

Correlation of Haematological Parameters with TNF- α and Interleukin (IL-6) in Seropositive HIV Patients on Highly Active Antiretroviral Therapy (HAART) and HAART Naïve patients.

ABSTRACT.

Objective: This study sought to establish the correlation between haematological parameters and inflammatory cytokines (TNF- α and IL-6) and also to compare the effects of these cytokines in HIV/AIDS patients on HAART and HAART naïve.

Place and Duration of Study: Komfo Anokye Teaching Hospital, Kumasi from June to November 2014

Methods: HIV patients' on HAART (n=50) and HAART naïve HIV (n=50) patients and the control group (n=50) were recruited for the study. Venous blood was drawn from each of the participant into K₃ EDTA tubes. XS-500i automatic blood cell analyzer (Sysmex Corporation Co., Ltd., Japan) was used to determine the blood parameters and ELISA Assay Kit (Greenstone Swiss Company limited, China) was used to determine TNF- α and IL-6 levels.

Results: This present study observed Hgb of 14.36 ± 0.20 g/dl in control group, 12.26 ± 0.28 g/dl and 12.13 ± 0.27 g/dl in on-HAART and HAART naïve group, RBC of $5.07 \pm 0.08 \times 10^6/\mu\text{l}$ in control, $4.20 \pm 0.09 \times 10^6/\mu\text{l}$ and $4.226 \pm 0.09 \times 10^6/\mu\text{l}$ in on-HAART and HAART naïve group. IL-6 levels of 17.66(13.00 - 24.10) ng/L and TNF- α levels of 318.3(123.0 - 497.9) ng/L.

24 **Conclusion:** There was a negative correlation between some haematological parameters
25 and the inflammatory cytokines (TNF- α and IL-6). This could be the basis for the continuous
26 reduction in the level of blood parameters which is likely to result in haematological
27 abnormalities such as anaemia, thrombocytopenia amongst others.

28 **Keywords:** Haematological parameters, HAART, HAART Naïve, HIV patients, TNF- α , IL-6

29 INTRODUCTION

30 Haematological parameters are very important in the determination of the severity of a
31 disease or the effectiveness of a treatment modality in some disease conditions [1]. The
32 levels of Haemoglobin, White Blood Cell, Red Blood Cell, Mean Cell Volume, MCHC,
33 Platelet amongst others are used for clinical diagnosis and prognosis. For instance, malaria
34 is associated with low haemoglobin levels and low platelet levels [2]. The use of these
35 haematological parameters in clinical diagnosis has made it prudent for clinicians to request
36 for the haematological profile of patients with conditions like anaemia, malaria, thrombosis
37 amongst others.

38 HIV infection is mostly transmitted through sexual intercourse whether homosexual or
39 heterosexual engagement from the body fluid of the infected individual to his/her partner.
40 Mothers having the virus can also transmit it to their babies during pregnancy, at the point of
41 delivery or through breastfeeding that is Mother-To-Child Transmission (MTCT). HIV-1 and
42 HIV-2 are the only types discovered with the same mode of transmission, progressing to
43 AIDS. However, transmission of HIV-2 is a little slower than HIV-1, which accounts for an
44 extended period between infection and the later stage of AIDS in HIV-2 patients. Drug users
45 can also be infected with the virus when they administer injections using a single infected
46 syringe. HIV destroys the immune system by invading the CD4 cells in the blood [3-5]. The
47 more CD4 cells that are destroyed by the virus, the weaker the immune system becomes.
48 Reduction in CD 4 T cell levels could be due to the cytotoxic effect of CD8 lymphocyte,
49 programmed cell death of infected cells and the viral attack [4, 5]. The virus may target

