
International Journal of Immunology

2016; 4(1): 1-5

<http://www.sciencepublishinggroup.com/j/iji>

doi: 10.11648/j.iji.20160401.11

ISSN: 2329-177X (Print); ISSN: 2329-1753 (Online)



Fas Mediated(CD95L) Periferal T-cell Apotosis Marker in Monitoring HIV-1 Disease Progression in Adults in Yaoundé, Cameroon

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To cite this article:

George Mondinde Ikomey, Atashili Julius, Graeme Brendon Jacobs, Martha Tongo Mesembe, Agnes Eyoh, Emilia Lyonga, Okomo Assoumou Marie Claire. Fas Mediated(CD95L) Periferal T-cell Apotosis Marker in Monitoring HIV-1 Disease Progression in Adults in Yaoundé, Cameroon. *International Journal of Immunology*. Vol. 4, No. 1, 2016, pp. 1-5. doi: 10.11648/j.iji.20160401.11

Received: November 21, 2015; **Accepted:** November 29, 2015; **Published:** March 22, 2016

Abstract: sFas (CD95) / FasL are hallmarks of apoptosis involvement in pathogenesis of HIV. We assess changes in soluble Fas /FasL, CD4 % and HIV-1 viral load in patients prior to the initiation of antiretroviral therapy (ART) and 6 months thereafter. A prospective longitudinal study on sixty consented HIV-1 positive adults. sFas and sFasL levels were measured by ELISA. CD4 cell counts and HIV-1 viralloads were measured using standard methods. Samples were analysed according to the manufacturers' guidelines. There was a significant positive correlation between HIV-1 viral load and FasL at six months (M6) on treatment [$r = +0.49$, (0.03)]. There were no correlation between sFas/FasL and CD4 cell counts [$r = -33$ (0.16), -31 (0.17) - 23 (0.03) respectively]. The significant correlation between sFasL and HIV-1 viral load at six months of ART suggests that sFasL could be a signal biomarker for HIV-1 disease progression. We have shown in this study that high levels of sFasL depict high HIV-1 viral loads and advance state of the HIV disease. These biomarker should be investigated further in other settings.

Keywords: sFasligands (CD95), Apoptosis, HIV, AICD, ART

1. Introduction

Monitoring treatment success of patients on ART is solely dependent on HIV viral load assays [1]. The CD4 absolute cell count and CD4 cell % are routinely used in Cameroon and many other Sub-Saharan African countries as a baseline prior to treatment. They are also used for monitoring treatment outcomes due to limited HIV viral load testing facilities available. Immunological and virological responses are required for better evaluation of patient success, especially after 6 months of ART initiation.

Fas-Ligand (FasL, CD95L) is a homotrimeric membrane-molecule. It is a type II membrane protein belonging to the Tumour Necrosis Factor (TNF) and the Nerve Growth

Superfamilies, (NGS). Their C-terminal regions are outside the cell, with a three cystein-rich domain (CRD) [3-4]. FasL are expressed only while the T-lymphocytes are activated. The expression of FasL is induced in T-cells through activation via their T-cell receptor (Fas, Apo-1, CD95) (4-6). The extracellular region of Fas has significant homology to the corresponding region of other members of the TNF family. Each FasL trimer binds three Fas receptor molecules on the surface of the targeted cells [4-5]. The Fas receptor (target cell) is the antagonist of FasLigand (effector cells). When they are cross-linked during the immune activation of T-Cells, it leads to an up-regulation of apoptosis of CD4. The Activated Induced Cell Death (AICD) pathways of apoptosis is involved in this up-regulation, leading to an uncontrolled depletion of CD4 T-cells, as witnessed in immune depressed

HIV patients [10-11].

Recent studies have highlighted the biological interaction of many viral and host factors, such as host genetics, immune response and their involvement in the pathogenesis of HIV disease. Members of the TNFR super family could mediate pleiotropic biological processes, such as cell proliferation, differentiation, apoptosis and cytokine production [4-9]. Several other studies had shown a significant increase of Fas receptors and ligand levels in plasma or serum of HIV-1 infected individuals, compared to their negative control subjects [10-17]. These studies also demonstrated the involvement of the Fas/FasL pathways in predicting HIV-1 disease progression. In our previous studies in resource limited settings in Cameroon we demonstrated that Fasligand (CD95L) were better alternatives, compared to the commonly used CD4 absolute or CD% for baseline and in monitoring disease progression in infants and adults on ART (23).

