Wydawnictwo UR 2021
ISSN 2544-1361 (online); ISSN 2544-2406
doi: 10.15584/ejcem.2021.1.5

http://www.ejcem.ur.edu.pl
European Journal of Clinical and Experimental Medicine
Eur J Clin Exp Med 2021; 19 (1): 33–39

ORIGINAL PAPER

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Changes in haematological parameters and serum beta-2-microglobulin levels in CD4⁺ T-cells-stratified Nigerian HIV patients

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ABSTRACT

Introduction. Reports have shown that there is a rise in beta-2-microglobulin (β2M) concentration in patients with HIV infection and that the degree of elevation correlates well with the extent of disease burden and could be an independent prognostic marker for death. However, there is the dearth of information on the interplay between alteration in haematological profile, a common cause of morbidity and mortality in HIV, and β2M.

Aim. Changes in selected haematological parameters and β2M in Nigerian HIV patients stratified based on CD4⁺ T-cells counts were thus assessed in this study.

Material and methods. Forty-eight asymptomatic, drug naïve HIV patients were enrolled into this cross-sectional study. Haemoglobin concentration (Hb), packed cell volume (PCV), total and differential white blood cell count, platelet count and CD4⁺ T-cells count were determined using standard methods while serum levels of β2M were determined using ELISA. Thereafter, the patients were stratified into three groups based on the CD4⁺ T-cells count.

Results. Hb and lymphocyte counts increased with increasing CD4⁺ T-cells count. In contrast, neutrophils percentage, MCV and MCH reduced with increasing CD4⁺ T-cells count. The mean lymphocytes percentage was significantly higher while the mean neutrophils percentage was significantly lower in patients with CD4⁺ T-cells count of 500–800 cells/μl compared with the patients with CD4⁺ T-cells count <200 cells/μl. Similarly, the mean MCV was significantly lower in patients with CD4⁺ T-cells count of 500–800 cells/μl compared with patients with CD4⁺ T-cells count of 200–499 cells/μl and patients with CD4⁺ T-cells count <200 cells/μl. β2M had significant positive correlation with WBC and neutrophils percentage but had a significant negative correlation with lymphocytes percentage and MCH in patients with CD4⁺ T-cells count <200 cells/μl. However, β2M had sig-

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Participation of co-authors: A – Author of the concept and objectives of paper; B – collection of data; C – implementation of research; D – elaborate, analysis and interpretation of data; E – statistical analysis; F – preparation of a manuscript; G – working out the literature; H – obtaining funds

Received: 23.12.2020 | Accepted: 15.01.2021
Publication date: March 2021

Olaniyi JA, Emeka GJ, Onifade AA, Adeyanju AO, Rahamon SK. *Changes in haematological parameters and serum beta-2-microglobulin levels in CD4⁺ T-cells-stratified Nigerian HIV patients*. Eur J Clin Exp Med. 2021;19(1):33–39. doi: 10.15584/ejcem.2021.1.5

nificant positive correlation with PCV, Hb, monocytes and morphology in patients with CD4+ T-cells count of 500–800 cells/µl. **Conclusion.** It could be concluded from this study that HIV infection is associated with alteration in haematological profile and the alteration is CD4+ T-cells count-dependent. Also, elevation in β2M concentration appears to be a marker of lymphopaenia in patients with low CD4+ T-cells count.

Keywords. beta-2-microglobulin, CD4+ T-cells count, haemocytometry, HIV, lymphopaenia

Introduction

Human immunodeficiency virus (HIV) is a pandemic affecting more than 35 million people worldwide.[1] Infection with HIV is associated with myriads of disorders including haematological complications which are considered the second most common cause of morbidity and mortality in HIV patients.[2] HIV replicates in CD4+ T-lymphocytes, macrophages and dendritic cells resulting in immune system depression and consequently, progression to life threatening opportunistic infections.[3-5]

The most common haematological abnormalities associated with HIV are anaemia and neutropaenia and both are associated with disease progression.[6,7] The anaemia, which ranges from mild-to severe, is significantly associated with reduced survival.[8] Neutropenia is observed in advanced stages of HIV infection after development of AIDS and has been associated with certain types of anti-retroviral medication. In fact, anaemia and leukopenia in ART naïve patients have been documented to result in poor ART-treatment outcome and otherwise, strongly predict mortality.[9] These haematological abnormalities are consequent to HIV mediated bone marrow myelosuppression caused by abnormal inflammatory cytokine expression and alteration of bone marrow micro-environment.[10]