immune cells like macrophages, and dendritic cells [6]. People diagnosed of HIV infection are placed on Highly Active Antiretroviral Therapy (HAART) to suppress viral replication thereby increasing the life expectancy of HIV/AIDS patients [6, 7]. TNF attracts more inflammatory cells to the site of infection and elevated levels causes heat, swelling, redness and pain at the site of infection. It also exerts systemic protective effect on the hypothalamus region of brain which causes fever. TNF has a suppressive effect on appetite and regenerative ability of the bone marrow stem cell division and a depressive effect on myocardial contractility through the perfusion of its tissues. High levels TNF- α is understood to speed up pathogenesis in HIV patients by supporting the NF- κ B pathway transcription of new virion [3, 4]. IL-6 is noted to be high in retroviral infection, autoimmune diseases and benign or malignant tumor patients and promotes HIV. It mediates T-cell activation, stimulates proliferation of keratinocytes and at the beginning of acute inflammation, mediates the acute phase responses. It is therefore pertinent to know the correlation existing between the haematological parameters and IL-6, TNF- α of HIV patients on this treatment modality and those who are not on the therapy. This study therefore sought to establish the correlation between haematological parameters and inflammatory cytokines (TNF- α and IL-6) and also to compare the effects of these cytokines in HIV/AIDS patients on HAART and HAART naïve.

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69 MATERIALS AND METHODS

This was a hospital-based, case control study conducted from June to November 2014 in the Komfo Anokye Teaching Hospital (KATH), Kumasi, Ghana. 150 participants in all were recruited for this study. Hundred participants were recruited from the HIV clinic at KATH out of which 50 were on ART and the other 50 participants were treatment naïve. The 50 control group was selected from client visiting the blood bank unit of KATH to make voluntary donation. Whilst HIV patients between 18-45years, HIV patient on HAART for at

76 most one year and HIV patients without Immuno-compromised infection (example
77 Tuberculosis, HCV, HBV) were included in the study, Patients with Hemoglobin levels less
78 than 8g/dl and those having any Immuno-compromised infection (example Tuberculosis,
79 HCV, HBV) were excluded from the study. Also, the exclusion criteria for the control group
80 were the same as those of the patients aside being negative for HIV.

81

82 **Sample Collection and Determination of Haematological parameters**

83 3 ml of venous blood was drawn from each of the participant by venepuncture into K₃ EDTA
84 vacutainer tube. XS-500i automatic blood cell analyzer (Sysmex Corporation Co., Ltd.,
85 Japan) was used in the determination of these parameters. The haematological parameters
86 that were analyzed were: haemoglobin concentration, total white blood cell count,
87 lymphocyte count, neutrophil count and platelet count. The XS-500i automatic blood cell
88 analyser determines the haemoglobin concentration just as the colorimetric method whiles
89 the platelets and the WBCs are counted similarly as the impedance method.

90 **Determination of TNF- α and Interleukin 6 (IL-6)**

91 All reagents were brought to stand on the bench for 30 minutes before it was used for the
92 analysis. The samples were prediluted in a 1:150 ratio with a zero standard. After the
93 dilution, 40 μ l of the standards (TNF- α or IL-6) and 10 μ l patient sample were pipetted into the
94 respective micro titre wells. The plate was tapped gently to mix the content; the plate was
95 sealed and incubated at 37 °C for 30 mins. The wells were washed five (5) times with diluted
96 wash buffer (diluted 30 folds with distilled water). After drying, 50 μ l horseradish peroxidase
97 (HRP)-Conjugate reagent was added to each well, except blank well. The plate was then
98 covered and incubated at 37°C for 30 minutes. The liquid was discarded after incubation and
99 washed with a buffer solution repeatedly for five times and dried by patting. After that 50 μ l
100 of tetramethylbenzidine (TMB)-peroxide substrate colour developer was added to each well

and incubated for 10 min at 37 °C. Finally 50 µl of stop solution is added to each well to stop the reaction. Absorbance was read at 450nm within 15min with Mindray MR-96A reader.

