LIFE: International Journal of Health and Life-Sciences
ISSN 2454-5872

Mugwe et al., 2019

Volume 5 Issue 1, pp. 105-118

Date of Publication: 20th April 2019

DOI-https://dx.doi.org/10.20319/lijhls.2019.51.105118

This paper can be cited as: Mugwe, J. N., Gicheru, M. M., & Mwatha, J. (2019). Circulatory Cytokines and Hematological Profiles: Possible Biomarkers of HIV/AIDS Disease Progression. LIFE: International Journal of Health and Life-Sciences, 5(1), 105-118.

CIRCULATORY CYTOKINES AND HEMATOLOGICAL PROFILES: POSSIBLE BIOMARKERS OF HIV/AIDS DISEASE PROGRESSION

Jane N. Mugwe
Kenyatta University, Nairobi, Kenya
jmugwe@laikipia.ac.ke

Michael M. Gicheru
Kenyatta University, Nairobi, Kenya
muitaku@gmail.com

Joseph Mwatha
Kenya Medical Research Institute, Nairobi, Kenya
josephmwatha@gmail.com

Abstract

Introduction: *This study sought to identify circulatory cytokines and hematological profiles measureable in blood plasma in newly diagnosed HIV patients as possible biomarkers that could predict the progression of HIV and AIDS disease in the course of acute HIV infection.*

Methodology: *A prospective cross sectional study design was used to recruit the participants at the Nakuru Provincial General hospital in Kenya. The study group included those who were HIV positive before and after commencing therapy and those who were HIV negative. The study group composed of male and female of different ages ranging from 7-72 years. Hematology auto analyzer system was used to analyze hematological parameters and indices. Types and concentrations of cytokines were determined using multiplex cytokine immunoassay by flow*

cytometry using Becton and Dickinsonfluorescence activated cell sorter (BD FACS) count. Descriptive statistics were applied and a p- value < 0.05 was considered statistically significant.

Results: *This study found a significant difference in mean Interleukin 12p70 (p<0.001), Tumor Necrosis Factor (p<0.05), Interleukin 10 (p<0.05), Interleukin 6 (p<0.005) and interleukin 1β (p<0.05) between HIV negative patients, treatment naïve HIV patients and HIV patients on highly active antiretroviral therapy (HAART). Among the treatment naïve HIV patients, significant associations were observed between IL-12p70 and HGB (p<0.05); between TNF and MPV (p<0.001); between IL-10 and PDW (p<0.005); between IL-6 and Gran# (p-0.05); between IL-1β and PDW (p<0.005).*

Conclusion: *The early period of infection with HIV is characterized by high circulatory cytokines levels and could be useful biomarkers and indicators of early immune activation of HIV infection. The results from this study also showed that acute HIV infection induces several hematological changes, involving all the blood parameters and indices, some of which may act as indicators of HIV/AIDS disease progression.*

Keywords

Circulatory Cytokines, Hematological Profiles, Human Immunodeficiency Virus, Biomarkers, HIV/AIDS Disease Progression

1. Introduction

The Human immunodeficiency virus (HIV) causes acquired immunodeficiency syndrome (AIDS) and has become one of the world's most serious health and development challenges. The earliest HIV cases were reported in 1981 and to date about 76.1 million persons have been infected with the virus (UNAIDS, 2017). It is estimated that in 2016, about 36.7 million people worldwide were living with HIV while about 1 million people died of AIDS related illnesses (UNAIDS, 2017). A huge majority of persons living with the HIV are found in middle and low income countries. About 25.5 million infected persons are said to be living in sub Saharan Africa with 19.4 million among them living in Eastern and Southern Africa accounting for 43% of people living with HIV globally; women and girls accounts for more that half (59%). Additionally, in 2016, an estimated 420,000 persons are said to have died of AIDS related illnesses in Eastern and Southern Africa (UNAIDS, 2017).

There have been reports of new cases of HIV from all regions of the world. In 2016, approximately 1.8 million people became newly infected worldwide with HIV. There were about

790,000 new HIV infections in Eastern and Southern Africa which accounts for about 44 % of the global new HIV infections (UNAIDS, 2017). Kenya's HIV epidemic is geographically diverse even .though reports indicate that about 44% of adults infected reside in 5 out of 47 counties (Kenya, 2014). Kenya is jointly ranked fourth largest HIV epidemic in the world (UNAIDS, 2017). New infections with HIV continue to be reported and about 36,000 adults and children are said to have died in 2016 of AIDS related illnesses in Kenya (UNAIDS, 2017).