The severity of anaemia in HIV infected persons is associated with CD4+ lymphocyte depletion and progression to AIDS and this serves as one of the strongest predictors of HIV mortality as well as poor responses to anti-retroviral therapy.[2] However, CD4+ T-cells counts have been reported not to always correlate with clinical outcome, possibly because they are not key players at all stages of infection and because CD4+ T-cells count do not reflect the totality of T cells functions.[11,12] In addition, technological problems are associated with lymphocyte phenotyping in CD4+ T-cells count and supplementary methods to improve the predictive information of CD4+ T-cells count are still required.[12,13] Therefore, the need for investigation of immunochemical markers such as beta-2-microglobulin (β2M) for their health monitoring potentials in HIV cannot be overemphasized.

Beta-2-microglobulin (β2M) is a component of the human major histocompatibility complex (HLA) class I molecule. It is coded on chromosome 17and expressed by nearly all nucleated cells.[14] Although β2M is expressed at a constant level in many cells, its formation is enhanced in the presence of IFN-α.[15] β2M has also been shown to induce cellular expression of interleukins 6, 8 and 10, regulate the expression of hormone/growth factor, and coordinate the interaction between cytokines and their receptors.[16-18] These activities could explain its rise in concentration in infections, malignancies and other pathological conditions.[19,20] Moodley and colleagues reported that HIV infected infants had elevated β2M levels with significant elevation at 1 month and 12 months compared with the uninfected infants.[21] Also, Chitra et al. reported elevated level of β2M during infections including cytomegalovirus and HIV.[22]

It has also been reported that the degree of elevation of serum β2M correlates well with the extent of disease burden i.e. ≥5µg/L, as against the normal level of 1.8 µg/L.[23] In addition, Mocroft et al. reported that β2M is an independent prognostic marker for death in patients infected with HIV.[24]

Aim

Although it has been reported that levels of some biomolecules are altered with changes in CD4+ T-cells count, there is still the dearth of information on the interplay between severity of HIV infection, changes in haematological parameters and β2M, this study was thus designed to assess changes in selected haematological parameters and β2M in Nigerian HIV patients stratified based on CD4+ T-cells count.[25]

Material and methods

Study Area

The study was conducted at Adeoyo Maternity Teaching Hospital in Ibadan North East Local Government Area, Ibadan, Oyo State, Nigeria.

Ethical consideration

Before the commencement of the study, the study was approved by the Oyo State Ministry of Health, Ibadan (AD13/479/468). Also, informed consent was obtained from each study participant.

Study Participants

Forty-eight asymptomatic, drug naïve HIV patients attending the HIV-PEPFAR Clinic at Adeoyo Maternity Teaching Hospital, Ibadan, Oyo State, Nigeria were enrolled into this cross-sectional study. Details about the age, gender and stages of HIV infection of the study participants have earlier been reported.[26] Patients with other viral co-infection and metabolic diseases such as diabetes were excluded from the study.

Sociodemographic data collection

A semi-structured questionnaire was used to obtain socio-demographic information as well as medical history of the patients.

Blood sample collection and laboratory analysis

Venous blood (5 ml) was obtained from each participant and dispensed into EDTA containing bottles. A complete blood count (CBC); consisting of total and differential white blood cell count and platelet count along with mean platelet volume (MPV), haemoglobin concentration (Hb), packed cell volume (PCV), Red cell indices including Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW); were determined using Swelabalfa 3 part haematology analyzer (9 version 2.1, series no SE12613, Boule medicals AB, Sweden).

$CD4^+$ T-cells count was done using flow cytometer (PartecCyFlow Counter®, Partec, Münster, Germany). About 20 ul of whole blood was dispensed into Partec test tube (Rohren tube). Then, 20 µl of $CD4^+$ T-cell antibody was added into the tube. The contents were mixed and incubated in the dark for 15 minutes at room temperature. After incubation, 800 µl of $CD4^+$ T-cells buffer was gently added to the mixture and mixed gently. The Partec tube was then plugged on the Cyflow counter and the $CD4^+$ T-cells were displayed as peaks and interpreted as figures.

Whole blood remaining after haematological analysis and $CD4^+$ T-cells count was centrifuged and plasma obtained was stored at -20^{0}C until analysed for β2M using a non-competitive (sandwich) enzyme linked immunosorbent assay (ELISA) kit (GenWay Biotech Inc., USA).

