).

Table 2 shows the haematological parameters of HIV-infected patients (who were on ART and who were ART-naïve). There was no significant difference ( $p>0.05$ ) in the mean values of the total white blood cells, red blood

cells, haemoglobin concentration and haematocrit between HIV patients (ART and ART-naïve) and HIV-negative patients. On the other hand, there was significant increase ( $p<0.05$ ) for MED (majorly, monocytes) and lymphocytes while the neutrophils and platelets showed significant decrease ( $p<0.05$ ) in HIV patients (on ART and ART-naïve) when compared with HIV-negative patients.

The comparison of the mean±standard deviation (SD) for these parameters showed no significant difference ( $p>0.05$ ) in mean values in mean values of all the haematological parameters when comparing HIV-infected males on ART with HIV-infected females on ART as shown in Table 3 but CD4 cell count was significantly higher in male than female ( $p=0.007$ ).

Table 1: Demographic and clinical characteristics of HIV-infected and HIV-negative patients in three hospitals, Rivers State, Nigeria

Characteristics	No of subjects on ART (%) (n=150)	No of ART-naïve subjects (%) (n=135)	No of control subjects (%) (n=90)	Total no of subjects (%) (n=375)	p value
<b>Gender</b>					
Male	58 (38.6)	59 (43.7)	34 (37.8)	151 (40.3)	0.5902
Female	92 (61.3)	76 (52.3)	56 (62.2)	224 (59.7)	
<b>Age group (years)</b>					
10-19	12 (8.0)	11 (8.2)	8 (8.8)	31 (8.3)	0.9940
20-29	37 (24.6)	38 (28.2)	25 (27.7)	100 (26.7)	
30-39	38 (25.3)	29 (21.5)	22 (24.4)	89 (23.7)	
40-49	30 (20.0)	24 (17.8)	15 (16.6)	69 (18.4)	
50-59	21 (14.0)	23 (17.0)	15 (16.6)	59 (15.7)	
60-69	12 (8.0)	10 (7.4)	5 (5.5)	27 (7.2)	
<b>Hospital</b>					
RSUTH	50 (33.3)	45 (33.3)	30 (33.3)	125 (33.3)	1.0000
ZHB	50 (33.3)	45 (33.3)	30 (33.3)	125 (33.3)	
ZHA	50 (33.3)	45 (33.3)	30 (33.3)	125 (33.3)	

ART=Antiretroviral therapy; RSUTH = Rivers State University Teaching Hospital; ZHB = Zonal Hospital Bori; ZHA = Zonal Hospital Ahoada

Table 2: Haematological Parameters of HIV- infected patients and HIV-negative patients in the three hospitals, Rivers State, Nigeria

Haematological parameters	HIV-infected on ART (n=150)	HIV-infected ART-naïve (n=135)	HIV-negative control (n=90)	F value	p value
WBC ( $\times 10^9$ /L)	5.69 ± 3.19	5.58±2.36	6.23±0.37	0.67	0.51
RBC ( $\times 10^{12}$ / $\mu$ L)	3.97 ± 0.93	7.09±3.64	4.19±0.89	0.76	0.47
HB (g/dL)	10.92 ± 2.47	10.76±2.11	10.87±2.43	0.16	0.83
HCT (%)	33.88 ± 7.57	35.63±6.30	33.53±7.35	2.58	0.77
MEB (%)	11.06 ± 5.55 <sup>b</sup>	11.03±4.77 <sup>b</sup>	8.49±3.25 <sup>a</sup>	21.01	<0.001*
LYM (%)	45.06 ± 10.76 <sup>b</sup>	41.77±13.46 <sup>b</sup>	36.70±13.25 <sup>a</sup>	16.78	<0.001*
NEUT (%)	44.07 ± 17 <sup>a</sup>	46.77±13.35 <sup>a</sup>	54.43±13.52 <sup>b</sup>	9.01	<0.001*
PLT ( $\times 10^9$ /L)	211.11±70.62 <sup>b</sup>	225.71±89.24 <sup>ab</sup>	261.40±85.59 <sup>a</sup>	4.45	0.012*