While there is still no cure for HIV, the majority of those that live with the virus or are at risk for HIV infection are not able to gain access to prevention, treatment and care (WHO, 2013). Various reports have indicated that in order to improve knowledge of HIV and HIV testing among young adults and adolescents, much more needs to be done. The young women are particularly at risk for HIVinfection. About 59% of new HIV infections occur in persons aged between 15- 24 years, an indication that the virus mainly affects those in their most productive years (UNAIDS, 2014). Whereas the immune systems of healthy individuals would normally clear a variety of infections, persons infected with HIV become more susceptible since the HIV weakens ones defense system and surveillance against infections (WHO, 2013).

Circulatory cytokines detectable in persons infected with HIV can be useful indicators of individuals' immune responses to HIV. The first weeks of HIV infection is characterized by circulatory cytokine production in the blood plasma (Stacey *et al.,* 2009). An increased production of pro-inflammatory cytokines is part of an immune response during HIV infection (Stacey *et al.,* 2009; Bebell*et al.,* 2008). These cytokines augment the replication of HIV and the loss of CD4 T cells through activation-induced apoptosis of by stander T cells, in activating and recruiting CD4 T cell targets for HIV infection and also by directly promoting proviral transcription (Osborne *et al.,* 1989; Lin *et al.,* 1997; Swingler*et al.,* 1999). As cytokines may suggest systemic inflammatory or a local milieu, they might be useful as biomarkers during the prognosis of early stages in HIV infection.

There are several hematological abnormalities that have been identified to be manifested throughout the period of infection with HIV that may consist of coagulation abnormalities thrombocytopenia, anemia and neutropenia (Attili*et al.,* 2008); cytopenias are the most common ones. Studies have shown that infection with HIV leads to a suppressed bone marrow due to changes of the bone marrow microenvironment and manifestations of abnormal cytokines that ultimately results in insufficient production, which generally cause anemia and neutropenia (Coyle, 1997).

2. Methodology

2.1 Study Site and Population

This study was done at Nakuru provincial general hospital (PGH), Kenya. Permission to carry out the study was sought from the hospital's administration. Enrolment of the study group was done at the Comprehensive Care Centre (CCC). Those that consented completed a written informed consent form.

2.2 Design of the Study

A prospective cross sectional study design was used which involved the selection of males and females who gave their consent, and were attending the Voluntary Counseling and Testing (VCT) centre at the hospital. The study involved 80 patients; subdivided into three subgroups comprising of 40 patients with early HIV infection before commencing therapy, 20 patients who were on antiretroviral therapy (HAART) and 20 HIV individuals who turned negative after testing for HIV. Blood samples for laboratory investigation from all the individuals was collected as they visited the VCT and labeled appropriately. Hematological profiles and cytokine assays of the study population were analyzed at the laboratories of Nakuru Provincial General Hospital.

2.3 Determination of Hematological Profiles

Blood parameters were determined using Quintus 5 – part hematology analyzer according to the manufacturer's protocol (BuoleMeidal, AB, Sweden).

2.4 Determination of Circulatory Cytokines

Flow Cytometry was performed using multiplex assay system which included Becton and Dickinson Cytometric Bead Array (BD CBA), Human Inflammatory Cytokine kit and the BD Fluorescence Activated Cell Sorter (FACSCalibur) flow cytometer (BD Biosciences, U.S.A) for cytokines detection. Recommended protocol by the manufacturers was followed.

2.5 Data Analyses

SPSS version 17 was used for the statistical analysis and descriptive statistics were applied for all the parameters that were measured. Analyses of variance were used to determine the differences in mean circulatory cytokines and mean hematological parameters between the subgroups forming the study population, while Chi-square were used to determine the relationships between plasma cytokines and hematological parameters. A p- value < 0.05 was considered to be significant.