HIV-1 viral load testing is the gold standard test recommended for monitoring patients' success with ART [18-22]. However, their availability, due to high cost, as demonstrated in our previous study in Cameroon [24] requires the need to identify alternative approaches for monitoring ART in resource-limited settings. The study objective was to assess the changes in sFasL, CD4 cell counts, CD4 % and HIV-1 viral load prior to and after the initiation of ART.

2. Materials and Method

2.1. Study Design

This was a prospective longitudinal study conducted between September 2012 and February 2013. Sixty HIV positive patients were recruited from the Center for the Study and control of Communicable Diseases (CSCCD), Faculty of Medicine and Biomedical Sciences, University of Yaoundé, Cameroon. Patients were screened for CD4 cell count and HIV viral load and were referred from different health units around the city of Yaoundé, Cameroon. All participants were those earmarked for ART with their baseline CD4 cell counts below 350 copies/ml. Initiation of ART was taken at time (M0) and all patients were followed-up after 6 months of treatment (M6). Demographic information was collected for each participant using a standard questionnaire and consent form. Ten milliliters of whole blood were collected under standard conditions in EDTA anticoagulant tubes and 50µl was used for CD4 absolute cell count and percentages. Plasma was obtained after centrifugation of whole blood at 12000 rpm for 5 minutes. The plasma was separated in less than four hours after blood collection in EDTA anticoagulant tubes.

2.2. Ethical Considerations

Ethical clearance was obtained from the Cameroon National Ethics Committee (Reg 049/CNE/SE/2012). Participation in this study was voluntary. A signed consent form was obtained from all participants. All procedures were standard and only involved minimal risk to the participants. Study results were returned to the patients and incorporated

into their medical records. The results were readily available to their medical practitioners.

2.3. Measurement of sFas Levels

Soluble Fas levels were assayed using a quantitative sandwich Enzyme-linked Immune-Sorbent Assay kit for both the receptors and the ligands (Quantikine®, R&D Systems, UK), strictly using the manufacturer's instructions. The wavelength and optical density were measured at 450 nm using an ELISA reader (Sunrise Tecan Austria GMBH 5082 Microplate Reader) and all samples were assayed in duplicate. The concentration of sFas in plasma samples were determined extrapolating the results from a standard curve.

2.4. HIV-1 Viral Load and CD4+ Cell Counts

We used the commercial kits manual (Amplicor 1.5, Roche diagnostics, Germany) an RT-PCR assay. This was performed with a prepared Master Mix containing oligonucleotide primers Specific to regions of the HIV-1 *gag* genes with upstream sense primers of (sk145) 5'(AGTGGGGGGACATCAAGCAGCCATGCAAAT3') and the downer stream antisense, (skcc1B) SKCC1B (5'-TACTAGTAGTTCCTGCTATGTCACTTCC-3'). The detection and quantification was done using coated hybridization probes. The optical density and wave lengths were measured and extrapolated curves generated to calculate results. The minimum detection limit of the kits was 2.3log10. CD4+ counts were done using the Automated FAScount machine from Becton Dickinson (Abbott France) both the CD4 and Viral loads were done simultaneously to generate both baseline values. Samples were analysed based on the manufacturers' guidelines.

2.5. Statistical Analysis

Data were analysed using STATA version 11 (STATA, Corps Texas, USA). Six month changes in Fas ligand levels were compared to six month HIV viral load changes using the Pearson correlation coefficient. Changes of HIV viral load were considered significant with a log difference of 0.5 between two viral load scores. The Mann Whitney test was used to compare association.

3. Results

3.1. Patient Demographics

Of the sixty participants enrolled in the study, 38 (63.3%; n=60) were female and 22 (36.6%; n=60) male. Their ages range from 20 to 61, with a mean of 34.3 (SD=7.8). The information is summarized in Table 1. All participants receive first line ART according to the WHO and Cameroon national guidelines.

3.2. Baseline Data (Mo)

3.2.1. HIV Viral Load and sFasLigands Levels

The HIV viral load levels ranged from 3.7 to 5.4 log10

with a mean value of 4.8, while the Fasligand concentration ranged from 51 to 321 pg/ml with a mean value of 153.7 (Table 2).

Table 1. Demographic data of study Population.