WBC= white blood cells; RBC=Red blood cell; HB=Haemoglobin; HCT=Haematocrit; MEB= Monocytes, Eosinophils, Basophils; LYM = Lymphocytes NEUT = Neutrophils; PLT= Platelets. Values with different subscripts are significantly different ( $p<0.05$ )

Table 3: Haematological parameters of HIV-infected patients on ART with respect to gender

Gender	WBC ( $\times 10^9$ /L)	RBC ( $\times 10^{12}$ / $\mu$ L)	HB (g/dl)	PCV (%)	LYM (%)	MEB (%)	NEUT (%)	PLT ( $\times 10^9$ /L)	CD4 (cells/ $\mu$ l)
Male	5.49 $\pm$ 2.59	3.91 $\pm$ 0.80	11.15 $\pm$ 2.6	34.59 $\pm$ 7.8	46.37 $\pm$ 10.3	11.76 $\pm$ 6.2	42.48 $\pm$ 10.9	231.52 $\pm$ 82.1	729.62 $\pm$ 244.64
Female	5.83 $\pm$ 3.77	4.01 $\pm$ 0.80	10.55 $\pm$ 2.2	32.74 $\pm$ 7.1	44.24 $\pm$ 11.0	10.61 $\pm$ 5.1	45.07 $\pm$ 11.2	214.42 $\pm$ 72.8	621.46 $\pm$ 303.68
p value	0.53	0.14	0.32	0.59	0.63	0.23	0.78	0.42	0.007*
T value	0.4	2.22	0.01	0.3	0.24	1.48	0.08	0.66	7.37

WBC=White blood cell; RBC=Red blood cell; HB=Haemoglobin; MEB=Monocytes, Eosinophils, Basophils; LYM = Lymphocytes; NEUT = Neutrophils; PLT = Platelet, HCT = Haematocrit; \* = statistically significant ( $p < 0.05$ )

Table 4: Comparison (ANOVA) of haematological parameters in HIV co-infections and HIV-negative controls

Haematological parameters	HIV/HBV	HIV/HCV	HIV/HBV/HCV	HIV	Control	F-value	p value
WBC ( $\times 10^9$ /L)	5.08 $\pm$ 1.69	4.28 $\pm$ 1.08	4.43 $\pm$ 1.24	5.69 $\pm$ 2.33	6.23 $\pm$ 2.37	1.67	1.50
RBC ( $\times 10^{12}$ / $\mu$ L)	3.88 $\pm$ 0.82	4.44 $\pm$ 1.21	3.57 $\pm$ 0.81	4.08 $\pm$ 0.97	4.19 $\pm$ 0.69	1.11	0.35
HB (g/dl)	10.99 $\pm$ 2.56	10.87 $\pm$ 2.43	9.17 $\pm$ 2.64	10.98 $\pm$ 2.11	11.53 $\pm$ 2.67	0.65	0.63
HCT (%)	35.52 $\pm$ 7.21	33.53 $\pm$ 7.35	29.60 $\pm$ 8.41	35.75 $\pm$ 6.19	36.21 $\pm$ 8.07	1.37	0.25
LYM (%)	47.64 $\pm$ 14.35 <sup>c</sup>	49.61 $\pm$ 5.81 <sup>d</sup>	46.53 $\pm$ 8.36 <sup>c</sup>	42.02 $\pm$ 12.75 <sup>b</sup>	36.69 $\pm$ 13.25 <sup>a</sup>	2.76	<0.001*
MEB (%)	10.85 $\pm$ 5.25	9.53 $\pm$ 3.74	9.23 $\pm$ 4.03	11.31 $\pm$ 5.10	8.84 $\pm$ 3.08	1.91	0.11
NEUT (%)	41.66 $\pm$ 12.94 <sup>a</sup>	40.86 $\pm$ 7.56 <sup>a</sup>	44.23 $\pm$ 9.30 <sup>b</sup>	46.33 $\pm$ 13.04 <sup>c</sup>	54.43 $\pm$ 13.52 <sup>d</sup>	3.66	0.007*
PLT ( $\times 10^9$ /L)	138.69 $\pm$ 56.25 <sup>c</sup>	152.25 $\pm$ 56.64 <sup>b</sup>	130.33 $\pm$ 79.51 <sup>a</sup>	229.26 $\pm$ 104.70 <sup>d</sup>	235.25 $\pm$ 109.52 <sup>e</sup>	5.02	0.001*
CD4 (cells/ $\mu$ l)	550.15 $\pm$ 311.72 <sup>b</sup>	590.63 $\pm$ 312.20 <sup>c</sup>	406.49 $\pm$ 261.75 <sup>a</sup>	619.67 $\pm$ 334.13 <sup>d</sup>	803.40 $\pm$ 211.24 <sup>e</sup>	15.73	<0.001*