3. Results

3.1 Circulatory Cytokine Profiles of the Study Population

The detectable cytokines in the study population were Interleukin 1β (IL1β), Interleukin 6 (IL-6), Interleukin 10 (IL-10), Interleukin 12p70 (IL-12p70) and Tumor Necrosis Factor (TNF). IL- IL-6, IL-10, 12p70 and TNF were detectable in treatment naïve HIV patients, in HIV positive patients on HAART and also in patients that were HIV negative. IL-1β was not detected in HIV negative patients; however, it was detectable in HIV positive patients on HAART and in treatment naïve HIV patients.

Table 1 shows that after analysis of variance (ANOVA), significant differences in the means of IL-12p70 [F (2, 79) = 10.376, p<0.001), TNF [F(2, 79) = 4.883, p=0.010], IL-10 [F(2,79) = 6.515, p=0.002], IL-6 [F(2,79) = 7.231, p=0.001] were observed and a significant difference in the means of IL-1β [F(2,79) = 5.253, p=0.007] between HIV negative patients, HIV patients on HAART and treatment naïve HIV patients.

Table 1: *Analysis of Variance for the Mean Circulatory Cytokines in HIV Patients on HAART, Treatment Naïve HIV Patients and HIV Negative Patients. Sample Sizes are in Brackets. Significant Differences are shown with Asterisks*

	HIV negative patients (20)	**treatment naïve HIV patients (40)**	**HIV patients on HAART (20)**		
Circulatory cytokines (pg/ml)	**Mean**	**Mean**	**Mean**	**ANOVA (F)**	**Difference (p value)**
IL-12p70	3.317	0.2815	0.5593	10.376	0.000*
TNF	0.7895	7.707	1.9750	4.883	0.010**
IL-10	0.1630	2.794	0.6918	6.515	0.002**
IL-6	0.5135	2.794	2.6020	7.231	0.001**
IL-1β	0.0000	5.401	0.7028	5.253	0.007**

*= Significant at p<0.001; **= Significant at p<0.05.

3.2 Blood Parameters of the Study Population

Analysis of variance showed no significant differences between the means of white blood cell count (WBC) [(F2, 79) = 2.615, p=0.080] and between the means of red blood cell count (RBC) [(F2, 79) = 1.153, p=0.321] in treatment naïve HIV patients, HIV patients on HAART

and in HIV negative patients. There were significant differences between the means of hematocrit (HCT) [(F2, 79) = 3.998, p=0.022]; hemoglobin (HGB) [(F2, 79) = 4.086, p=0.021] and platelets (PLT) [(F2, 79) = 3.555, p=0.033] in HIV negative patients, treatment naïve HIV patients and in HIV patients on HAART (Table 2).

Table 2: *Analysis of variance for the means of blood parameters in HIV negative patients, HIV patients on HAART and treatment naïve HIV patients. Significant levels are shown with asterisks*

	HIV Negative patients (n=20)	**treatment naïve HIV patients (n=20)**	**HIV patients on HAART (n=20)**		
Blood parameters	**Mean**	**Mean**	**Mean**	**ANOVA (F)**	**Difference (p value)**
WBC (10^9/L	9.41	6.91	9.30	2.615	0.080
RBC (10^{12}/L)	4.15	4.11	4.45	1.153	0.321
HCT (%)	39.08	36.16	36.13	3.998	0.022**
HGB (g/dl)	13.37	11.43	11.49	4.086	0.021**
PLT (10^9/L)	269	335.70	242.85	3.555	0.033**

Key: PLT = Platelets, HCT = Hematocrit; RBC = Red Blood Cells; HGB =Hemoglobin; WBC = White Blood Cells.
**= Significant at p<0.05

Table 3 show the differences between the means of the blood indices: Erythrocyte indices Platelet indices, Leukocyte indices of the subgroups

Table 3: *Analysis of Variance for Blood Indices means of HIV Patients on HAART, Treatment Naïve HIV Patients and HIV Negative Patients HIV. Significant differences are indicated with asterisks*

	HIV Negative patients (n=20)	**treatment Naïve HIV Patients (n=20)**	**HIV Patients on HAART (n=20)**		
Blood indices	**Means**	**Means**	**Means**	**ANOVA (F)**	**Difference (p value**
Erythrocyte indices					
MCV	89.53	81.13	83.55	4.108	0.020**
MCH	30.03	27.64	27.74	3.359	0.040**

