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Research Article

The Interleukin-6 and Interleukin-10 Plasma Levels the Driver of Immune Dysfunction and Chronic Inflammation in HIV Infection

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ABSTRACT

Background: Cytokines network drive Human Immunodeficiency Virus (HIV) inflammation and pathogenesis. Highly Active Antiretroviral Therapy (HAART) maintains low HIV viral load (VL) but chronic inflammation remains high in HIV subjects compared to HIV naïve population. To date, there is no surrogate biomarker that gives the hallmark picture of immune activation, chronic inflammation in HIV programs. This study aimed at determining the plasma cytokine levels and their association with HIV VL at initiation and after six months on HAART in Rwanda.

Methods: We used a matching groups approach based on sex and systematic sampling within the groups was used to enrol fifty (50) patients in each group. Fifteen (15) HIV naive people were included in the study as control group. BD Flow cytometry was used to determine cytokine levels while revised WHO questionnaire was used to collect socio-demographic and clinical data. Independent sample T tests (T) and Wilcoxon rank (W) were used to compare cytokine mean levels while Pearson's Product Moment Coefficient (PPMC) r was used for correlation of parameters at HAART initiation and after six months of treatment. The statistically significant differences and the correlation between assessed parameters were determined at $p \leq 0.05$.

Results: At the initiation of HAART, Interleukin (IL)-10, IL-6 plasma levels were higher while IFN-γ and TNF-α were lower compared to its levels after six months of HAART. The present study found a positive association between IL-10, IL-6 and HIV VL.

Conclusion: Proinflammatory and antinflammatory cytokines levels change differently following HAART and correlate with the HIV VL. The IL-10, IL-6 plasma levels are an alternative biomarker for assessment of hallmark of immune activation and inflammation in HIV infection.

Keywords: HIV; Viral load; Cytokine; Active antiretroviral therapy

Abbreviations: CBA: Cytometric Bead Array; HAART: Highly Active Anti-Retroviral Therapy; HIV: Human Immunodeficiency Virus; VL: Viral Load; IFN-γ: Interferon Gamma; IL: Interleukin; IREC: Institutional research and ethics committee for Moi University College of Health Sciences/Moi Teaching and Referral Hospital; NRL: National Reference Laboratory; RBC: Rwanda Biomedical Centre; TNF-α: Tumour Necrosis Factor alpha

BACKGROUND

HIV/AIDS continues to be a global challenge. Despite the effort made in its diagnosis and treatment, the HIV/AIDS burden, morbidity, and mortality remain problematic. The magnitude seems to be high in Africa compared to the developed countries due to the delay in diagnosis and HAART initiation. Although the evidence highlights the correlation between inflammatory markers with a higher morbidity and mortality rates [1]. Cytokines coordinate immunological processes and demonstrated the ability to interfere with the molecular mechanisms responsible for HIV

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latency [2,3]. Cytokines imbalance is key in HIV pathogenesis by harmonizing viral replication and latency formation, innate immune response and adaptive immune responses [4]. Immune dysfunction in HIV infected/AIDS individuals had been characterized by inadequate CD4+T cell responses to antigens; more activated and differentiated T-cell markers of all sub phenotypes including regulatory T cells [5]. The immune disturbance observed in HIV infection had been argued in different studies to be responsible of chronic inflammation as well as HIV replication and infection reservoir establishment [6,7]. However, limited data is available for other routine immunological markers to monitor HIV infection and HIV disease progression in the advent of high morbidity and mortality rate attributed to chronic inflammation and immune system dysfunction in HIV infected/AIDS patients [3,4]. To address the hallmark of immune dysfunction, the use of groups of soluble biomarkers have been recommended rather than use of individual biomarkers [3,4,8]. This study aimed at describing and determining the relationship between plasma levels of TNF-α, IFN-γ, IFN-α, IL-2, IL-4, IL-6, IL-10 and HIV VL with a view of understanding cytokine networks during HIV infection before HAART and after six months of treatment and its power as immunological biomarker. This study found increased interleukin (IL)-10, IL-6 plasma levels and lower IFN-γ and TNF-α plasma levels compared to its levels after six months of HAART. IL-10, IL-6 were mostly associated with HIV VL (r=0.8 and 0.7 p-value=0.001).

