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Pro-and Anti-Inflammatory Cytokines in Persistent Low-Level Viremia HIV-1 Patients on Combination Antiretroviral Therapy in Western Kenya

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Abstract

Background: Persistent low-level viremia leads to activation of the immune system in HIV-1 patients on combination antiretroviral therapy (cART) and is a consequence of virologic rebound. The prominence of cytokine storm in HIV-1 patients could serve as a pointer to persistent low-level viremia in previously cART suppressed patients. Very little has been done to relate the cytokines IL-17, IFN-γ, IL-10 and TGF-β with low-level viremia in HIV-1 patients on cART in Kenya. Compared to viral load testing, cytokine assays are relatively cheaper and may be an option to predicting of possible virologic failure in HIV-1 patients on cART. The aim of this study was to determine pro-inflammatory (IL-17, IFN-γ) and anti-inflammatory (IL-10, TGF-β) cytokines in peripheral blood of HIV-1 patients with and without low-level viremia, as potential predictors of viral rebound in HIV-1 patients on cART in Western Kenya.

Objective: To determine the levels and ratio of pro- and anti-inflammatory cytokines in HIV-1 patients on first line cART with and without lw-level viremia in Western Kenya.

Methods: This was a cross-sectional comparative study of 82 HIV-1patients comprising of 41 HIV-1patients on first line cART with persistent low-level viremia as the test group (persistent viral load of 50–1000 copies/ml) and 41 HIV-1 patients on first line cART without low-level viremia (consistently suppressed viremia (<50 copies/ml) from start of therapy), as the the comparison group. Clinical and demographic information was obtained from the patients files. A blood sample was collected from each participant and levels of IL-17, IL-10, TGF-β, and IFN-γ were determined by ELISA (Zeptometrix, Buffalo, NY, USA) 6 h. while viral load levels was determined by polymerase chain reaction (PCR)- COBAS TaqMan real-time HIV-1 RNA assay, version 1.0 (Roche Diagnostics, Indianapolis, Indiana) in HIV-1patients on first line with and without low-level viremia. Data analysis was done by STATA version 17. Median (IQR) was used to summarize the data. Mann-Whitney U test was used to compare medians between the two groups. P-value ≤ 0.05 was considered significant.

Results: The age of participants in the study ranged from 18 to 57 years. The majority of the participants (30 patients) were between the ages of 38-47. The least represented age group was 18-27, with 12 patients. Matching of age and gender was done for HIV-1 patients on first line cART with and without persistent low-level viremia. The median levels of IL-17, IFN-γ, IL-10 and TGF-β (pg/ml) in patients on first line cART with and without persistent low-level viremia were 23.8 vs 15, 5 (P-value <0.001) 28.3 vs 11.4, 45.2vs 28.4 (P-value <0.001) and 56.9 vs 27.7 (P-value <0.001) respectively. The median (IQR) viral load in HIV-1 patients with persistent low-level viremia was 407.5 (IQR) copies/ml.

Conclusion and recommendation: The high levels of IL-17, IL-10, TGF-β, and IFN-γ which were found in HIV-1 patients with persistent low-level viremia signify HIV-1 viral rebound,

Recommendation: Close monitoring of HIV-1 patients without low level viremia should include pro-inflammatory and anti-inflammatory cytokine analysis as potential predictors of persistent low-level viremia.

Keywords: HIV-1 patients; Antiretroviral therapy

Introduction

Substantial human mortality and morbidity from human immunodeficiency virus-1 (HIV-1) infection poses a significant health burden worldwide even as the medical science strives to make considerable strides towards better management practices in the past two decades. According to the 2023 HIV/AIDS Joint United Nations Program (UNAIDS) fact sheet epidemiological estimates, as at 2022, 39.0 million people were living with HIV-1 world-wide, the Eastern and Sountern Africa (ESA) region being highly affected with 20.8 million of the HIV-1 positive population. The report further indicated that the highest incidence of AIDS-related mortality estimated at 260,000 (200,000-370,000) occurred within the ESA region. Kenya being part of the ESA region was reported to be having 1.4 million people living with HIV-1, while deaths due to AIDS in Kenya was 18,000 people. Fortunately, it has been noted from the same report that, combination antiretroviral therapy (cART) introduction and coverage across the region has improved greatly, with 94% of the patients in Kenya being on treatment out of which, only 1.2 million of the people had suppressed viral loads. Viral load suppression is a critical component of HIV-1 treatment and prevention efforts, and it plays a significant role in achieving the goal of ending the HIV/AIDS epidemic by 2023, as outlined in Sustainable Development Goal 3.3. The primary goal of cART is to achieve and maintain undetectable viral loads, as this is associated with improved clinical outcomes such as immune reconstitution, sustained virus suppression, a significantly reduced risk of HIV-1 transmission, and reduction in HIV/AIDS morbidity and mortality [1]. Despite the great improvement in HIV-1 care management, low-level viremia still occurs in HIV-1 patients on cART, as some patients on cART exhibit virological rebound of 50- 500 copies/ml, partly due to the existence of persistent virus in reservoirs [2-4]. Low-level Viremia in HIV-1 patients on cART as a result of viral rebound has been associated with virological failure characterized by viral loads >1000 copies/ml after previous attainment of viral loads of <1000 copies/ml [5]. This has a consequence of morbidity and mortality in HIV-1 patients [6]. Persistent low-level viremia and virological failure has been associated with HIV-1disease progression, and therefore patients with persistent low-level viremia may require individualized management which can include drug regimen adjustments, adherence counselling, and close monitoring to determine whether there is an increasing trend in viral load or evidence of treatment failure [7,8].