MCHC	33.62	33.02	34.27	4.988	0.009**
RDW-CV	14.32	15.36	14.78	0.992	0.376
RDW-SD	41.08	47.49	44.95	2.423	0.095
Platelet indices					
MPV(fL)	9.03	10.16	9.55	9.600	0.000*
PDW	14.62	15.03	14.77	30.793	0.000*
PCT (%)	0.24	0.25	0.30	1.536	0.222
Leukocyte indices					
Lymph# (10^9/L	2.69	2.42	2.43	0.198	0.821
Mid# (10^9/L)	0.48	0.69	0.68	3.116	0.048**
Gran# (10^{10}/L)	6.19	3.87	6.05	2.908	0.061
Lymph%	35.76	28.91	29.67	1.492	0.231
Mid%	7.18	9.06	8.24	3.641	0.031**
Gran%	55.72	64.04	62.09	1.776	0.176

Key: RDW-SD = relative distribution width of red blood cells by volume, standard deviation; RDW-CV = relative distribution width of red blood cells by volume, coefficient of variation; MCHC = mean concentration of hemoglobin; MCH = mean content of hemoglobin; MCV = mean volume of erythrocytes; GRAN% = the relative (%) content of granulocytes; MID% = the relative (%) content of the mixture of monocytes, basophils and eosinophils; LYMPH% = the relative (%) content of lymphocytes; GRAN# = the absolute content of granulocytes; MID# = absolute content of the mixture of monocytes, basophils and eosinophils; LYMP# = the absolute content of lymphocytes; PCT (%) = platelet crit; PDW = the relative width of the distribution of platelets; MPV(f/L) = mean platelet volume;

* = Significant at p<0.001; ** = Significant at p <0.05

3.3 Relationships between CirculatoryCytokines and Hematological parameters

Table 4 show relationships between circulatory cytokines and hematological parameters.

Table 4: *Chi-square test (X^2) for the relationships between circulatory cytokines and blood parameters of HIV patients on HAART, treatment naïve HIV patients and HIV negative patients. Similar associations are indicated with asterisks and significant ones are shown by superscripts.*

Cytokines:		**IL-12p70**	**TNF**	**IL-10**	**IL-6**	**IL-1β**
Groups of patients	**Blood parameters**	**Association of cytokines and blood parameters: p values**				
HIV Negative patients	WBC*	0.331*	0.137*	0.137*	0.331*	-
	RBC*	0.412*	0.367*	0.579*	0.412*	-

	HCT*	0.412*	0.367*	0.579*	0.412*	
	HGB*	0.395*	0.208	0.390	0.395*	-
	PLT*	0.412*	0.579	0.579*	0.412*	-
Treatment naïve Patients	WBC	0.500	0.414	0.754	0.460	0.221
	RBC	0.500	0.473	0.531	0.381	0.381
	HCT	0.806	0.908	0.500	0.992	0.960
	HGB	0.030[a]	0.017[a]	0.353	0.194	0.129
	PLT	0.352	0.346	0.359	0.330	0.426
HIV patients on HAART	WBC**	0.309**	0.115**	0.157	0.115**	0.309**
	RBC**	0.500**	0.652**	0.772	0.652**	0.500**
	HCT**	0.221	0.267**	0.297	0.267**	0.500**
	HGB**	0.127	0.182**	0.221	0.182**	0.353
	PLT**	0.343	0.368**	0.381	0.368**	0.500**

KEY: ** Similar associations in HIV patients on HAART. * Similar associations in HIV negative patients.
[a] = Significant at $p<0.05$

Table 5 show relationships between circulatory cytokines and erythrocyte indices: volume of erythrocytes (MCV), the mean content of hemoglobin (MCH), the mean concentration of hemoglobin (MCHC)**,** the relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV) and the relative distribution width of red blood cells by volume, standard deviation (RDW-SD) in HIV negative patients, treatment naïve HIV patients and in HIV patients on HAART.