Statistical Analysis

The data was analysed using the Statistical Package for Social Scientist (SPSS) Statistical Software (version 17.0, SPSS Inc., Chicago, IL, USA). The confidence intervals were calculated using the binomial test and correlations were evaluated using the Pearson's correlation analysis. For all statistical comparisons, the level of significance was set at $p < 0.05$.

RESULTS

Demographic characteristics of Study population

The mean age of the HIV patients (38.57 ± 1.13 for those on treatment and 37.25 ± 1.19 for treatment naïve) were significantly higher ($P < .0001$) respectively than that of the controls (22.35 ± 1.03). The female participants (42 for those on treatment and 36 for the treatment naïve) far outnumber the male participants (8 for on-treatment and 14 for treatment naïve). However for the controls the number of males (37) was almost three times the number of females (13). (Table 1.0).

118 **Table 1.0: Socio-demographic Characteristics of study participants**

	Control (n=50)	HIV Patients		P-value
		On HAART (n=50)	HAART Naïve(n=50)	
Age (years)	22.35 ± 1.03	38.57 ± 1.13†††	37.25 ± 1.19†††	< .0001
Gender n (%)				
Male	37(74.0%)	8(16.0%)	14 (28.0%)	
Female	13(26.0%)	42(84.0%)	36(72.0%)	

119 *Values are presented as mean ± standard deviation (SD) and frequency (percentage)*

120 **Haematological Profile of studied participants**

121 Full blood count analysis indicated that White Blood Cells (WBC), Red Blood Cells(RBC),
122 Haematocrit (HCT), and Haemoglobin (Hb) were significantly lower among treatment and
123 treatment naïve HIV infected participants compared to control ($P=.05$). Meanwhile Mean
124 Platelet Volume (MPV) were significantly higher among treatment and treatment naïve HIV
125 infected participants compared to control ($P=.05$). Absolute and differential eosinophil count
126 was significantly lower while absolute and differential monocyte count were significantly
127 higher in treatment naïve HIV infected participants compared to control ($P=.05$) (Table 2.0).

128 **Table 2.0: Hematological Profile of studied participants**

FBC	Control (n=50)	HIV		P value
		On HAART (n=50)	HAART Naïve(n=50)	
WBC ($10^3/\mu\text{L}$)	6.12 ± 0.24	5.19 ± 0.24††	5.35 ± 0.23†	.01

RBC (10 ⁶ /μL)	5.07 ± 0.08	4.20 ± 0.09†††	4.226 ± 0.09†††	< .0001
Hb (g/dl)	14.36 ± 0.20	12.26 ± 0.28†††	12.13 ± 0.27†††	< .0001
HCT (%)	44.85 ± 0.73	37.41 ± 0.73†††	36.74 ± 0.96†††	< .0001
MCV (fL)	88.97 ± 1.22	90.27 ± 1.62	87.31 ± 1.65	.38
MCH (pg)	28.50 ± 0.35	29.55 ± 0.65	28.80 ± 0.44	.30
MCHC(g/dl)	32.11 ± 0.28	32.69 ± 0.28	33.18 ± 0.33†	.04
PLT (10 ³ /μL)	241.0 ± 7.80	244.2 ± 12.05	261.2 ± 18.21	.52
RDW-SD (fL)	47.68 ± 0.82	49.66 ± 1.02	47.11 ± 1.24	.20
RDW-CV (%)	15.17 ± 0.24	14.82 ± 0.32	14.78 ± 0.32	.59
PDW (fL)	13.86 ± 0.34	14.47 ± 0.29	13.77 ± 0.36	.28
MPV (fL)	10.99 ± 0.14	10.18 ± 0.18††	13.77 ± 0.36†††	< .0001
P-LCR (%)	32.32 ± 0.97	32.08 ± 1.83	28.73 ± 2.06	.20
PCT (%)	0.26 ± 0.01	0.2513 ± 0.01	0.2592 ± 0.02	.90
WBC differential count				
NEUT (10 ³ /μL)	2.24 ± 0.13	2.083 ± 0.19	2.202 ± 0.16	.79
LYM (10 ³ /μL)	3.020 ± 0.13	2.37 ± 0.10	4.274 ± 1.73	.41
MONO (10 ³ /μL)	0.5784 ± 0.03	0.57 ± 0.04	0.65 ± 0.05††	.35
EOSIN (10 ³ /μL)	0.26 ± 0.05	0.36 ± 0.10	0.12 ± 0.03†	.05
BASO (10 ³ /μL)	0.04 ± 0.003	0.05 ± 0.009	0.03 ± 0.008	.07
Absolute WBC count				
NEUT (%)	36.10 ± 1.52	37.30 ± 2.03	39.17 ± 2.21	.52