It's essential to have effective methods for monitoring low-level viremia in HIV-1 patients, especially in regions like Africa and Kenya, where the HIV-1 epidemic is a significant public health concern. Monitoring viral load is crucial to assess the effectiveness of cART, to prevent the spread of the virus, and to make informed clinical decisions. Access to and availability of technologies or tests for specific monitoring of low-level viremia in Africa, including Kenya, may be limited [9]. This can present challenges in accurately tracking treatment responses and potential viral rebound. In some regions, there may be a primary focus on achieving and maintaining viral suppression (an undetectable viral load) as a key measure of treatment success [10]. This is crucial for the health of individuals living with HIV-1 and for preventing transmission. There may be a need for more research and focus on understanding and addressing viral rebound. In the context of healthcare in Africa and Kenya, it's important to address the challenges associated with HIV-1 management, including the availability of monitoring tools, research efforts, and treatment strategies. HIV-1 care and treatment programs in these regions can benefit from a multi-faceted approach that addresses viral non-suppression, viral rebound, and the specific needs of the local population. Frequent viral load testing is indeed essential for monitoring the effectiveness of antiretroviral therapy and for early detection of virological rebound, which is crucial for preventing treatment failure and optimizing HIV-1 care [11]. Frequent viral load testing can be expensive as this cost may pose a barrier to regular testing for some individuals living with HIV-1 [12]. Routine viral load testing may also face challenges in public hospitals, especially in low-income settings. Public healthcare systems in resource-constrained areas may struggle to provide comprehensive testing services due to limited resources. In the Western region of Kenya, access to viral load testing is limited with only a few laboratories offering these services [13]. This situation can be particularly challenging for individuals living in geographically remote areas. Efforts to reduce the cost of viral load testing, can help make this crucial monitoring tool more accessible. Public hospitals in low-income settings may require additional support, both in terms of resources and infrastructure, to ensure routine testing is more widely available. The challenges and limitations regarding frequent viral load testing in the context of HIV-1 patients on cART do indeed necessitate the development and exploration of alternative testing methods for predicting and monitoring virological rebound. Monitoring specific biomarkers related to inflammation and immune activation, such as cytokine profiles, may offer insights into the patient's immune response and potential for virological rebound. Incorporating this alternative approach into HIV-1 care can enhance the ability to predict and monitor virological rebound and treatment failure, especially in regions where frequent viral load testing is challenging.

Extensive studies reveal the essential role of the host immune system in attempts to control HIV-1 replication through interaction of pattern recognition receptors (PRRs) and pathogen associated molecular patterns (PAMPs) [14,15]. On sensing of virus invasion by pattern recognition receptors (PRRs), the innate immune system produces pro-inflammatory and anti-inflammatory cytokines as early phase antiviral host response. These Pro-inflammatory and anti-inflammatory cytokines could serve as potential pointers to persistent low-level viremia occurrence and could provide insights into immune response to HIV-1 and how it might be impacted in HIV-1 patients on cART with and without low-level viremia. Additionally, just as in other infections where a balance in the pro-inflammatory and anti-inflammatory state is of essence in clearing the infections, IL-17/ IL-10 ratio in HIV-1 patients on cART critically allows for safe clearing of infection while preventing tissue injury resulting from the pro-inflammatory state [16]. This study investigated the levels and ratios of pro-inflammatory cytokines (IL-17 and IFN-γ), and anti-inflammatory cytokines (IL-10 and TGF-β) in HIV-1 patients on first line with and without low-level viremia. The study aimed to determine the relationship of these cytokines with HIV-1 rebound. The focus on viral load in HIV-1 patients on cART is crucial for understanding the changes in these patients and the implications for HIV-1 disease progression. The study also focused on comparing the pro-inflammatory and anti-inflammatory cytokine responses in HIV-1 patients on cART, both those with and without low-level viremia. The aim of this research was to investigate how these cytokines interact and differ in the context of HIV-1infection, to gain a better understanding of the immune responses in HIV-1 patients on cART, identify potential markers or patterns in cytokine responses that could serve as predictors for the occurrence of low-level viremia in patients on cART, provide information about potential monitoring and therapeutic interventions, and to contribute to the better management and treatment of HIV-1 patients on cART. Finally, the IL-17/IL-10 ratio was of interest because it represents the balance between pro-inflammatory (IL-17) and anti-inflammatory (IL-10) cytokines in the immune system. An imbalance in this ratio can have implications for immune function, inflammation, and disease progression.

Materials and Methods

Study site

The study was carried out at AMPATH Module 1clinic at Moi Teaching and Referral Hospital, Eldoret, Kenya.

Study design

This was a cross sectional comparative study in which 41 HIV-1patients on first line cART with persistent low-level viremia and 41 cART adherent and HIV-1 patients with viral suppression were enrolled.

Study population

HIV-1 patients on first line cART attending AMPATH Module 1 clinic, Moi Teaching and Referral Hospital -Eldoret, Kenya over the study period from 1st January 2019 to 31st December, 2022.The test group comprised of HIV-1 Patients on first line with low-level and with at least two consecutive episodes of persistent low-level viremia. The comparison group included HIV-1 patients who were without persistent low-level viremia.

Sampling technique

Consecutive sampling was done. Patients who were HIV-1 positive, on cART, and had experienced persistent low-level viremia during the study period were identified. The review was done retrospectively, meaning it looked back in time from the present (the date of testing) to an earlier point when viral load suppression had been achieved. The primary source of information for this retrospective review was the patients' medical records.

Data collection

Patient's records were used in data collection. After patient consenting and recruitment process, phlebotomy for VL, and cytokines IL-17, IFN-γ, IL-10 and TGF-β analysis was done for the study patients. 4 ml blood samples from each participant was collected into EDTA tubes. Separation of plasma was done within 2 hrs of sample collection at 3000 rpm for 3 minutes and kept at -800 till the day of analysis. Cytokines analyses was done on the stored plasma samples by ELISA (Zeptometrix, Buffalo, NY, USA) 6 h, while viral load was analyzed by COBAS TaqMan real-time HIV-1 RNA assay, version 1.0 (Roche Diagnostics, Indianapolis, Indiana). The levels of IL-17, IFN-γ, IL-10 and TGF-β was determined in HIV-1 infected patients on cART with experienced two episodes of persistent low-level viremia, and controls comprising of HIV-1 patients on cART without low-level viremia.