HIV Staging

The World Health Organisation staging criteria were adopted.[27]

Statistical analysis

Data analysis was carried out using the Statistical Package for Social Sciences (SPSS Inc., Chicago) version 16.0. Differences in mean of variables in different groups were determined using ANOVA followed by post-hoc and results presented as mean ± standard deviation. Pearson correlation was used to determine the correlations between variables while the association between the parameters and HIV stage was determined using the Chi-square test. *P*-values less than 0.05 were considered as statistically significant.

Results

As shown in Table 1, there was significant progressive rise in Hb count, lymphocyte count and morphology as the $CD4^+$ T-cells count increases. In contrast, neutrophils percentage, MCV and MCH reduced progressively as the $CD4^+$ T-cells count increases. Also, in Table 1, the mean lymphocytes percentage was significantly higher in patients with $CD4^+$ T-cells count of 500–800 cells/µl compared with the patients with <200 cells/µl. In contrast, the mean neutrophils percentage in patients with $CD4^+$ T-cells count of 500–800 cells/µl was significantly lower compared with the patients with $CD4^+$ T-cells count <200 cells/µl. Similarly, the mean MCV was significantly lower in patients with $CD4^+$ T-cells count of 500–800 cells/µl compared with patients with $CD4^+$ T-cells count of 200–499 cells/µl and patients with $CD4^+$ T-cells count <200 cells/µl.

Considering the proportion of patients with altered levels of the haematological parameters, it was observed that anaemia is significantly associated with

Table 1. Haematological parameters and beta-2-microglobulin in HIV patients grouped by CD4+ T-cells count

Parameters	CD4 < 200(cells/µl) (n = 10)	CD4 200-499(cells/µl) (n = 13)	CD4 500-800(cells/µl) (n = 17)	*P*-value
PCV (%)	32.14±5.13	32.87±5.60	35.29±7.36	0.084
Hb (g/l)	9.55±1.69	9.96±1.69	10.66±2.30	0.047*
WBC (x10^3)	6.28±3.77	5.33±2.19	6.70±2.25	0.101
Neutrophils (%)	55.36±15.72	48.21±11.89	44.00±12.03^a	0.002*
Lymphocyte(%)	34.68±15.82	42.04±12.08	45.14±11.63^a	0.003*
Monocytes (%)	9.95±3.88	9.58±3.33	10.86±3.35	0.319
Platelets (x10^3)	226.45±95.40	241.17±72.56	263.36±100.36	0.228
MCV (FL)	87.71±7.52	86.33±5.22	82.36±5.45^{a,b}	0.002*
MCH (pg)	26.26±2.88	25.92±2.18	24.71±2.23	0.036*
MCHC (g/dl`)	29.81±1.27	30.13±1.10	29.93±0.90	0.409
Morph. (x10^{12}/L)	3.76±0.73	3.84±0.61	4.31±0.84	0.004*
β2M (µg/mL)	13816.28±19584.08	12034.74±10148.51	13871.19±15819.20	0.914
CD4 (cells/µl)	116.95±59.50	333.20±81.49^a	669.79±124.8^{a,b}	0.000*

*Significant at p<0.05, acompared with CD4 <200 (cells/µl), bcompared with CD4 200 – 499 (cells/µl)

CD4⁺ T-cells count as the proportion of patients with low Hb was significantly lower in patients with CD4⁺ T-cells count above 500 cells/μl compared with patients with CD4⁺ T-cells count <500 cells/μl and <200 cells/μl (Table 2). Furthermore, the proportion of patients with neutrophilia and elevated MCV was significantly higher in patients with CD4⁺ T-cells count <200 cells/μl compared with patients with ≥200 cells/μl. In contrast, the proportion of patients with lymphocytosis was significantly higher in patients with CD4⁺ T-cells ≥500 cells/

Table 2. Proportions of HIV patients with haematological parameters within and outside reference interval in various CD4+ T-cells count groups