LYM (%)	49.96 ± 1.57	48.53 ± 2.19	46.86 ± 2.30	.55
MONO (%)	9.282 ± 0.31	11.56 ± 0.54†††	10.70 ± 0.58††	.001
EOSIN (%)	3.70 ± 0.53	5.88 ± 1.43	2.06 ± 0.39†	.01
BASO (%)	0.65 ± 0.05	0.98 ± 0.19	0.51 ± 0.11	.02

129 Values are presented as mean ± SD. † $P=.05$; †† $P<.001$; ††† $P<.0001$ HIV patients (on-
130 treatment and treatment naïve groups) compared to control

131

132 Inflammatory Cytokines

133 Table 3.0 shows median levels of inflammatory cytokines in the study population. There was
134 a significantly higher median levels of IL-6 and TNF- α in the order of Control<on-treatment
135 group< treatment naïve group ($P<.0001$) (Table 3.0).

136 **Table 3.0: Level of Inflammatory markers in general studied population**

Cytokines	Control (n=50)	HIV Patient		P-value
		On HAART (n=50)	HAART Naïve (n=50)	
IL-6 (ng/L)	4.45(1.3 - 8.7)	14.98(5.9 - 24.9)†††	17.66(13.0 - 24.1) †††	< .0001
TNF- α (ng/L)	40.28 (24.7 - 81.0)	297.40 (150.2 - 419.7)†††	318.3(123.0 - 497.9)†††	< .0001

137 Values are presented as Median (25th to 75th quartile range). † $P=.05$; †† $P<.001$;
138 ††† $P<.0001$ HIV patients (On-treatment and treatment naïve groups) compared to
139 control

140 CORRELATION OF INFLAMMATORY CYTOKINES WITH HB, HCT, RBC AND WBC

141 Table 4.0 shows correlation of inflammatory cytokines with Hb, HCT, RBC and WBC count
142 among HAART naïve HIV participants. TNF-alpha was significantly higher and positively

correlated with Hb ($r=0.310$; $P=.03$); HCT ($r=0.307$; $P=.03$) and RBC ($r=0.323$; $P=.02$) but not WBC ($r=0.117$; $P=.42$). Meanwhile Hb, HCT, RBC and WBC were not significantly associated with both IL-6 ($P=.05$) (**Table 4.0**).

Table 4.0: Correlation of IL-6 and TNF- α with Hb, HCT, RBC and WBC

	IL-6	TNF- α
HB	$r=-0.020$; $p=0.891$	$r=-0.310$; $P=.03$
HCT	$r=0.025$; $p=0.865$	$r=-0.307$; $P=.03$
RBC	$r=-0.043$; $p=0.768$	$r=-0.323$; $P=.02$
WBC	$r=0.012$; $p=0.932$	$r=0.117$; $P=.42$

r =correlation coefficient

DISCUSSION

The study observed that lower levels of some haematological parameters were associated with seropositive HIV group than the control group (Table 2.0). This study also observed a significant increase in TNF- α and IL-6 in HIV patients with a strong negative correlation between the inflammatory cytokines and haematological parameters (Tables 3.0 and 4.0). In effect, the cycle of HIV infection, immunosuppression, opportunistic disease, high TNF levels, and further induction of HIV expression could explain the progression of disease in HIV infected individuals. Generally, HIV ailments have been associated with haematological abnormalities [8, 9] with prevalence and severity of the anaemia correlating with the progression of HIV infection [10, 11]. An HIV patient with CD4 count of 200 cells/ μ L or less and anaemic has a low survival rate [5]. Increase in viral load results in low CD4 count and high levels of pro-inflammatory cytokine. The suppressive effect of some of the cytokines