Laboratory Procedures

The level of IL-17, IFN-γ, IL-10 and transforming growth factor beta, HIV-1 patients on first line cART with and without persistent low-level viremia was analysed by ELISA (Zeptometrix, Buffalo, NY, USA) 6 h. Blood samples were taken from patients with and without persistent low-level viremia.

Determination of IL-17, IFN-γ, IL-10 and Transforming Growth Factor Beta

Genway's ELISA (Zeptometrix, Buffalo, NY, USA) 6 h was used. The avidin-biotin enzyme complex (ABC) working solution and Tetramethylbenzidine (TMB) colour developing agent was be kept warm at 370C for 30minutes before use. 0.1 ml of samples and standards were be added to each well and incubated at 370C for 90minutes. Bionylated antibodies will be added and incubation done at 370C for 60minutes. The plate was washed 3 times with 0.01M TBS. The ABC working solution was added and the plate incubated at 370C for 30minutes. It was then washed 5 times with 0.01M TBS. MB colour developing agent was added and incubation of the plate was done at the optical density (OD) absorbance at 450nm in a micro plate reader within 30minutes after adding the stop solution. In addition to use of the Beer-Lambert's formula for calculation of the concentration of cytokines in pg/ml, a standard curve generated was used for OD v/s pg/ml.

Determination of viral load

HIV-1 RNA quantitation was done by COBAS TaqMan real-time HIV-1 RNA assay, version 1.0 (Roche Diagnostics, Indianapolis, Indiana). The ultrasensitive procedure was performed according to the manufacturer's instructions, and has a detection range of 20-750,000 HIV-1 copies/ml. During the specimen preparation procedure, the HIV-1 viral particles in plasma were concentrated by high speed centrifugation, followed by lysis of the virus with Tris-HCL buffer, 3% Dithiothreitol, less than 1 % glycogen, and Guanidine thiocyanate. Precipitation of the HIV-1 RNA with alcohol was done. Quantitation standard RNA molecules was mixed with a lysis reagent. The quantitation standard was carried through sample preparation, reverse transcriptase, amplification and detection steps in each sample.

Data management and analysis

Filled data forms were checked for completeness, forms that had missing information or gaps were taken back to the point of filling for verification and re-filling. Data was entered in an excel Microsoft® Excel® 2019 MSO (16.0.11328.20156)32-bit spreadsheet, and later exported to STATA version 17 for analysis. Normality test was performed using the Shapiro Wilk test. The measure of central tendency median (IQR) was used to summarize the data. Mann-Whitney U-test was used to compare median cytokines between the HIV-1 patients on first line with and without persistent low-level viremia. P< 0.05 was considered statistically significant. Data were presented in form of tables and box plots. Median viral loads were determined to establish the levels in relation to levels viremia status in HIV-1 patients with and without low-level viremia patients.

Ethical considerations

The study protocol was approved by the Institutional Research and ethics Committee (IREC) of Moi University/Moi Teaching and Referral Hospital (MTRH).

Results

A total of 82 participants (41 HIV-1 patients on first line cART with low-level viremia and 41 HIV-1 patients on first line cART without low-level viremia age and gender matched patients attending AMPATH Module-1 clinic, Moi Teaching and Referral Hospital -Eldoret, Kenya were enrolled. The age group range was between 18-57 years with the majority of the population being between ages of 38-47 with a total of 30 patients. The least age group was 18-27 years with a total of 12 patients. All patients sampled had been on first line cART with and without persistent low-level viremia of at least two consecutive episodes in one year proceeding recruitment. The level of IL-17, IFN-γ, IL-10, and TGF-β in HIV-1 patients on first line with and without persistent low-level viremia were as indicated in (Figures 1,2).

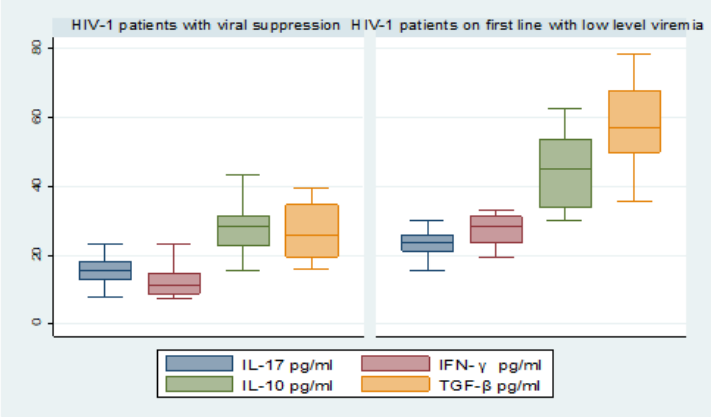

Figure 1: *Median Cytokines pg/ml in HIV-1 patients on first line with and without low-level viremia.*

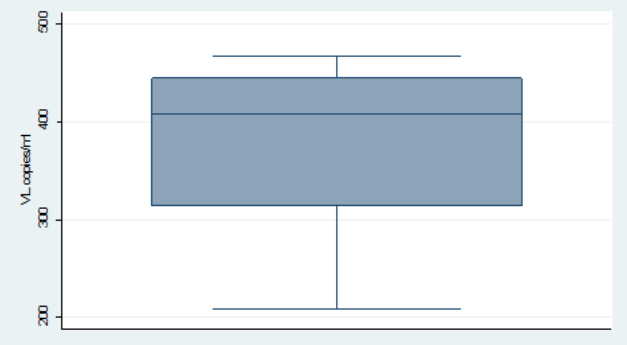

Figure 2: *Viral load in HIV-1 patients with persistent low-level viremia.*

The main findings of this study indicated higher levels of IL-17, IFN-γ, IL-10 and TGF-β in HIV-1 patients on first line with persistent low-level viremia. Among the HIV-1 patients on first line with low-level viremia, the median (IQR) IL-17, IFN-γ, IL-

10 and TGF-β were 23.8(21.325.8), 28.3(23.531.2), 45.2(34.0, 53.7) and 56.9(50.0 67.8) pg/ml respectively. Among the HIV-1 patients without low-level viremia, the median (IQR) IL-17, IFN-γ, IL-10 and TGF-β were 15.5 (12.7, 18.2), 11.4(8.8, 14.8), 28.4(22.6, 31.6) and 25.7(19.5, 34.7) respectively. The median (IQR) viral load in HIV-1 patients with persistent low-level viremia was 407.5 (314.5, 445.0). The level of IL-17, IFN-γ, IL-10 and TGF-β in HIV-1 patients on first line cART with persistent low-level viremia compared to HIV-1 patients without persistent low-level viremia was as indicated in (Tables 1,2).