Gender	N = (60)	%
Male	38/60	63.3
Female	22/60	36.6
Age Group(Years)		
20-25	2/60	3.3
26-31	6/60	10
32-37	33/60	55
<38	19/60	31
Marital Statue		
Married	23/60	38
Single	37/60	61

Table 2. Baseline and Six months Data.

Base line values	Log10/copies/ml/pg/ml	Means
HIV-1 viral load	3.7 - 5.4	4.8
FasLigand (CD95L/127) concentrations	51 - 321	153.7
Six months on ART		
HIV viral loads	1.3 - 3.4	3.1
FasLigand (CD95L/127) concentrations	27 - 231	88.1

3.2.2. sFasR, CD4 Cell Counts

sFas, CD4 levels ranged from 95 to 300 pg/ml, 200 to 500/mm³ and 30 - 40%, respectively. At six months HIV viral loads, sFas, sFasL and CD4 levels were 1.3 to 3.4 log₁₀, 80 to 290 pg/ml, 27 to 231 pg/ml, 200 to 650 mm and 30 to 45%.

3.3. Data at (M6)

3.3.1. HIV-Viral Load and FasL(M6)

At six months on ART the HIV viral load and Fasligand levels showed a significant fall with their ranged of 1.3 to 3.4 log₁₀ with a mean of 3.1, while the Fasligand concentration ranged from 27 to 231 pg/ml, with a mean of 88.1 (Table 2).

3.3.2. sFasR, CD4 Absolute and CD4% (M6)

At six months sFas, CD4 levels ranged from 1.3 to 3.4 log₁₀, 80 to 290 pg/ml, 200 to 650mm³ and 30 to 45%. These changes were not statistically significant.

3.4. Substantial Change After Six-Month in HIV Viral Load and Fasligands Levels

The values show substantial change at after six-month with ranged from -3.5 to +0.3 log₁₀ with a mean of -1.7, while the FasL concentration ranged from -260 to +81pg/ml. Twenty patients had <1log change in HIV-1 viral load and a mean decrease of FasL levels of -5.4 pg/ml, while the forty patients had >1log decrease in HIV-1 viral load and a mean decrease

in Fas ligand concentration of - 87.2 pg/ml with a (p-value = 0.06) (Table 2).

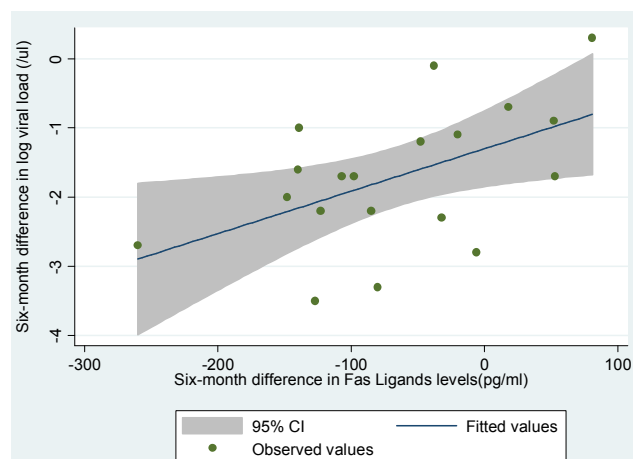


Figure 1. The scatter plot illustrates the correlation between six-month changes in HIV-1 viral and sFasligand Levels. The X-axis shows the difference in HIV Viral load while the Y-axis shows the sFasligand concentration. ($r=+0.49$, $p\text{-value}=0.03$).

3.5. Substantial Change After Six-Month in HIV Viral Load and sFasligands Levels

The values show substantial change at after six-month with ranged from -3.5 to +0.3 log₁₀ with a mean of -1.7, while the FasL concentration ranged from -260 to +81pg/ml. Twenty patients had <1log change in HIV-1 viral load and a mean decrease of FasL levels of -5.4 p g/ml, while the forty patients had >1log decrease in HIV-1 viral load and a mean decrease in Fas ligand concentration of - 87.2 pg/ml with a p-value=0.06.