(TNF- α and IL-6) on the bone marrow can lead to anaemia [12]. The results of this study analyses on anaemic condition of seropositive HIV patient were consistent with the study of anaemia as a prognostic factor in HIV infection [13].

Haemoglobin (HB) level was lower in seropositive HIV patient compared to control group. There was not much difference between on-treatment and treatment naïve group. Ledru et al in his study in West Africa identifies haemoglobin as a diagnostic indicator in the management of HIV patients [14]. Clinicians managing HIV patient normally lookout for the Hb level of their client to decide the next action to take. Regular checking of Hb level, therefore, gives a clue of HIV progression and also helps the Clinician to determine which patient needs or may require further treatment. The suppressive effect of the pro-inflammatory cytokine on the bone marrow, internal bleeding, lack of proper diet intake are some but few causes of low haemoglobin recorded in HIV patients.

Red Blood Cell (RBC) decreased in HIV infected patient as compare to the control group. But there was no significant difference between on-treatment and treatment naïve patients. This may be due to the decrease in RBC production as result of opportunistic infection, direct effect of the HIV infection, myelosuppressive medication and decrease in production of erythropoietin. It can also be the inefficient production of RBC as a result of folic acid and vitamin B12 deficiencies. And finally increased RBC destruction resulting from autoimmune haemolytic anaemia, thrombotic microangiopathy, disseminated intravascular coagulation may have accounted for the low RBC observed [15].

There were lower white blood cells (WBC), recorded in HIV positive individuals as compared to the seronegative group. The immune system of a host in a bid to defend itself against infection and intrusion of foreign particle employs white blood cells in it defence mechanism. Therefore during inflammation, bacterial or viral infection one would expect some changes in the WBC level. The decrease in white blood cells observed in HIV patients may be due to the deleterious effect of the HIV virus on the immune system.

187 Meanwhile MPVs were significantly higher among treatment and treatment naïve HIV
188 infected participants compared to control (p=.05).

189 **CONCLUSION**

190 There was a negative correlation between some haematological parameters and the
191 inflammatory cytokines (TNF- α and IL-6). This could be the basis for the continuous
192 reduction in the level of blood parameters. As the disease progresses, haematological
193 parameters gradually decrease and this may lead to some haematological abnormalities
194 such as anaemia, thrombocytopenia, amongst others. The net effect expected is a reduction
195 in the life expectancy of people living with HIV/AIDS since these haematological
196 abnormalities are capable of triggering further complications.

197 **Ethical Approval**

198 The study protocol was sent for review and approval from the Committee on Human
199 Research, Publications and Ethics, School of Medical Sciences, Kwame Nkrumah University
200 of Science and Technology and Komfo Anokye Teaching Hospital, in Kumasi. Permission
201 was sought from the Head of Diagnostic Directorate to undertake the study at the serology
202 unit of the Komfo Anokye Teaching Hospital.

203 **Consent Disclaimer:**

204 As per international standard or university standard written patient consent has been
205 collected and preserved by the author(s).

206 **REFERENCES**

- 207 1. Malenica M, Prnjavorac B, Bego T, Dujic T, Semiz S, Skrbo S et al Effect of
208 Cigarette Smoking on Haematological Parameters in Healthy Population. *Med Arch*
209 2017; 71(2): 132-136.