Table 1: *Demographic data of the study population.*

Age range (years)	Males Freq. (%)			Females Freq.(%)			Total Freq. (%)	P-value
	/HIV-1 low-level viremia Patients	HIV-1 Suppressed patients	**Total**	HIV-1 low-level viremia Patients	HIV-1 Suppressed patients	**Total**		
18-27	3(3.63)	3(3.63)	6(8.37)	3(3.63)	3(3.63)	6 (8.37)	12(16.74)	0.001
28-37	7(8.83)	6(8.37)	13(10.40)	7(8.83)	6(8.37)	13(10.40)	25(20.8)	0.001
38-47	7(8.83)	8(8.37)	15(16.00)	7(8.83)	8(8.37)	15(16.00)	30(32.00)	0.001
48-57	4(4.71)	3(3.63)	7(8.83)	4(4.71)	3(3.63)	7(8.83)	15(17.66)	0.001
TOTAL	21(26)	20(24)	41(50)	21(26)	20(24)	41 (50)	82(100)	

Table 2: *Cytokines (pg/ml) in HIV-1 patients with and without persistent low-Level viremia.*

Cytokine	HIV-1 patients with viral suppression	HIV-1 patients on first line with persistent low-level viremia	Z-value	p-value
IL-17	15.5 (12.7, 18.2)	23.8(21.3, 25.8)	7.462	<0.001
IFN-γ	11.4(8.8, 14.8)	28.3(23.5, 31.2)	8.277	<0.001
IL-10	28.4(22.6, 3.6)	45.2(34.0, 53.7)	7.354	<0.001
TGF-β	25.7(19.5, 34.7)	56.9(50.0, 67.8)	8.296	<0.001

Table 3: *IL-17/ IL-10 ratio in HIV-1 patients on first line with and without Persistent low-level viremia.*

Cytokine	HIV-1 patients without persistent low-level viremia	HIV-1 patients with persistent low-level viremia	Z-value	p-value
Median IL-17/ IL-10 ratio (IQR)	0.549 (0.450, 0.590)	0.552(0.442, 0.677)	0.784	0.433

Generally, HIV-1 patients without persistent low-level viremia had lower levels of IL-17, IFN-γ, IL-10 and TGF-β compared to HIV-1 patients on first line with persistent low-level viremia. There was a significant difference in the median IL-17, IFN-γ, IL-10 and TGF-β between HIV-1 patients on first line with persistent low-level viremia and HIV-1 patients without persistent low level viremia (p<0.001). That is 23.8(21.3, 25.8) vs 15.5 (12.7, 18.2), 28.3(23.8, 31.2) vs 11.4(8.8, 14.8), 45.2(34.0, 53.7) vs 28.4(22.6, 31.6) and 56.9(50.0, 67.8) vs 25.7(19.5, 34.7) for IL-17, IFN-γ, IL10 and TGF-β respectively. HIV-1 patients on first line with persistent low-level viremia had significantly higher levels of IL-17, IFN-γ, IL-10 and TGF-β compared to HIV-1 patients with viral suppression. Present this only once not twice! The IL-17/ IL-10 ratio in HIV-1 patients on first line with and without low-level viremia. HIV-1 patients without persistent low-level viremia had slightly higher IL-17/ IL-10 ratio compared to HIV-1 patients on first line with persistent low-level viremia, however, the difference was not statistically significant 0.549 (0.450, 0.590) vs 0.552(0.442, 0.677), Z=0.784, p=0.433) as indicated in (Table 3).

Discussion

To determine the pro-inflammatory and anti-inflammatory cytokine response in HIV-1 patients on cART, a total of 82 patients on first line with and without persiostent low-level viremia were analyzed. The results indicated higher levels of IL-17, IFN-γ, IL-10 and TGF-β with a median of 23.8, 28.3, 45.2 and 56.9 pg/ml respectively, in HIV-1 patients with persistent low-level viremia. The higher levels of these cytokines in HIV-1 patients with persistent low-level viremia may indicate a complex immune response involving both pro-inflammatory and anti-inflammatory components. The immune response may be influenced by the ongoing presence of the virus, the need to control viral replication, and the body's efforts to balance the inflammatory and regulatory aspects of the immune system [17].

The increased levels of cytokines, including IL-17 and IFN-γ (pro-inflammatory) and IL-10 and TGF-β (anti-inflammatory), in HIV-1 patients on first line cART with persistent low level viremia may have distinct roles in the immune response. Elevated levels of pro-inflammatory cytokines could be seen as an attempt by the immune system to strengthen inflammation. In the context of HIV-1 infection, this pro-inflammatory response may be necessary for the functioning of innate immunity, which plays a crucial role in the early defense against pathogens including viruses [18]. On the other hand, the presence of anti-inflammatory cytokines could be interpreted as an effort to counterbalance the pro-inflammatory response. These anti-inflammatory cytokines may help regulate and slow down the activities of the pro-inflammatory cytokines. This balance may be important to prevent excessive inflammation and tissue damage. The interpretation suggests a dynamic interplay between pro-inflammatory and anti-inflammatory cytokines in response to persistent low-level viremia in HIV-1 patients. Such a balance is essential for the proper functioning of the immune system, as an overly aggressive immune response can be harmful, while an insufficient response may allow the virus to replicate-unchecked. Elevated levels of IL-17 and IFN-γ may indicate immune activation and inflammation, while increased levels of IL-10 and TGF-β suggest regulatory mechanisms to counterbalance the inflammation. The levels of these cytokines could potentially reflect the extent of HIV-1 proliferation and disease progression. Higher levels of pro-inflammatory cytokines might be indicative of a more active viral replication and immune response, while elevated anti-inflammatory cytokines could represent an attempt to mitigate excessive inflammation [19]. Higher levels of IL-17 were found in HIV-1patients on first line with persistent low-level viremia (median 23.8pg/ml; $p< 0.001$) compared to HIV-1patients with viral suppression (median 15.5pg/ul; $p<0.01$).

