Original Article

Raised Interleukin 6 Levels: A Risk Factor for Cardiovascular Associated Complications in HIV Positive Zambians before Initiation of Antiretroviral Therapy

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ABSTRACT

Objectives: The objectives of the study were to compare plasma levels of IL-6 in HIV positive and HIV negative individuals and to correlate them with CD4 count

Materials and methods: A cross-sectional study was carried out at the University Teaching Hospital in Lusaka, Zambia. IL-6 and CD4 were assessed in HIV positive on ART, HIV positive ART- naïve and HIV negative control participants.

Results and Conclusion: Our study showed that HIV ART naïve participants had higher IL-6 concentrations (2.83 ± 1.60 ng/ml) than those on ART (2.49 ± 1.21 ng/ml) p = 0.020. HIV negative control participants however, had higher concentrations of IL-6 (3.24 ± 1.33 ng/ml) than HIV positive participants on ART (2.49 ± 1.21) p = 0.002. HIV positive ART naïve individuals therefore, had the highest IL-6 levels. The results also showed that ART lowers inflammation in HIV and this may explain why ART reduces the risk of developing opportunistic tumours and other infections in HIV.

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INTRODUCTION

Interleukin 6 (IL-6) is an interleukin that acts as both a pro-inflammatory cytokine and an anti-inflammatory cytokine. In humans, it is encoded by the *IL6*gene[1] and it is secreted by T cells and macrophages to stimulate immune response, e.g. during infection and after trauma, especially burns or other tissue damage leading to inflammation. It also plays a role in fighting infection, as it has been shown in mice to be required for resistance against bacterium *Streptococcus pneumoniae.*[2] It is an important mediator of fever and of the acute phase response. The release of pro-inflammatory cytokines which include IL-6 is caused by IL-2,[3, 4] these cytokines are known to increase acute phase reactants such as C-reactive protein (CRP) and activate pro-thrombotic pathways.[5, 6] With regard to the relationship of this cytokine to HIV, in vitro infection of normal monocyte/macrophages with HIV-1 has been found to induce gene expression and secretion of IL-6.[7] In addition, increased levels of IL6 have been recently reported both in the serum[8] and in the cerebrospinal fluid[9] of HIV-infected patients. It has been demonstrate that IL-6 directly stimulates HIV replication in primary human macrophages acutely infected in vitro.[10] IL-6 also induces HIV expression in chronically infected

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promonocytic cells and synergizes with TNF-α in this effect[10]. Furthermore, at the molecular level, it is evident that IL-6 induction of HIV expression involves multiple and diverse mechanisms.[10] Proinflammatory cytokines are important mediators of activation of coagulation. Infusion of tumour necrosis factor (TNF)-α into healthy humans induces not only signs of a systemic inflammatory response, but also activation of coagulation as indicated by an increase in plasma concentrations of the prothrombin fragment F1+2.[11] However, blocking TNF-α with monoclonal antibodies does not neutralise coagulation activation during endotoxaemia in chimpanzees.[12] Rather, blocking interleukin (IL)-6 attenuated activation of coagulation in the same model of endotoxaemia, both systemically and locally in the bronchoalveolar compartment.[12] This suggests that IL-6 is the most important mediator in inflammation-induced coagulation. Hence, the proinflammatory cytokines IL-6 and TNF-α establish a procoagulant shift in the haemostatic balance, promoting fibrin generation in severe inflammatory states, both systemically and locally. The purpose of the study was to determine levels of IL-6 in HIV positive individuals. The study will contribute to the body of knowledge so that if our population has high levels, measures to monitor them should be implemented so that those with high levels of IL-6 can be treated to reduce mortality

METHODS

Selection of participants and specimen collection- Blood was collected with informed consent from HIV positive individuals reporting for ART management at the Adult Infectious Diseases Centre (AIDC) at the University Teaching Hospital (UTH), Lusaka, Zambia. HIV negative participants were recruited from the Voluntary Counselling and Testing (VCT) centre at skin clinic after testing negative for HIV and consenting to participate in the study. Blood samples were collected from research participants via venepuncture using the Evacuated Tube System (ETS) following the Clinical Laboratory Standards Institute (CLSI) order of draw. Ethical clearance was granted by ERES CONVERGE I.R.B No. 00005948, F.W.A No. 00011697.