- 210 2. Edzeamey FJ, Awuah M, Ako AK, Wiredu OK, Sampong BB, Gborgblorvor D et al.
211 Thrombocytopenia amongst patients: A comparison of Malaria and Non- Malaria
212 patients. *Int J Pharm Biol Sci* 2018, 8(1): 303-307
- 213 3. Hagaa T, Efird JT, Tugizov S & Palefsky JM. Increased TNF-alpha and sTNFR2
214 levels are associated with high-grade anal squamous intraepithelial lesions in HIV-
215 positive patients with low CD4 level. *Papillomavirus Research* 2016; 3(2017) 1-6.
- 216 4. Pasquereau S, Kumar A and Herbein G. Targeting TNF and TNF Receptor Pathway
217 in HIV-1 Infection: from Immune Activation to Viral Reservoirs. *Viruses* 2017; 9:64
- 218 5. Wiredu OK, Edzeamey FJ, Dompok A, Awuah M, Ako AK, Sampong BB, et al.,
219 Lymphocyte Subset (CD3, CD4 and CD8 Cells) and their Ratios in HIV patients on
220 Highly Active Antiretroviral Therapy (HAART) and HAART Naïve Patients. *Int J*
221 *Microbiol Adv Immunol.* 2018;06(2):92-95
- 222 6. Ngala RA, Opoku D & Asare G. Effects of HIV infection and Highly Active
223 Antiretroviral Therapy (HAART) on the liver of HIV Patients. *Trends in Medical*
224 *Research* 2015; 10 (1), 1-11
- 225 7. Passaes CP, Sáez-Ciríó A. HIV cure research: Advances and prospects. *Virology*
226 2014; 454-455 (2014) 340–352.
- 227 8. Harbol AW, Liesveld JL, Simpson-Haidaris PJ & Abboud CN. Mechanisms of
228 cytopenia in human immunodeficiency virus infection. *Blood Rev* 1994 8, 241-51.
- 229 9. Ballem PJ, Belzberg A, Devine DV, Lyster D, Spruston B, Chambers H et al. Kinetic
230 studies of the mechanism of thrombocytopenia in patients with human
231 immunodeficiency virus infection. *N Engl J Med* 1992; 327: 1779-84
- 232 10. Shive CL, Biancotto A, Funderburg NT, Cooper HAP, Valdez H, Margolis L, et al
233 HIV-1 is not a major driver of increased plasma IL-6 levels in chronic HIV-1 disease.
234 *J Acquir Immune Defic Syndr.* 2012; 61(2): 145–152.
- 235 11. Sullivan PS, Hanson DL, Chu SY, Jones JL & Ward JW. Epidemiology of anemia in
236 human immunodeficiency virus (HIV)-infected persons: results from the multistate

- 237 adult and adolescent spectrum of HIV disease surveillance project. *Blood*.1998, 91,
238 301-8
- 239 12. Borges AH, O'Connor JL, Phillips AN, Rönsholt FF, Pett S, Michael J. Vjecha MJ et
240 al. Factors Associated With Plasma IL-6 Levels During HIV Infection. *The Journal of*
241 *Infectious Diseases* 2015; 212:585–95.
- 242 13. Morfeldt-Manson L, Bottiger B, Nilsson B & von Stedingk LV. Clinical signs and
243 laboratory markers in predicting progression to AIDS in HIV-1 infected patients.
244 *Scand J Infect Dis* 1991; 23, 443-9.
- 245 14. Ledru E, Diagbouga S, Meda N, Sanou PT, Dahourou H, Dembele A et al. A
246 proposal for basic management of HIV disease in west Africa: use of clinical staging
247 and haemogram data. *Int J STD AIDS* 1998; 9, 463-70
- 248 15. Antelman G, Msamang GI, Spiegelman D, Urassa EJ, Narh R, Hunter DJ et al.
249 Nutritional factors and infectious disease contribute to anemia among pregnant
250 women with human immunodeficiency virus in Tanzania. *J Nutr* 2000; 130, 1950-7.