MATERIALS AND METHODS

A follow up study design with the matching approach based on sex before and after six months of HAART and systematic sampling among the groups were used to enrol fifty patients before and after six months of HAART.

Study participants

The study included Fifty (50) HIV positive patients before and after Six months and Fifteen (15) healthy, non-HIV positive as controls with no malaria, Hepatitis B and C and Tuberculosis (TB) from five health facilities (Kigali city, East; North; South) representing five provinces of Rwanda from April 2018 to June 2018, Eligible to start HAART and willing to sign consent to participate in the study and data publication.

Sample size calculation

The sample size was estimated using the two groups comparison formula by Lwanga et al., (Lemeshow and Lwanga,; Lwanga and Lemeshow,) as follows whereby: SD is the Standard deviation of the mean of cytokines the two populations. The IL-4 showed to be highly varied in HIV and its SD was used in the sample size estimation formula 38 pg/ml and 41 pg/ml for ART naïve and patients on HAART respectively (Gori et al.,) d=Expected precision. Mean difference of variables between the groups that is associated with clinical difference. The mean IL-4 for ART naïve patients (μ1) was estimated at 138 pg/ml for ART naïve patients and 116 pg/ml for HIV patients on treatment (μ2). Precession (d)=μ1-μ2=22 pg/ml (Gori et al.,). Z1-α/2=Standard normal variate for level of significance. At 5% type1 error p value ≤ 0.05, is 1.96 as from normal distribution curve. The minimum sample size (N) for each group equals 49 patients. The sample size was rounded up to fifty patients for each matching group based on cytokines kit size that is fifty test samples and the generation of two standard curve sets.

Sampling process

Considering the five sites distributed countrywide, incidence in Rural (0.22) and urban (0.65), a following assumption was made: N=3X+4X=50 HIV+People where N=sample size and X=equal distribution of patients per site was used to estimate 28 patients for rural hospitals and 22 HIV+People for Kigali city. The Kth=four (4) was calculated Based on ground registers and HMIS data and it was used to recruit 7 patients per site in rural and 22 HIV+People in Kigali city. A matching group by sex was recruited between 31st May to 15th June 2018, as they are coming for biological follow up after six months on HAART. Each selected client was screened for Malaria, TB, HBV, and HCV using appropriate rapid tests. If he or she is positive for one of the screened conditions, he or she was excluded and looked for the next client.

Procedure for cytokines level determination

Cytokines were measured using Flow cytometry technology powered with Cytometric Bead Array (CBA). CBA was improved analysis tool compared to conventional ELISA using BD™ Cytometric Bead Array (CBA) and allowed analyst to discriminate different particles based on size and color in a single sample. The BD CBA system used the sensitivity of amplified fluorescence detection by flow cytometry to measure soluble analytes in a particle-based immunoassay. Each bead in a CBA provided a capture surface for a specific protein.

The cytokine capture beads were mixed with the PE-conjugated detection antibodies and then incubated with recombinant standards or test samples to form sandwich complexes. Following acquisition of sample data using the flow cytometry, the sample results were generated in graphical and tabular format using the BD™ CBA Analysis Software.

Demographic and HIV VL data

The demographic data and HIV VL level data were collected using the customized WHO questionnaire to fit for the purpose by checking the medical file and interview

Statistical analysis

R studio, descriptive statistics package was used to compute the mean, standard deviation, and 95% confidence interval. The relationship between cytokines and HIV VL was determined using Graph pad prism 7 package.

Ethical consideration

This study was conducted under the ethical clearance IREC/2017/125 obtained from Moi University (IREC)

RESULTS

TThe changes in plasma cytokines levels following HAART and their relationship with HIV VL before and after six months of HAART treatment were investigated. In the above context, the study performed and determined relationship between IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, plasma levels and HIV VL at initiation and after six months of HAART for one hundred study participants and fifteen participants HIV naïve as controls. Our findings showed statistically significant differences in plasma cytokines levels at initiation and after six months of HAART. A positive correlation was found between IL-10, IL-6 with HIV VL and a negative correlation was found between IFN-γ, TNF-α and HIV VL.