Table 5: *Chi square test (X^2) for the relationships between circulatory cytokines and erythrocyte indices of HIV patients on HAART, treatment naïve HIV patients and HIV negative patients. Similar associations are indicated with asterisks and those significant are shown by superscript*

	Cytokines:	**IL-12p70**	**TNF**	**IL-10**	**IL-6**	**IL-1β**
Groups of patients	**Erythrocyte indices**	**Association of cytokines and blood parameters: p values**				
HIV Negative	MCV	0.500	0.267*	0.297	0.267*	-

patients	MCH	0.343	0.368*	0.381	0.368*	-
	MCHC	0.224	0.201*	0.390	0.201*	-
	RDW-CV	0.803	0.850*	0.494	0.850*	-
	RDW-SD	0.496	0.478*	0.841	0.478*	-
Treatment naïve Patients	MCV	0.363	0.305	0.432	0.181	0.984
	MCH	0.463	0.668	0.209	0.303	0.258
	MCHC	0.544	0.145	0.224	0.292	0.105
	RDW-CV	0.355	0.245	0.494	0.381	0.946
	RDW-SD	0.237	0.009[a]	0.819	0.767	0.920
HIV patients on HAART	MCV	0.263**	0.367**	0.367**	0.263**	0.500
	MCH	0.412**	0.367**	0.367**	0.412**	0.221
	MCHC	0.126**	0.171**	0.171**	0.126**	0.221
	RDW-CV	0.460	0.208**	0.208**	0.210	0.224
	RDW-SD	0.283	0.813	0.405	0.543	0.247

KEY: ** Similar associations in HIV patients on HAART; * Similar associations in HIV negative patients. [a] = Significant at $p<0.05$.

Table 6 show relationships between circulatory cytokines and platelet indices.

Table 6: *Chi-square test (X^2) for the relationships between circulatory cytokines and platelet indices of HIV negative patients, HIV patients on HAART and treatment naïve HIV patients. Similar associations are indicated with asterisks and significant associations are shown by superscripts*

Cytokines:		**IL-12p70**	**TNF**	**IL-10**	**IL-6**	**IL-1β**
Groups of patients	**Platelet indices**	**Association of cytokines and blood parameters: p values**				
HIV negative patients	MPV	0.090	0.502*	0.909	0.502*	-
	PDW	0.940	1.000*	0.999	1.000*	-
	PCT	0.221	0.267*	0.297	0.267*	-
Treatment naïve Patients	MPV	0.003[b]	0.000[a]	0.114	0.011[c] ($p<0.05$)	0.011[c]
	PDW	0.162	0.022[c]	0.001[b]	0.006[c]	0.001[b]

	PCT	0.232	0.454	0.268	0.552	0.297
HIV patients on HAART	MPV	0.285**	0.521**	0.521**	0.285**	0.097
	PDW	0.723**	0.585	0.978	0.723**	0.678
	PCT	0.246**	0.208	0.208	0.246**	0.353

KEY: ** Similar associations in HIV patients on HAART; * Similar associations in HIV negative patients.
[a] = Significant at p<0.001; [b] = Significant at p<0.005. [c] = Significant at p<0.05

Table 7 show relationships between circulatory cytokines and leukocyte indices:the absolute content of lymphocytes (Lymph#), the absolute content of the mixture of monocytes, basophils and eosinophils (Mid#), the absolute content of granulocytes (Gran#), the relative (%) content of lymphocytes (Lymph %), the relative (%) content of the mixture of monocytes, basophils and eosinophils (Mid%) and the relative (%) content of granulocytes (Gran%) in treatment naïve HIV patients, HIV negative patients and in HIV patients on HAART.

Table 7: *Chi-square test (X^2) for the relationships between circulatory cytokines and leukocyte indices of treatment naïve HIV patients, HIV patients on HAART and HIV negative patients. Similar associations are indicated with asterisks those that are significant shown by superscripts.*

	Cytokines:	**IL-12p70**	**TNF**	**IL-10**	**IL-6**	**IL-1β**
Groups of patients	**Leukocyte indices**	**Association of cytokines and blood parameters: p values**				
HIV negative patients	LYMPH#	0.324	0.125*	0.021[a]	0.125*	-
	MID#	0.308	0.092*	0.044[a]	0.092*	-
	GRAN#	0.127	0.182*	0.221	0.182*	-
	LYMPH%	0.343	0.368*	0.381	0.368*	-
	MID%	0.223	0.115*	0.157	0.115*	-
	GRAN%	0.343	0.368*	0.381	0.368*	
Treatment naïve patients	LYMPH#	0.130	0.330	0.942	0.564	0.011[a]
	MID#	0.391	0.175	0.881	0.077	0.034[a]