WBC=White blood cell; RBC=Red blood cell; HB=Haemoglobin; MEB=Monocytes, Eosinophils, Basophils; LYM = Lymphocytes; NEUT = Neutrophils; PLT = Platelet, HCT = Haematocrit. Values with different superscripts are significantly different ( $p < 0.05$ )

In Table 4, comparison of haematological parameters among HIV-negative (control), HIV-infected, and HIV/HBV/HCV, HIV/HCV and HIV/HBV co-infected patients showed significant increase ( $p < 0.05$ ) in mean lymphocyte counts of 36.69 $\pm$ 13.25, 42.02 $\pm$ 12.75, 46.53 $\pm$ 8.36, 47.64 $\pm$ 14.35, and 49.61 $\pm$ 5.81, and a significant decrease ( $p < 0.05$ ) in mean neutrophil counts of 54.43 $\pm$ 13.52, 46.33 $\pm$ 13.04, 44.23 $\pm$ 9.30, 41.66 $\pm$ 12.94 and 40.86 $\pm$ 7.56 respectively. The mean platelet count ( $10^9$ /L) in HIV-negative control, HIV-infected, and HIV/HCV, HIV/HBV and HIV/HBV/HCV co-infected patients showed significant decrease ( $p < 0.05$ ) of 235.25 $\pm$ 109.52, 229.26 $\pm$ 104.70, 152.25 $\pm$ 56.64, 138.69 $\pm$ 56.25, and 130.33 $\pm$ 79.51, as well as a significant decrease in CD4 cell counts (cells/ $\mu$ l) of 803.40 $\pm$ 211.24, 619.67 $\pm$ 334.13, 590.63 $\pm$ 312, 550.15 $\pm$ 311.72, and 406.49 $\pm$ 261.75 respectively.

## Discussion:

Although there have been drastic improvements in level of survival and HIV progression due to the use of combination antiretroviral

therapy (ART), complications resulting from co-infections which could injure major organs of the body such as the liver, heart, kidney and others are now the major cause of death among HIV-infected patients. This study was conducted to compare the haematological parameters of HIV-patients co-infected by hepatitis B and C viruses with HIV-negative controls.

Our data showed that 40.0% of the recruited patients were HIV-infected on ART, 36.0% were HIV-infected but ART-naive while 24.0% were HIV-negative control, and male (40.2%) to female (59.8%) ratio was 1:1.5. The high ratio for females may be indicative of the fact that females tend to access hospital care more commonly than males, and not necessarily that more females are HIV-infected. The age groups 20-29 and 30-39 years were most commonly HIV-infected, with 26.7% and 23.7% of the total study population respectively. These age groups are sexually active and are usually involved in risky behaviors that could expose them to sexually transmitted infections such as HIV, HBV and HCV.

The mean values of some haematological parameters (total white blood count, red

blood cell, haemoglobin and haematocrit) were not significantly different between HIV-infected patients (on ART and ART-naive) compared to HIV-negative controls ( $p>0.05$ ). However, there was significant increase in the mean values of MEB (monocytes, eosinophils and basophils) and lymphocytes, and a decrease in the mean values of neutrophils and platelets ( $p<0.05$ ) between HIV-infected patients and HIV-negative controls. These findings agree with those of other studies (2,15) on the types of cytopenia commonly observed among HIV-infected individuals. The significant decrease in neutrophil and platelet counts in HIV co-infections indicate that co-infection suppresses the bone marrow and may increase the chances of cytopenia. This could be the result of increased oxidative stress caused by cytokines and reduction of oxidant status of co-infected patients. Our findings agree with others (16) showing that neutropenia and thrombocytopenia are common in viral infections such as HIV and hepatitis.