Social demographic characteristics of participants

Among HIV positive clients, 50 clients were HAART naïve, while

50 clients were on HAART for at least 6 months. Based on sex, there were no statistically significance differences in male 24 (48%)/23 (46%) and female 26 (52%)/27 (54%) at initiation and after 6 months of HAART respectively, Chi-square p-value 0.8411 (Table 1). The dominant groups of study participants (56% and 62%) were between 25 and 44 years old at initiation and after 6 months, respectively.

Cytokines levels before and after initiation of HAART

This study found that the mean concentration and standard deviation of the mean (SD) in pg/ml for TNF-α, IFN-γ were increasing, 11.9 (5.21) versus 35.3 (16.2), 23.8 (10.4) versus 47.1 (21.6), respectively. IL-2, IL-4, IL-6 and IL-10 were decreasing, 34.7 (9.43) versus 28 (9.98), 10.7 (15.1) versus 7.72 (6.19); 78.6 (13.8) versus 54.8 (17.0) and 45.7 (15.1) versus 17.4 (6.79), respectively following HAART (Figure 1).

In this study, we noticed a significant difference in plasma cytokines concentration following HAART as follows: IL-2 (t (98)=3.3098 with a p-value=0.0013), TNF-α (W=-9.770 with a p-value=0.0001); IFN-γ (t (98)=-6.9009 with a p-value=0.0001); IL-4 (W=1721, p-value=0.0118); IL-6 (t (98)=5.78 with a p-value 0.0001); and IL-10 (W=2413 with a p-value<0.0001) (Figure 2).

The same difference was observed when compared with healthy controls, HAART naïve sub-population and a sub-population after six months of HAART.

Cytokines plasma levels did not show any statistically difference upon stratification based on age and sex at initiation and after six months with ANOVA. Although the data for this study revealed the high concentration and data overlap mostly for IL-2 and IL-6 in the strata of 25-44 years old (Figure 2).

Relationship between cytokines and HIV VL

The linear relationship of IL-2, IL-4, IL-6, IL-10, and TNF-α and IFN-γ and HIV_VL were assessed using Pearson's Product Moment Coefficient (r). The positive relationship was observed for HIV_VL and IL-2 (r=0.4148 p-value <0.0001), HIV_VL and IL-6 (r=0.8164 p-value <0.0001), HIV_VL and IL-10 (r=0.8667 p-value<0.0001) while HIV_VL was associated negatively with TNF (r=-0.3483 p-value 0.0005) and IFN (r=-0.4835 p-value<0.0001) (Table 2). IL-4 did not show statistically significant relationship with HIV VL (r=0.0839 p-value=0.41)

Table 1: Number of participants: 50 participants at initial of HAART and 50 participants after 6 months on treatment. N=100 (%) unless otherwise indicated; HIV: Human Immunodeficiency Virus HAART: Highly Active Antiretroviral Treatment.

Parameters	Health Group Control N=15	HIV+HAART Naïve N=50 (%)	HIV+ HAART after 6 months N=50 (%)	p-value
Sex				
Males	7	24(48)	23(46)	0.8411
Females	8	26(52)	27(54)	
Age				
18- 24		6 (12)	7 (14)	
25-44		28 (56)	31 (62)	0.0032
45-64		16 (32)	12 (24)	
Smoking				
Yes		5 (10)	1 (2)	0.2123
No		45 (90)	49 (98)	
Alcohol consumption				
Yes		4 (8)	2 (4)	0.3997
No		46 (92)	48 (96)	
Meals per day				
One per day		5 (10)	3 (6)	0.0004
Two per day		43 (86)	45 (92)	
Three per day		2 (4)	2 (4)	
Eating fruits and vegetables				
At least once week		35 (70)	28 (56)	
Not all		15 (30)	22 (44)	0.147