Specimen Preparation and Storage – In the laboratory, blood specimens in the plain tubes were centrifuged at 1500 rpm for 15 minutes to separate serum from the blood cellular components. Only serum was meticulously collected from the vacutainers using pasture pipettes and transferred to 2ml cryovials with sealable screw caps, which were stored in a freezer at -80°C until the specimens were required for analysis.

IL-6 Estimation - Serum IL-6 concentration was assessed using the Abcam IL-6 High SensitivityHuman ELISA Kit (Abcam plc, United Kingdom); a quantitative immunoassay for measurement of Human IL-6 in supernatants, buffered solutions, serum, plasma and other body fluids. This assay employed an antibody specific for Human IL-6 coated on a 96-well plate. ELISA plates were read using the VersaMaxPLUS Rom v1.23 ELISA plate reader (manufactured by Molecular Devices LLC, United States of America).

CD4 Estimation – CD4 counts were obtained from the Laboratory Information Systems at the University Teaching Hospital (UTH) Department of Pathology and Microbiology.

Data Processing - Data were expressed as mean ± SEM for normally distributed continuous variables. Normality was assessed using the Shapiro and Wilk statistic and the normality plots. Skewed variables were log – transformed prior to analysis. The one way Anova and Tukey post hoc test was used to compare mean values of Serum IL-6, concentrations between the three groups (HIV+ ART naïve, HIV+ on ART and HIV- control). The data was cleaned and thereafter showed no violation of normality as assessed by use of the Shapiro and Wilk statistic.

RESULTS

This study had 3 groups of participants; 50 HIV+ ART naïve, 50 HIV+ on ART and 50 HIV- control participants.

The study showed that IL-6 concentration was significantly higher in ART naive participants (2.83 ± 1.60 ng/ml) compared to HIV positive participants on treatment (2.49 ± 1.21 ng/ml, p = 0.020).There was no significant difference between this group and HIV negative control group (3.24 ± 1.33 ng/ml, p = 0.721). However, the HIV negative control group had a significantly higher IL-6 concentration (3.24 ±1.33 ng/ml) than the HIV positive treatment group (2.49 ± 1.21 ng/ml, p = 0.002).

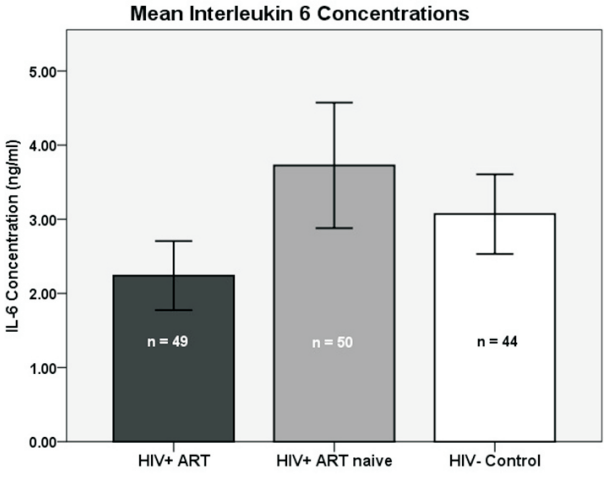

Fig 1: Mean IL-6 concentration for HIV+ ART naïve (3.24 ± 1.33 ng/ml) was higher than for the HIV+ on ART (2.49 ± 1.21 ng/ml) and HIV- control participants (2.83 ± 1.60 ng/ml).

Table 1: Multiple Comparisons for IL-6 Between groups

Variable	HIV+ ART	HIV+ ART Naive	HIV- Control	P-Value
IL-6	2.49 ± 1.21	3.24 ± 1.33		0.020
	2.49 ± 1.21		2.83 ± 1.60	0.002
		3.24 ± 1.33	2.83 ± 1.60	0.721

Multiple comparisons of p-values for IL-6 Concentrations between HIV+ on ART, HIV+ ART naïve and HIV- control groups significant at the 0.05 level. IL-6 in HIV+ ART naïve was significantly higher than in HIV+ on ART. IL-6 in HIV- control was significantly higher than in HIV+ on ART.

The results also showed that the CD4 count between HIV positive participants on treatment (339.57 ± 89.45 cells/µl) and HIV positive treatment naïve participants (311.83 ± 95.35 cells/µl) was not significantly different (p = 0.501).