	GRAN#	0.014	0.053	0.018[a]	0.038[a]	0.221
	LYMPH%	0.352	0.346	0.359	0.330	0.426
	MID%	0.021[a]	0.197	0.116	0.637	0.105
	GRAN%	0.230	0.220	0.241	0.405	0.381
HIV patients on HAART	LYMPH#	0.229	0.159	0.485	0.295	0.334
	MID#	0.978	0.912	0.967	0.994	0.850
	GRAN#	0.716**	0.890**	0.890**	0.716**	0.859
	LYMPH%	0.263**	0.367	0.171	0.263**	0.221
	MID%	0.210**	0.390	0.208	0.210**	0.127
	GRAN%	0.280**	0.312**	0.312**	0.28**0	0.343

KEY: ** Similar associations in HIV patients on HAART; * Similar associations in HIV negative patients. [a] = Significant at $p<0.05$.

4. Discussion

Levels of circulatory cytokines of persons infected with human immunodeficiency virus (HIV) are altered when compared to cytokine profiles found in those without the infection. Infection with the HIV along with the viral proteins is capable of disturbing the cytokines productions as well as disrupting their normal interactions which results in the normal immune function being disrupted (Vishwanath*et al.,* 2011). The altered levels of circulatory cytokines in individuals infected with HIV are likely to have a direct impact on the course of HIV disease by enhancing or suppressing HIV replication, and can affect the function of the immune system (Breen, 2002). This study sought differences in circulatory cytokine levels by measuring plasma cytokines in individuals infected with HIV, without treatment and on treatment, and comparing the cytokine levels with those found in individuals without HIV. According to this study, statistically significant differences were observed in the levels of the pro-inflammatory circulatory cytokines, which stimulates the immune system, and the anti-inflammatory cytokines which suppresses the immune system among those infected and not infected with HIV.

Recognition of hematological features during the various stages of human immune deficiency virus (HIV) infection, particularly in recently diagnosed HIV patients, is very

important with the continuing challenges of monitoring HIV disease progression. Disorders of hematopoietic system are common but often overlooked complications of HIV infection which manifest at any stage of the disease (Sujata *et al.*, 2013).

5. Conclusion

The early stages of HIV infections are characterized by varying levels of different types of circulatory cytokines, both pro-inflammatory and anti-inflammatory. The inflammatory responses may be as a result of a complex interplay of many cell types using chemical messengers to communicate among themselves, hence forming heightened chains of command and feedback loops.

Blood parameters that include the white blood cells (WBC), hematocrit (HCT), hemoglobin (HGB) and platelets (PLT) may act as predictive biomarkers during early or acute HIV infection. Other parameters that may also act as indicators of the disease progress during this period includes: erythrocyte indices: the mean content of hemoglobin (MCH), the mean concentration of hemoglobin (MCHC) and the mean volume of erythrocytes (MCV); leukocyte indices: the absolute content of the mixture of monocytes, basophils and eosinophils (Mid#) and the relative content of the mixture of monocytes, basophils and eosinophils (Mid%); platelet indices: the absolute content of the mixture of monocytes, basophils and eosinophils (Mid#); the mean platelet volume (MPV) and the relative width of the distribution of platelets (PDW).

The effects of the virus on the immune system may not be the only attribute to the conditions seen during HIV infection but might also be due to the immune system's responses to the virus. Since the immune system uses hundreds of signaling chemicals, some of which may buildup in the bloodstream, they can be measured as biomarkers to provide clues about immune system activities.

6. Recommendations

i) In a set up where CD4 T cell counts analysis is difficult or where it is not possible to conduct diagnostic tests for HIV patients including circulatory cytokine levels, the hematological parameters and indices could be useful biomarkers in low socio-economic setups.

ii) The determinants of inflammation in HIV infected persons can be considered when investigating circulatory cytokines as biomarkers; while the measurements of blood parameters: white blood cells, hematocrit, hemoglobin and platelets plus the erythrocyte indices, platelet

indices and leukocyte indices may expose possible diseases or conditions that go unnoticed in recently diagnosed HIV individuals.

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