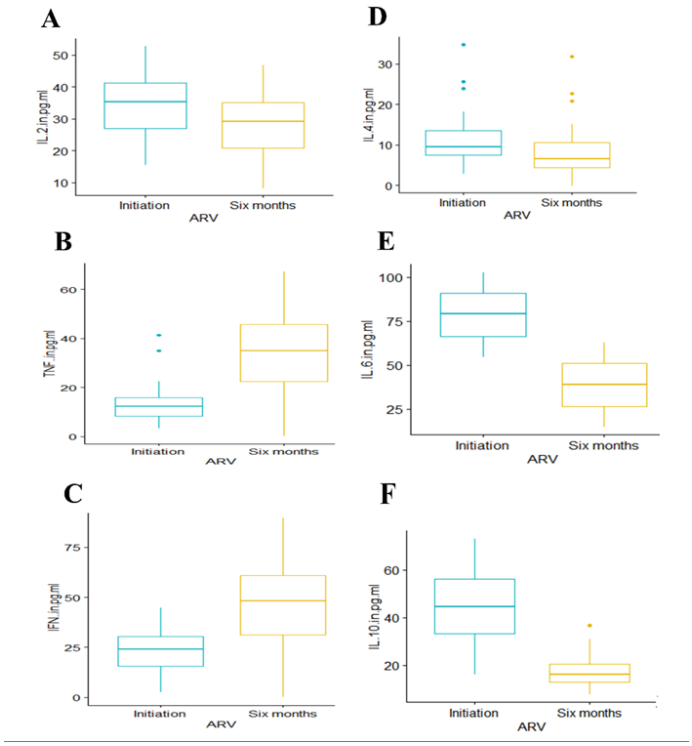

Figure 1: Cytokines levels before and 6 months after initiation of HAART compared to 15 healthy controls.
Note: ARV; (▭) Initiation; (▭) Six months

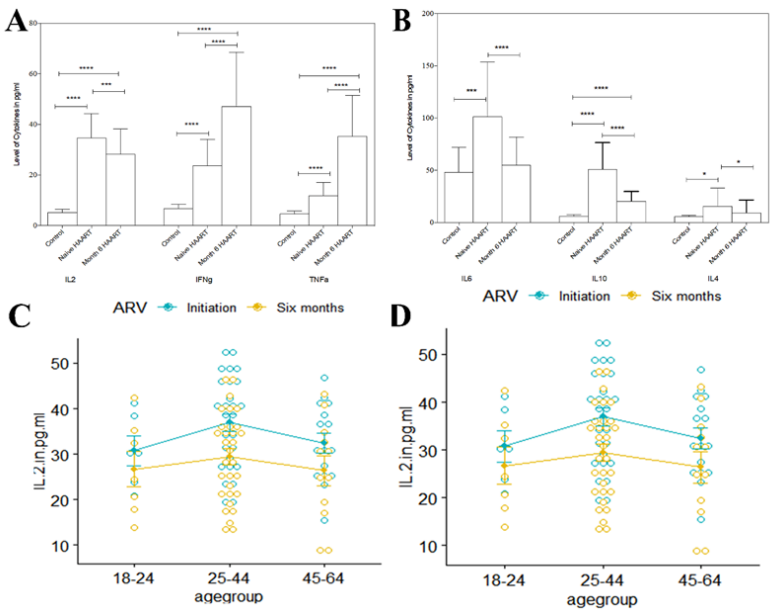

Figure 2: Cytokines levels before and 6 months after initiation of HAART compared to 15 healthy controls.

Table 2: The correlation analysis of pro inflammatory cytokine and anti inflamatory cytokine with HIV viral load.

	Viral Load vs. IL2	Viral Load vs. IFN-γ	Viral Load vs. TNF-α	Viral Load vs. IL4	Viral Load vs. IL6	Viral Load vs. IL10
Pearson r	0.4148	-0.3483	-0.4835	0.08392	0.8164	0.8667
95% CI	0.2327 to 0.5688	-0.5138 to -0.1579	-0.6242 to -0.3124	-0.1196 to 0.2807	0.7359 to 0.8741	0.8059 to 0.9094
p-value	<0.0001	0.0005	<0.0001	0.4188	<0.0001	<0.0001