IL-17, also known as interleukin-17, is a pro-inflammatory cytokine associated with the activation of immune responses, and plays a key role in the body's defense against infections to recruit other immune cells to sites of infection or inflammation, and to promote inflammation, which is a crucial part of the immune response [20]. The findings of high levels of IL-17 in this study suggest that there may have been a pro-inflammatory state in HIV-1 patients with persistent low-level viremia. This pro-inflammatory state could be attributed to increased immune activation in response to HIV-1 infection typically implying that the immune system is actively trying to combat the virus, even in patients with low-level viremia (low levels of viral replication). The presence of low-level viremia in HIV-1 patients indicates that the virus is not fully suppressed by antiretroviral therapy (ART). The elevated IL-17 levels may be a reflection of the body's continued efforts to control the virus, even when it's not replicating at high levels. This could suggest that the virus may be escaping immune surveillance to some extent. Similar to findings of this study, Noted higher levels of IL-17 in HIV-1 patients on cART [21]. In the study Zhang et al., it was observed that higher IL-17 levels were found in HIV-1 patients with low CD4 cell counts (less than 200cells/ml). CD4 cell count is an important prognostic marker in HIV-1 infection and can help predict the risk of disease progression and complications [22]. In the context of HIV-1 infection, there is typically an inverse correlation between CD4 cell count and HIV-1 viral load. This means that as the HIV-1 viral load increases (indicating more active viral replication), the CD4 cell count tends to decrease, and vise versa. As viral load decreases due to successful treatment, the CD4 cell count often stabilizes or increases, reflecting a healthier immune system [23]. The comparison of IL-17 levels in HIV-1 patients with different CD4 cell counts reported by Zhang. Revealed that high IL-17 levels were associated with the end stage of acquired immunodeficiency syndrome (AIDS). The findings of this study reporting high levels of interleukin-17 (IL-17) in HIV-1 patients with persistent low-level viremia suggest that IL-17 levels could have clinical significance in the context of HIV-1 transmission and disease progression.

Findings from the multicentre study conducted by J. M. Rocco and colleagues in 2020 reinforces the earlier findings by suggesting a correlation between high levels of IL-17 and viral load, CD4 cell count and IL-17 in HIV-1 patients. The statistical significance of these findings was indicated by the p-values ($P=0.02$; $p<0.0001$). A viral load greater than 1000 copies/ml suggests an elevated level of viral replication in the bloodstream. When HIV-1 patients with both a low CD4 count and a high viral load exhibit high levels of IL-17, it underscores the link between immune activation and the presence of the virus. The observation of high IL-17 levels in these patients further supports the idea that IL-17 may be associated with an active and robust immune response in individuals with advanced HIV-1 infection. This could be a reflection of the immune system's attempt to control the virus. The implication is that IL-17 levels might serve as an immunological marker for disease progression in HIV-1 patients as the observations in the current study appear to align with the findings from Rocco's study, since both studies found higher levels of IL-17 in HIV-1 patients, the current study having the median IL-17 level of 15.5pg/ml with a p-value of <0.01. In summary, both studies suggest a correlation between high IL-17 levels and disease progression in HIV-1 patients. The findings of the study conducted by Mlambo, which suggest a correlation between high levels of interleukin-17 (IL-17) and high viral load ($p=0.012$) in HIV-1 infection, provide further support for the association between IL-17 and HIV-1 disease characteristics [24]. High levels of IL-17 correlating with high viral load in HIV-1 infection indicate that there may be a connection between IL-17 and the extent of viral replication in the body. High viral load

signifies a greater amount of HIV-1 replication, which typically indicates more active infection and a potentially higher risk of disease transmission to others. The current study's results indicated that IL-17 levels were increased in HIV-1 patients with a median viral load of 407.5 copies/ml. The correlation between IL-17 and high viral load suggests that IL-17 may be produced in response to the presence of the virus. In HIV-1 infection, IL-17 might play a role in the immune response to control or combat the virus. This finding underscores the potential clinical significance of monitoring IL-17 levels in HIV-1 patients. If high IL-17 levels are consistently associated with high viral loads, healthcare providers may consider IL-17 as a potential marker for assessing disease activity and the effectiveness of treatment. Overall, the study by Mlambo and colleagues adds to the growing body of evidence that IL-17 is linked to HIV-1 disease characteristics, particularly in relation to viral load. Understanding this association can aid in monitoring and managing HIV-1 infection more effectively, as well as potentially informing strategies for disease control and prevention.

The investigation of pro-inflammatory cytokines, such as interferon-gamma (IFN-γ), in HIV-1 patients receiving combination antiretroviral therapy (cART) is important for understanding the inflammatory processes associated with HIV-1 infection and the impact of treatment. IFN-γ is a key cytokine in the immune response and plays a role in antiviral defense. Higher levels of IFN-γ were found in HIV-1patients on first line with persistent low-level viremia (median 28.3;$p<0.001$) compared to IFN-γ levels in HIV-1patients with viral suppression (median 11.4; $p<0.001$). Elevated levels of IFN-γ can indicate ongoing immune activation or inflammation, which is a characteristic feature of HIV-1 infection. Even when viral replication is suppressed by cART, chronic immune activation may persist, leading to potential long-term health complications. The elevated levels of IFN-γ found in this study could be indicative of ongoing immune activation or inflammation in HIV-1 patients on cART. IFN-γ is a critical component of the immune system's antiviral response as the cytokine helps protect the host by inhibiting viral entry, promoting antiviral activity, and regulating the immune response to ensure an effective defense against viral infections [25]. The high levels of IFN-γ in patients with persistent low-level viremia found in this study may indeed have been part of the immune response aimed at inhibiting HIV-1 entry at various levels, reflecting the immune system’s efforts to control the virus. The presence of replicating virus, as indicated by the high levels of IFN-γ, suggests an ongoing immune response against HIV-1, which aligns with the idea that the immune system is actively trying to control the infection. The finding of higher levels of IFN-γ in HIV-1 patients who were on combination antiretroviral therapy (cART) can be interpreted in the following way: HIV-1 infection leads to chronic immune activation and inflammation in the body. Even though cART is effective in suppressing viral replication and reducing the viral load, it may not completely normalize the immune system. In some cases, individuals on cART may still have residual immune activation, which could result in higher levels of immune response markers like IFN-γ.