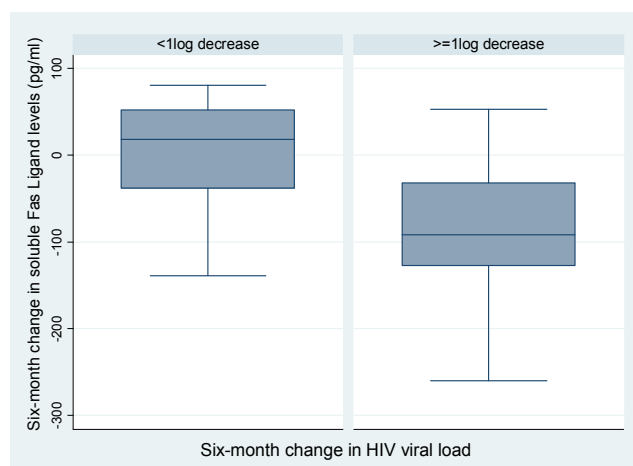


Figure 2. Box plot illustrates the substantial change in HIV-1 viral load and Fasligand using a < and >1 Log. The middle horizontal lines represent the median concentrations of sFas ligands and HIV-1 viral loads >1log and <1log.

4. Discussion

Fas and FasL play critical roles in the immune system, including the killing of pathogens in infected cells, causing death of absolute and potentially dangerous lymphocytes. They have also been shown to play critical roles in HIV-1

pathogenesis [1-4]. This study aimed at assessing the six-month (M6) change in both FasL levels in comparison with HIV-1 viral load. Limited studies have been performed to evaluate the correlation between the apoptotic immunologic marker FasL (CD95L) and the gold standard virologic marker (HIV-1 viral load) used in monitoring patients on ART [5-9]. We have previously done a cost analysis to show the potential of using Fas and FasL as a cheaper alternative to evaluate ART outcomes (Ikomey *et al.*, 2012; Ikomey *et al.*, 2013).

The mean age of the study population was 34.4 (SD \pm 7.8), with 63.3% of patients being male and 36.6% female. Our results show that the majority of patients at baseline, before ART, had HIV-1 viral loads above 3.5 log₁₀ and FasL levels above 300 ng/dl. Other studies have also shown high FasL levels of above 3log for treatment naïve patients [6]. The elevated levels of both biomarkers at baseline could be as a result of the high viral burden and immune activation of T-cells observed in naïve to early treated HIV-1 positive patients. These high levels of FasL could potentially indicate an increase in HIV-1 viral load and an advanced state of disease progression.

After six months on ART the HIV-1 viral load and FasL levels were significantly reduced, from 1.3 to 3.4 log₁₀ and 27 to 231 pg/ml, respectively. The significant decrease in both the FasL and HIV-1 viral load levels could be interpreted as the result of the reduced viral burden due to ART. ART reduces the pressure of immune activation exerted on the cells, thereby reducing the apoptotic action of the Fas/FasL.

We have previously shown study results that demonstrated a positive correlation between HIV-1 viral loads and Fas ligand levels in HIV positive adults and children. This is the first longitudinal study we have done to evaluate FasL and HIV-1 viral loads over time [10-14]. We have shown the potential use of FasL in monitoring HIV disease progression in children, especially in resource limited settings.

We hypothesize that high levels of FasL in HIV-1 positive patients depict a high and clear signal of CD4 T-cell depletion. The results show that a high level of T-cell depletion could indicate a high HIV viral load and visa-versa.

The strength of this study is the fact that it was performed in Yaoundé, Cameroon, a resource limited setting in which alternative and cheaper methods are required to monitor HIV positive patients. The limitation of this study was the non inclusion of children and patients failing ART treatments. This will be done in a follow-up study.

5. Conclusion

The significant correlation between sFasL and HIV-1 viral load at six months of ART suggests that sFasL could be a better and more affordable alternative biomarker of HIV disease progression compared to CD4 absolute and CD%, currently being used in resource limited settings. We have shown in this study that high levels of sFasL depict high HIV-1 viral loads. And advance state of the disease. These biomarker should be investigated further in other settings.

Authors' Contributions

GMI conceived and designed the study, implemented sample collection and laboratory analysis, and wrote the first draft of the manuscript. MCOA, MJ contributed in the design and supervised laboratory analysis. JA participated in the design of the study, performed the statistical analysis and substantially revised the draft of the manuscript. MM, GJ contributed in the design, draft and wrote the manuscript. AE contributed in the design and participated in laboratory analysis. EL participated in laboratory analysis. BB participated in laboratory analysis. All authors read and approved the final manuscript.

Acknowledgements

The CSCCD laboratory, and the Chantal Biya reference hospital where this study and participants were recruited. The study was partially sponsored by a generous grant from the HIV Research Trust (United Kingdom) in 2015.

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