Parameters		CD4<200 cells/μl	CD4 200-499 cells/μl	CD4 500-800 cells/μl	χ2	*P*-value
PCV	Normal	15.0	26.3	40.0	4.571	0.102
	<Normal	85.0	73.7	60.0		
Hb	Normal	15.0	15.0	40.0	6.188	0.045*
	<Normal	85.0	85.0	60.0		
WBC	Normal	72.7	91.6	78.6	4.703	0.095
	<Normal	9.1	4.2	0.0		
	>Normal	18.2	4.2	21.4		
Neutrophil	Normal	81.8	87.5	64.3	7.773	0.021*
	<Normal	13.6	12.5	35.7		
	>Normal	4.6	0.0	0.0		
Lymphocytes	Normal	68.2	54.2	42.9	10.229	0.006*
	<Normal	9.1	4.2	0.0		
	>Normal	22.7	41.7	57.1		
Monocytes	Normal	68.2	83.3	64.3	0.612	0.736
	<Normal	4.5	0.0	7.1		
	>Normal	27.3	16.7	28.6		
Platelets	Normal	77.3	79.2	71.4	4.962	0.084
	<Normal	9.1	0.0	0.0		
	>Normal	13.6	20.8	28.6		
MCV	Normal	85.7	95.8	71.4	9.969	0.007*
	<Normal	9.5	4.2	28.6		
	>Normal	4.8	0.0	0.0		
MCH	Normal	42.9	41.7	35.7	1.334	0.513
	<Normal	52.4	58.3	64.3		
	>Normal	4.7	0.0	0.0		
MCHC	Normal	4.8	12.5	7.1	1.798	0.407
	<Normal	95.2	87.5	92.9		

*Significant at p<0.05

Table 3. Correlation between β2M and haematological parameters in HIV patients grouped by CD4⁺ T-cells counts

β2M	CD4<200 cells/μl	CD4 200-499 cells/μl	CD4 500-800 cells/μl
	r-value, *P*-value	r-value, *P*-value	r-value, *P*-value
PCV	-0.132, 0.457	0.231, 0.328	0.903, 0.000*
Hb	-0.199, 0.258	0.183, 0.440	0.898, 0.000*
WBC	0.416, 0.015*	0.396, 0.084	0.392, 0.166
Neutrophils	0.427, 0.012*	0.276, 0.239	0.119, 0.685
Lymphocytes	-0.478, 0.004*	-0.031, 0.896	-0.331, 0.248
Monocytes	0.134, 0.450	-0.504, 0.024*	0.660, 0.010*
Platelets	0.321, 0.065	0.348, 0.132	-0.159, 0.586
MCV(FL)	-0.250, 0.168	0.103, 0.667	0.210, 0.471
MCH (pg)	-0.349, 0.049*	-0.034, 0.887	0.230, 0.429
MCHC (g/dL)	-0.342, 0.056	-0.245. 0.298	-0.116, 0.693
Morphology	-0.012, 0.945	0.134, 0.573	0.668, 0.010*

*Significant at p<0.05

μl compared with patients with CD4+ T-cells <500 cells/μl (Table 2).

As shown in Table 3, β2M had significant positive correlation with WBC and neutrophils percentage but had a significant negative correlation with lymphocytes percentage and MCH in patients with CD4+ T-cells count <200 cells/μl. In patients with CD4+ T-cells count of 200–499 cells/μl, β2M had significant negative correlation with monocytes percentage. However, β2M had significant positive correlation with PCV, Hb, monocytes and morphology in patients with CD4+ T-cells count of 500–800 cells/μl (Table 3).

Discussion

Haematologic abnormalities are the most common complications of HIV infection and are strong independent predictors of morbidity and mortality in infected individuals.[28,29] These abnormalities are associated with a number of complex and multi-factorial factors including infection of the multipotent haematopoietic progenitor cells by HIV, establishment of latent cellular reservoirs, activation of the reticulo-endothelial system resulting in increased blood cell destruction, and disturbance in the bone marrow microenvironment causing immune dysregulation with its associated cytokine imbalances and disruption of factors essential for normal haematopoiesis.[30] Anaemia is one of the most frequent haematological abnormalities in individuals with HIV infection including those on highly active antiretroviral therapy (HAART).[6,31] The observed progressive rise in Hb with increasing CD4+ T-cells count and the low prevalence of anaemia in patients with CD4+ T-cells count of ≥500 cells/μl indicate that anaemia is associated with CD4+ T-cells depletion and that the anaemia resolves with improvement in CD4+ T-cells count. This observation supports the report of Mata-Marín et al. which showed that anaemia is associated with CD4+ T-cells depletion and progression to AIDS.[32] Anaemia in HIV infection is caused by inadequate blood cell production due to bone marrow suppression by HIV infection mediated by abnormal cytokine expression and alteration of the bone marrow microenvironment.[33,34]