Although there was a significant increase ( $p<0.05$ ) in the mean values of lymphocytes and monocytes in HIV-mono and co-infected patients in our study compared to HIV-negative controls, there was no significant difference ( $p>0.05$ ) in the values of the red blood cells. It has been reported that both HIV and HBV/or HCV infections exert significant effect on the white blood cells rather than the red blood cells (15). In spite of the initiation of ART, alteration in haematological parameters can impact negatively on HIV-infected patients especially in those with co-infection of hepatitis B or C virus (10). The decrease in neutrophils and platelets among co-infected individuals could be attributed to synergistic effects of HIV and HBV/or HCV on the bone marrow with resultant effect of bone marrow failure, destruction of peripheral cells and invasion by opportunistic pathogens. Thrombocytopenia in co-infected patients can lead to immune complex-mediated platelet clearance and anti-platelet HIV antibodies that can cross react with platelet membrane glycoprotein, creating a situation could lead to serious bleeding (17).

In this study, there was no significance difference in all the haematological parameters HIV-infected patients on ART with respect to gender ( $p>0.05$ ). Although, the mean haemoglobin concentration in males ( $11.15\pm 2.6$  g/dl) was higher than the value in females ( $10.55\pm 2.2$  g/dl), this difference was not statistically significant ( $p=0.32$ ). This contradicts previous findings of studies conducted in Nigeria and Uganda where significantly higher haemoglobin values were reported in HIV-infected females on ART than their than male counterpart (18,19).

The lower but insignificant mean haemoglobin concentration in HIV-infected females in our study could be associated with iron deficiency commonly experienced during the menstrual cycle especially as most of the participants in our study were within child-bearing age.

The mean CD4 cell count in males (729 cells/ $\mu$ l) was however significantly higher than in females (621 cells/ $\mu$ l) ( $p=0.007$ ). It is expected that CD4 cell counts will drop as HIV infection progresses, and initiation of ART is meant to increase the CD4 cell count, but in the presence of other co-infections, CD4 cells are further reduced. In our study, there was statistically significant reduction in the mean CD4 cell count in HIV mono-infected (619 cells/ $\mu$ l) patients, and in HIV/HCV (590 cells/ $\mu$ l), HIV/ HBV (550 cells/ $\mu$ l), and HIV/ HBV/HCV (406 cells / $\mu$ l) co-infected individuals, compared to HIV-negative controls (803 cells/ $\mu$ l). This finding is at variance with the mean CD4 count of 141.6 cells/ $\mu$ l and 121 cells/ $\mu$ l reported in studies conducted in South Africa and Nigeria respectively (provide reference!!). This disparity may be due to differences in the immune status of the individuals in the study or due to viral hepatitis. In individuals who have both HIV and HBV infections, there may be high HIV and HBV viral replication that may further contribute to impairment of the immune system of the patients.

The mean CD4 count (590 cells/ $\mu$ l) in HIV/HCV co-infected patients reported in our study was comparably higher than the mean CD4 count of 274 cells/ $\mu$ l, 260 cells/ $\mu$ l and 288.6 cells/ $\mu$ l reported from studies conducted in Nigeria, Ethiopia and India respectively (18,19). The reason for this disparity is unclear, however, this finding agrees with other study in Nigeria (20), indicating that those with HIV/HCV co-infection tend to present at earlier stages of the disease when immunity may still be strong.

## Conclusion:

Our study showed that levels of white blood cells such as lymphocytes, neutrophils, platelets, and CD4 cells, which are markers of some haematological disorders like leukaemia and bleeding, were significantly affected by HIV co-infection with hepatitis B and C viruses. Screening for HBV and HCV should be routinely done for every HIV-infected patient, and these haematological parameters should be monitored regularly for better management of the patients.

## Conflict of interest:

Authors declare no conflict of interest

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