When individuals with HIV-1 start cART, their immune system begins to recover as viral replication is controlled in a process known as immune reconstitution. As the immune system becomes more functional, it can mount a more robust immune response against the virus, which could lead to increased levels of cytokines like IFN-γ. Even on cART, there can be low-level viral replication or viral reservoirs that are not completely eliminated. The observation of elevated IFN-γ levels in HIV-1 patients, possibly in response to viral replication or viral reservoirs that are not completely eliminated, aligns with the findings of this study, in which there could have been immune activation as a result of the presence of viral reservoirs. This observation underscores the complexity of the interaction between the immune system and HIV-1, as well as the potential for ongoing immune responses even when viral replication is suppressed. Monitoring such immune responses is important for understanding the long-term effects of HIV-1 infection and the impact of cART on the immune system. The contrasting findings between the current study (which observed high IFN-γ levels in HIV-1 patients on cART with persistent low-level viremia) and the sampled studies (which found reduced levels of IFN-γ in HIV-1 patients without viremia) highlight the complexity of immune responses and the variability that can occur among individuals with HIV-1. Possible explanations for variations may be HIV-1 is a highly heterogeneous virus, and individuals infected with it can have varying immune responses and genetic factors that influence how their immune system responds to the virus and treatment. Differences in the patient populations studied could explain the contrasting results. The timing of the studies and the stage of HIV-1 infection in the patient cohorts can also play a significant role. IFN-γ levels and their impact on HIV-1 infection may change as the disease progresses. The use of cART and the level of viral suppression can vary among individuals, affecting the immune response. Variations in the methods and assays used to measure IFN-γ levels can also contribute to different study results. Different laboratories may use different techniques or assays, and the sensitivity and specificity of these methods can vary. The size of the study population and statistical power can influence the ability to detect differences. Larger sample sizes may provide more robust and generalizable results. HIV-1 strains and subtypes can vary geographically, and the immune responses may differ accordingly. Differences in the study populations, such as geographic location and demographic characteristics, may contribute to varying results.

To elucidate the anti-inflammatory activity of cytokines in HIV-1infection, the levels of IL-10 were sought. Higher levels of IL-10 were found in HIV-1 patients with low-level viremia (median 45.2; $p<0.001$), compared to IL-10 levels in HIV-1 patients without low-level viremia. Interleukin-10 (IL-10) is an anti-inflammatory cytokine that plays a crucial role in regulating the immune response and reducing inflammation. In the context of HIV-1 infection, the measurement of IL-10 levels is an important component of understanding the immune response and the balance between pro-inflammatory and anti-inflammatory factors. The presence of a high level of interleukin-10 (IL-10) in this study suggests a role in regulating the immune response and reducing inflammation. High levels of IL-10 may indicate the body's attempt to regulate and dampen excessive immune response as well as controlling and limiting inflammation, thereby reducing the risk of immune-mediated damage to the host. In their multicentre cross-sectional study, Rocco and colleagues found the HIV-1 patients with low CD4 counts (less than 200cells/mm3) and high viral loads (HIV-1 RNA >100copies/ml) had higher levels of IL-10 (J. M. Rocco et al., 2020). High viral loads are often associated with HIV-1 disease progression, and in some cases, elevated IL-10 levels could be a result of disease progression. High viral loads typically indicate a greater degree of viral replication and activity in the body. As HIV-1 replicates and infects more cells, it can lead to a decline in CD4 T-cell counts and an increased risk of disease progression. High viral loads are a hallmark of uncontrolled HIV-1 infection. In response to the elevated viral load and ongoing viral replication, the immune system may become more activated, leading to inflammation. In an effort to regulate and dampen this excessive immune response, the body may produce higher levels of IL-10. IL-10 acts as a counterbalance to limit the inflammatory response and immune-mediated damage. The presence of high IL-10 levels may represent the immune system's attempt to control the inflammatory response that results from high viral replication. While high viral loads often signify HIV-1 disease progression, the relationship between viral load, IL-10 levels, and disease progression is multifaceted. High IL-10 levels may be a component of the body's response to regulate inflammation and immune activation in the face of high viral replication.

This suggests that the high IL-10 levels found in this study may have been elevated in patients with more advanced HIV-1 disease and higher viral replication. Zhang and colleagues found high levels of IL-10 in HIV-1 patients who were on cART. This is interesting because cART is typically used to suppress viral replication. Similar to findings of this study of high levels of IL-10 in HIV-1 patients on cART with persistent low-level viremia, the elevated IL-10 levels in these patients may indeed indicate the presence of an anti-inflammatory response, possibly as a counterbalance to the pro-inflammatory state created by both the virus itself and the immune response to it. Collectively, these findings suggest that IL-10 might be involved in regulating the balance between pro-inflammatory and anti-inflammatory responses in HIV-1 infection. The additional findings mentioned from studies conducted by Gorenec and Kahle further support the idea that high levels of IL-10 may be a relevant marker in HIV-1 infection with potential implications for disease progression and management. In their study, Gorenec and colleagues found that HIV-1 patients with low-level viremia who were on cART during the chronic stage of HIV-1 infection had elevated levels of IL-10. Kahle and colleagues associated high levels of IL-10 with seroconversion in HIV-1 patients. This association suggests that IL-10 levels may be involved in the transition from acute to chronic infection. Taken together, these findings suggest that IL-10 may serve as a marker for HIV-1 disease progression and could potentially be used to predict persistent low-level viremia. Similar to the high levels of IL-10 found in this study, the study findings reported by Ngangali and colleagues in 2021, which indicated a positive correlation between IL-10 and viral load in HIV-1 patients, align with the broader pattern of elevated IL-10 levels in the context of HIV-1 infection. This positive correlation suggests that IL-10 may indeed be associated with the level of viral replication in these patients. IL-10 is known to be an anti-inflammatory cytokine that can limit the immune response to prevent excessive inflammation. In the case of HIV-1, this suppression of pro-inflammatory responses could be a double-edged sword.