Neutrophils, the most abundant cell type in human blood, play important effector roles in immune response to pathogens.[35] It has been shown that there is decreased peripheral blood neutrophil counts and reduced activities, including chemotaxis, phagocytosis, bactericidal activity, and oxidative burst abilities in individuals with HIV infection.[36] The observed reduction in neutrophils percentage with increasing CD4+ T-cells count is in contrast to the report of De Santis et al. which showed that the mean neutrophil count was lower in HIV patients with low CD4+ T-cells count.[31] Observation from this study could indicate that increase in CD4+ T-cells count resulted in enhanced activities of the adaptive arm of the immune system facilitating protection against opportunistic infections which would result in down regulation of neutrophil activities. Our observation is buttressed by the observed significant reduction in neutrophils percentage in patients with CD4+ T-cells count of 500–800 cells/μl compared with those with <200 cells/μl, the observed higher proportion of patients with neutrophilia in patients with CD4+ T-cells count <200 cells/μl compared with patients with ≥200 cells/μl. Observations from this study suggest that observation of neutrophilia in patients with HIV is a possibility especially in those with low CD4+ T-cells count. Musubire et al. reported elevated neutrophil counts in HIV-infected patients with cryptococcal meningitis and the elevation was associated with mortality.[37]

T lymphocytes are white blood cells with important roles in adaptive immunity. The observed progressive rise in lymphocyte count as the CD4+ T-cells count increases and the elevated lymphocyte count in patients with CD4+ T-cells count of 500–800 cells/μl compared with those with <200 cells/μl corroborate the report of Bhardwaj et al.[29] These observations are not surprising as CD4+ T-cells count are lymphocytes. Obirikorang et al. reported positive correlation between TLC and CD4+ T-cells count and that total lymphocyte count can serve as a surrogate marker for CD4+ T-cells count in drug naïve HIV patients.[38]

Although low haemoglobin level symbolizes anaemia, other indices of red blood cell including mean corpuscular volume (MCV) provide information on the aetiology of the anaemia which could be morphologic (normocytic, microcytic or macrocytic) or pathophysiologic (excessive destruction, loss or diminished production).[39] The observed progressive reduction in MCV and mean corpuscular haemoglobin (MCH) concentration as the CD4+ T-cells count increases indicates that low CD4+ T-cells count is associated with hypochromic microcytic anaemia. This observation is alluded to by the observed improvement in Hb concentration with increasing CD4 count and the significantly lower MCV in patients with CD4+ T-cells count of 500–800 cells/μl compared with patients with CD4+ T-cells count <500 cells/μl. Our observation is indicative of progressive reduction in the degree of inflammation and better utilization of iron in the attempt to correct co-existing anaemia of chronic disease at CD4+ T-cells count of 500–800 cells/μl.

In addition, there was significant association between MCV and low CD4+ T-cells count. MCV, the average size of the circulatory erythrocyte, is an index for the differential diagnosis of anaemia and has been associated with mortality in many diseases.[40,41] On the other hand, MCH is the average concentration of Hb in erythrocytes and anaemia is the most common cause of its low concentration.

Elevated level of β2M has been reported in patients with HIV.[22] The observed inverse relationship between β2M and lymphocytes indicates that elevated level of β2M could suggest lymphopaenia in HIV patients with low $CD4^+$ T-cells count. This observation is in line with previous reports showing that the degree of elevation of serum β2M correlates well with the extent of disease burden.[23] Similarly, the observed inverse relationship between β2M and MCH suggests that elevated level of β2M could be an indication of microcytic anaemia in HIV patients with low $CD4^+$ T-cells count. This observation corroborates the earlier observed low MCH concentration in the group with low $CD4^+$ T-cells count.

Improved $CD4^+$ T-cells count is the hallmark of favourable response to anti-retroviral therapy.[42] The observed positive correlation between β2M and PCV, Hb, monocytes and morphology in patients with $CD4^+$ T-cells count of 500–800 cells/μl suggests that increase in β2M level might indicate improvement or restoration of HIV-induced alteration in haematological profile as the $CD4^+$ T-cells count improves. It has been shown that β2M plays important roles in expression of hormones/growth factors which coordinate various cellular activities.[16-18]

It could be concluded from this study that HIV infection is associated with alteration in haematological profile and the alteration is $CD4^+$ T-cells count-dependent. Also, elevation in β2M concentration appears to be a marker of lymphopaenia in patients with low $CD4^+$ T-cells count but a marker of normal haematocrit level in patients with optimal $CD4^+$ T-cells count.

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