DISCUSSION

In our days, persistent immune activation and chronic inflammation constitute the most patho-immunological status in HIV/AIDS patients and predisposes them more to non-AIDs diseases. In our HIV protocols, there is no biomarker to assess the above state. This study detailed and correlated cytokine levels with HIV viral load to assess the cytokines potentials to monitor the immune activation and chronic inflammation. The data showed that IL-4, IL-6 and IL-10 were statistically high at initiation of HAART and tended to reduce following HAART at 6 months. The same statistically significant changes were observed between HAART naïve subpopulation, after six months HAART subpopulation and heath controls groups in progressive manner but still high in HIV positive groups. These data supported the facts that HAART reduces partially inflammation in HIV infected/AIDS patients following successful treatment and highlight on mechanisms by which HIV infection causes inflammation (IL-6) and immune modulation (IL-10) via inhibition of pro-inflammatory cytokines. Our study findings concurred with other studies urged that for HIV to survive, it is directly subvert specific immune responses by IL-10, other anti-inflammatory cytokines induction, and inhibitory effects on other inflammatory cytokines [6,9,10]. The similar findings were observed in single cells study which showed that high frequency of circulating of CD4+T cell from HIV infected/AIDS patients produced more of IL-10 compared to HIV naïve and the same results were highlighted in HIV infected/AIDS individuals with rapid HIV infection progression and in active HIV viral replication cases [11]. IL-2, IFN-γ and TNF-α plasma levels were statistically low at initiation and tended to increase after six months of HAART. These data showed that antiviral mechanisms increase with HAART treatment and support the idea that HIV inhibit normal release of IFN-γ and TNF-α for host colonization. This research findings support other research evidences that had demonstrated a shift from Th-1 into Th-2 cytokines during HIV/AIDS and the trend from Th-2 immune milieu in active HIV infection to Th-1 immune milieu following successful HAART [2,12]. IL-2, IL-6, IL-10 showed a positive relationship with HIV VL while TNF-α, IFN- γ showed a negative relationship with HIV VL. From these results, we demonstrated that IL-2, 6 and 10 increased as HIV VL increased too. These results were not suppressing due to inhibitory mechanism of IL-10 to favour viral colonization and persistent inflammation. This study results concur with another study titled, asymptomatic HIV people on HAART and HAART naïve present different profiles of sCD14, sRAGE, DNA damage, and vitamins where by CD4+T increases was associated with decreased levels of IL-10 in long-term suppressor group compared short-term suppressor group [13]. The results of this study showered a positive relationship between HIV VL and IFN-γ and TNF-α too, despite difficult correlation in many studies due to the double role of IFN-γ as an inflammatory and antiviral cytokine. These results concurred with the findings from other previous studies that detailed cytokines storm during HIV 1 infection before and after HAART [14]. The increased level of IFN-γ and TNF-α probably increases the onset of immune activation and immune exhaustion.

CONCLUSION

Cytokines levels differ from initiation of HAART to hit levels after six months in HIV patients. IL-4, IL-10 and IL-6 decreased while IFN-γ and TNF-α increased following HAART. Before HAART, HIV/AIDS immune response is mediated by Th-2 milieu while it tended to shift to Th-1 milieu following successful HAART treatment. IL-10 and IL-6 plasma level strongly correlated with HIV VL while inflammatory cytokines (IFN-γ and TNF-α) showed a negative relationship with HIV VL. The IL-10, IL-6 plasma levels are an alternative biomarker for assessment of hallmark of immune activation and inflammation in HIV infection.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was conducted under the ethical clearance IREC/2017/125 obtained from Moi University (IREC) and Rwanda biomedical Centre (RBC) endorsed the approval. Eligible HIV positive patients received details of study procedures including anonymous sample collection, analysis, and confidentiality of results and all right to withdrawn them self from study any time as they want. Blood sample collection was performed for those who signed the consent.

CONSENT FOR PUBLICATION

The Clients in this study consented for participation and data publication by signing the consent form..

COMPETING INTERESTS

All authors declare no competing interests.

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AUTHORS' CONTRIBUTIONS

Jean Pierre Ngangali, Alex K. Chemtai, Patel Kirtika and Emonyi Ingera Wilfred conceived the study protocol and wrote the first manuscript. Jean Pierre Ngangali, Alex K. Chemtai, Emonyi Ingera Wilfred, Leon Mutesa drafted the implementation and analysis plan. Jean Pierre Ngangali, Isabelle de Valois and JB Munyemana did sample collection and laboratory sample analysis and storage. Jean Pierre Ngangali and Lilian Nkinda performed the statistical analysis. Edouard Ntagwabira, Robert Rutayisire, Swaibu GATARE and Sabin Nsanzimana reviewed the manuscript. All authors contributed to data analysis, review, and interpretation of the results. All authors read, revised, and approved the final manuscript.

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None.

AVAILABILITY OF DATA AND MATERIALS

Apart of the data provided in this manuscript, the lower data used and/or analysed during the implementation and analysis of this study are available from the corresponding author upon request.

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