The virus's ability to persist and hide in reservoirs within the body is a major challenge. Elevated IL-10 levels may represent an attempt by the immune system to modulate the inflammatory response to avoid excessive tissue damage and immune activation without necessarily eliminating the virus completely. This may contribute to the persistence of low-level viremia in some patients. The findings reported by Twizerimana, where higher levels of IL-10 were observed in HIV-1 patients prior to the initiation of combination antiretroviral therapy (cART), provide valuable insights into the dynamics of IL-10 in the context of HIV-1 infection. These elevated IL-10 levels could be indicative of an active immune response is reasonable. The elevation of IL-10 levels in HIV-1 patients prior to cART initiation suggests that the immune system is actively responding to the presence of the virus, which triggers the production of IL-10 as a countermeasure to dampen excessive inflammation. IL-10's ability to regulate the immune response is indeed beneficial in certain contexts, such as preventing excessive inflammation and immune-mediated damage. However, in the context of HIV-1 infection, it can also contribute to the chronicity of the infection and the persistence of the virus. The high levels of IL-10 found in this study may be indicative of the immune system's response to chronic HIV-1 infection, where regulation and limiting inflammation are

important. However, this balance may also have implications for the persistence of the virus in the body. Contrary to findings of this study, Pina, demonstrate that IL-10 can be produced in HIV-1 patients who are treatment-naïve and have undetectable viral loads load [26]. This is an interesting and somewhat contradictory observation compared to the previous studies that associated high IL-10 levels with active viral replication. However, these findings may be explained by the complexity of the host-virus interactions in HIV-1 infection. This could be explained; in some HIV-1 patients, the virus may be slowly proliferating but still effectively controlled by the immune response and low-level viremia may not be detectable. In this scenario, IL-10 production could be an adaptive response to counteract proinflammatory effects associated with the presence of the virus. This demonstrates the dynamic nature of the immune response in HIV-1 infection. To further analyze the immunosuppressive activity of cytokines on HIV-1 patients on cART, the levels of TGF-β were sought. This study found higher levels of TGF-β in HIV-1 patients on first line with persistent low-level viremia compared to HIV-1 patients with viral suppression. Transforming growth factor-beta (TGF-β), an immunoregulatory cytokine showed higher levels, with a median of 56.9pg/ml. Similar to findings of this study, Dickinson and colleagues found higher levels of TGF-β in HIV-1 patients across various stages of infection (acute, sub-acute, and chronic) when compared to negative controls [27]. The findings of higher levels of transforming growth factor-beta (TGF-β) in HIV-1 patients across various stages of infection (acute, sub-acute, and chronic) compared to negative controls are interesting and indicative of the complex interplay between the virus and the immune system in the context of HIV-1 infection. In the acute phase, the body may produce TGF-β as part of the initial response to the virus. In the chronic phase, elevated TGF-β levels might represent a regulatory mechanism to counterbalance the immune activation and inflammation associated with long-term infection.

TGF-β also plays a role in tissue repair and fibrosis. In the context of HIV-1, tissue damage and fibrosis can be significant, especially in long-term infection. Elevated TGF-β levels may indicate ongoing tissue repair processes or, in some cases, excessive fibrosis, which can be detrimental. The presence of higher TGF-β levels across different stages of HIV-1 infection suggests that the immune response to the virus is dynamic and multifaceted. TGF-β may be produced to regulate the immune response and limit excessive inflammation, but it can also have implications for immune suppression and immune exhaustion. Musa and colleagues observed elevated TGF-β levels in HIV-1 non-adherent patients but not in cART-adherent patients or healthy controls. This finding suggests that TGF-β levels may be linked to cART adherence and treatment response. It implies that the virus may be more active in non-adherent patients, leading to higher TGF-β levels [28]. These findings, along with the findings from the current study regarding TGF-β levels in HIV-1 patients with low-level viremia, collectively indicate that high TGF-β levels may be associated with HIV-1 progression. TGF-β is known for its role in regulating immune responses and promoting tissue repair. However, in the context of HIV-1 infection, the virus's ability to persist and evade the immune system can lead to ongoing immune activation and inflammation, possibly resulting in the elevation of TGF-β. The implications of these findings may suggest that monitoring TGF-β levels could be useful in assessing HIV-1 disease progression and treatment response. However, further research is needed to establish the specific mechanisms and clinical utility of TGF-β in HIV-1 management. The findings from Osuji, which show elevated TGF-β levels in HIV-1 patients both before the initiation of combination antiretroviral therapy (cART) and even 12 months into treatment, are in line with the notion that TGF-β may play a role in chronic immune stimulation and response to residual HIV-1. Elevated TGF-β levels in HIV-1 patients prior to starting cART may be indicative of ongoing immune activation in response to the virus.