TABLE 2: Linear correlation between IL-6 and Cd4

Independent Variable	Dependent Variable	R	R^2	P-Value
IL-6 HIV+ on ART	CD4 count HIV+ on ART	0.019	0.000	0.904
IL-6 HIV+ ART naive	CD4 count HIV+ ART naïve	0.64	0.004	0.744

There was no correlation between high IL-6 and CD4 count in the HIV+ ART naïve and the HIV+ on ART

The study also showed that there was no correlation between IL-6 and CD4 in the HIV positive ART naive and those on ART with r^2 = 0.004 and 0.000 respectively.

DISCUSSION

IL-6 is an interleukin that acts as a pro-inflammatory cytokine and is therefore used as an inflammatory biomarker. The results therefore, indicated that HIV positive ART naive individuals underwent a significant inflammatory process than their counterparts on ART. As revealed by most researchers, this cytokine when elevated has negative implications especially on the health of HIV positive individuals. Persistent systemic inflammation as denoted by elevated levels of IL-6 has been associated with an increased risk of serious non-AIDS defining illnesses such as cardiovascular disease (CVD) in HIV-positive adults despite effective treatment with antiretroviral therapy.[13, 14] IL-6 is a major mediator of the acute-phase response that is expressed by antigen-presenting cells and non-hematopoietic cells. It is an important growth factor for B cells while also promoting CD4 T cell proliferation and survival.[15] During the acute stage

of an infection, relatively high levels of IL-6 are produced and this, together with other cytokines, help to activate T cells, increase the number of antibody producing B cells and stimulate the release of hormones. Such a response during an acute infection is useful. However, prolonged production of relatively high levels of IL-6 may weaken the immune system over the long- term. This weakness can occur because higher than normal levels of IL-6 could cause the premature death of immune cells, increase the susceptibility of the liver to injury and raise the risk of cardiovascular disease. IL-6 production has been shown to be an important component of autocrine[16] and paracrine[17] circuits that fuel the growth of solid tumours. A review of data from 5023 HIV positive individuals statistically found that participants with elevated IL-6 levels in their blood had the strongest risk of developing cancer.[18]

Results from this study showed that IL-6 levels in HIV positive ART naive individuals were significantly higher than in the HIV positive individuals on ART. Our findings agreed with those of Klein et al[19] who found that IL-6 dropped by 32% over the first 24 weeks on ART $p<0.0001$. The low IL-6 levels in the ART group were because the initiation of ART improved the immune system which caused a reduction in the inflammatory processes caused by many organisms such as bacteria. This finding also agreed with a study that was conducted by Hamlyn et al which found that at week 12 after commencement of ART, IL-6 levels were significantly lower compared to pre-ART levels in the same group of individuals[20] thereby leading to a reduced risk of associated opportunistic infections and tumour development. The study also revealed that there's no relationship between interleukin 6 and CD4 count in both the HIV positive ART naïve and the HIV positive on ART implying that regardless of the CD4 count, an individual can still have high levels of IL-6 and be prone to the effects of high levels of this biomarker.

The finding that HIV negative controls had a significantly higher mean IL-6 concentration than the HIV positive participants on ART could have been due to factors relating to participant recruitment. All HIV negative control participants were recruited from the skin clinic at UTH. The skin clinic at UTH provides medical services for the management of various skin conditions and is also a walk-in HIV testing site. In this study, we did not distinguish between those that were attending the skin clinic just for an HIV test and that were attending the clinic due to a skin condition. Patients attending the skin clinic are encouraged also to take an HIV test. Studies have actually proved that IL-6 is essential in the skin wound healing process as demonstrated by delayed wound healing in IL-6 deficient mice.[21] These participants were also not screened for many other ailments that could also activate the inflammatory process compared to HIV positive on ART who report to the clinic for periodic reviews and receive treatment not only for HIV but also for many other ailments.

The results showed that ART lowers inflammation in HIV and this explains why ART reduces the risk of developing opportunistic tumours and why ART naïve individuals are more at risk of cancers and cardiovascular associated disease which contribute to a high mortality rate in these individuals. These results further support the need for early initiation of HIV therapy for HIV positive individuals.

The limitation of the study is that it did not have a follow up programme to assess the implications of high levels of IL-6 concentrations in HIV positive individuals. A follow up programme could have provided morbidity and mortality data for our participants. Follow up studies may be warranted to determine whether for our population pre ART levels of IL-6 are really important as far as mortality and prognosis is concerned in HIV positive individuals.

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COMPETING INTERESTS

No conflicts of interest declared.

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