To evaluate the viral load in HIV-1 patients with and without persistent low-level viremia, the viral load was determined. This study found patients with persistent low-level viremia to have viral loads of varying levels. The median (IQR) viral load in HIV-1 patients with persistent low-level viremia was 407.5 (314.5, 445.0) copies per ml. The lowest viral load considered was 209 copies/ml while the highest had 467 copies/ml (both values presenting as outliers). Patients without persistent low-level viremia had undetectable viral loads (below 50 copies/mL). The viral load data obtained suggests that even among these patients, there can be a wide range of viral load levels. This variability might be influenced by factors such as the specific antiretroviral medications used, individual patient characteristics, and potential drug resistance, among other factors. A median viral load of 407.5 copies per ml in found in this study is indeed a notable finding. While it's not possible to definitively conclude virological failure based solely on this information, such a viral load level would raise concerns and warrant further investigation and clinical assessment. In the context of antiretroviral therapy (ART), one of the primary goals is to achieve and maintain an undetectable viral load, typically defined as fewer than 50 copies per ml. To determine the cause of the elevated viral load and whether it indicates virological failure, clinical evaluation is necessary. This may involve assessing treatment adherence, checking for drug resistance, and making adjustments to the treatment regimen if needed. In clinical practice, individuals with HIV-1 are regularly monitored for viral load, CD4 T-cell counts, and other relevant markers. An elevated viral load should prompt healthcare providers to conduct a thorough evaluation, as it may have implications for the individual's health and the effectiveness of their treatment. To gain a deeper understanding of the immune

response and its balance in HIV-1 patients on first line cART with and without low-level viremia, the IL-17/IL-10 ratio was explored. The findings from this study, which indicate a slight difference in the IL-17/IL-10 ratio between the two groups of HIV-1 patients (those without low-level viremia and those with low-level viremia), are important to report. In this case, the slight difference is not statistically significant, as indicated by the Z-score and p-value. As seen earlier, the IL-17/IL-10 ratio is an indicator of the balance between pro-inflammatory (IL-17) and anti-inflammatory (IL-10) responses within the immune system. The study revealed that patients without low-level viremia had a slightly higher IL-17/IL-10 ratio compared to patients with low-level viremia. This indicates that the pro-inflammatory component may be slightly more prominent in the group without low-level viremia. Patients without low level viremia had a slightly higher IL-17/IL-10 ratio 0.556pg/ml (with a range of 0.450 to 1.178), compared to patients with low-level viremia 0.552 (with a range of 0.442 to 0.882), Z- score: 0.264, p-value: 0.792 as indicated in Table 2.

The Z-score and p-value confirm that the observed difference could have occurred by chance and is not a statistically meaningful difference. While the findings do not suggest a significant difference in the IL-17/IL-10 ratio between the two groups, they still contribute to the overall understanding of the immune response in these patients. This information provides insights into the immune balance but does not indicate a clear trend or association between low-level viremia and the IL-17/IL-10 ratio in this particular study. The IL-17/IL-10 ratio is a measure of the balance between pro-inflammatory (IL-17) and anti-inflammatory (IL-10) cytokines in the immune system. An imbalance in this ratio can have implications for the immune response. In the study by Li, it was found that there is an imbalance in the IL-17/IL-10 ratio in HIV-1 patients. This suggests that HIV-1 infection may lead to alterations in the immune system's cytokine profile [29-85]. The study indicates that this imbalance could promote HIV-1 replication. In the context of HIV-1 infection, an imbalance towards pro-inflammatory cytokines (such as IL-17) could potentially enhance viral replication by creating a more permissive environment for the virus. This study results, which found a slight increase in the IL-17/IL-10 ratio in suppressed HIV-1 patients, suggests that there might be ongoing HIV-1 replication or immune activation in these individuals. An elevated IL-17/IL-10 ratio indicates an imbalance in the pro-inflammatory (IL-17) and anti-inflammatory (IL-10) milieu. The immune response to HIV-1 is multifaceted, and these cytokines play a role in modulating that response. Further research is needed to fully elucidate the mechanisms involved and their clinical implications.

Conclusions and Recommendations

Conclusion

The findings of this study highlight key insights into the relationship between cytokines analyzed, low-level viremia, and HIV-1 progression. The study indicates that cytokines such as IL-17, IFN-γ, IL-10, and TGF-β play a significant role in the progression of HIV-1 infection. Varying levels of these cytokines suggest their importance in influencing the course of the disease. The study demonstrates that HIV-1 infection can lead to long-term immune activation marked by elevated cytokine levels in some patients. This chronic immune activation can result in a loss of immune function in T cells, making it difficult for the immune system to control the virus. This phenomenon may contribute to immune system degradation and the development of AIDS. The study observed low-level viremia in HIV-1 patients on combination antiretroviral therapy (cART), which was evidenced by the levels of cytokines in the sampled patients over the study period. Higher levels of cytokines in patients with low-level viremia are linked to viral replication and subsequent virologic failure. This implies that cytokine levels may be useful in predicting HIV-1 replication and progression and suggests that monitoring cytokine levels can provide valuable information regarding the effectiveness of cART and the potential for virological rebound. The study demonstrates a relationship between HIV-1 rebound and the levels of both pro-inflammatory and anti-inflammatory cytokines, suggesting that HIV-1 proliferation can impact cytokine production. HIV-1 progression may be influenced by the balance between pro-inflammatory and anti-inflammatory cytokines. The study suggests that pro-inflammatory and anti-inflammatory cytokines can be used to monitor the prognosis of HIV-1 disease during therapy, especially in resource-limited settings with limited access to regular viral load monitoring. This can help identify persistent low-level viremia, which could be an early indicator of potential virological failure. This balance can play a role in determining the course of disease. The slight imbalance in IL-17/IL-10 ratio suggests that the immune system is simultaneously trying to clear the infection while balancing the effects of inflammation by releasing immunoregulatory cytokines. This points to the complex immune response in HIV-1 patients. The study underscores the importance of cytokines in HIV-1 progression and the potential for using cytokine measurements as a valuable tool for monitoring disease prognosis and treatment effectiveness, particularly in settings with limited resources. These findings have implications for improving HIV-1 patient care and treatment strategies.

Recommendation

Findings of this study that provides valuable insights into the role of cytokine levels in HIV-1 patients on combination antiretroviral therapy (cART) can indeed inform policy makers and health care

providers on robust grounds to: Consider regular cytokine measurements as revised monitoring benchmarks for HIV-1 patients on cART, Provide a platform for exploring other immune markers and consider validation and further research on the study.

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