IMPROVED CD4$^+$ CELL RESPONSE TO SHORT TERM TREATMENT IN HUMAN IMMUNODEFICIENCY VIRUS INFECTED SUBJECTS ATTENDING AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, ZARIA

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Received: 25th June, 2024 **Accepted**: 23rd November, 2024 **Published**: 31st December, 2024

ABSTRACT

Background: The replication and life cycle of HIV within CD4 T cells is understood in molecular detail, less is known about how this human retrovirus promotes the loss of CD4 T lymphocytes. It is this cell death process that drives clinical progression to acquired immune deficiency syndrome (AIDS). However, CD4$^+$ T cell proliferation is rapidly delay by a functional interplay of regulatory T cells and CTLA4. The interplay paralyzes CD4$^+$ T cell differentiation, redirecting metabolic circuits, and reducing their accumulation in the site of infection. The mechanisms are actively maintained throughout HIV progression and CD4$^+$ T cells rapidly recommence proliferation and functional differentiation. We demonstrated the significant increase in CD4$^+$ cells count in six (6) months on cART. This indicates an increase in the number of CD4$^+$ T cell is a central part of immune improvement phase and lead to immune defense ability to kill the HIV virus. In this study, we aimed to determine the CD4$^+$ T cell count recovery in HIV-infected subjects on cART attending ABUTH within six months.

Materials and Methods: We employed quasi experimental design, where non-probability sampling techniques was used in recruiting thirty-eight (38) naive HIV patients up to six (6) months on Combine Antiretroviral Therapy (cART) and ten (10) controls. We quantified the CD4$^+$ T cells count using Partec CyFlow Counter; Germany.

Results: The median CD4$^+$ T cell counts using Friedman test was increased significantly with p-value of 0.0001 in treated HIV-infected participants after six (6) months and Controls. The median and interquartile range (IQR) of the CD4$^+$ T cell counts of HIV-infected ART naive participants at baseline, on treatment, and controls were 199 (92.75-402.8) Cell/µL, 379.5 (265.5-569.8) Cell/µL and 1316 (1082-1480) respectively. The result shows the increase of CD4$^+$ T cell counts recovery within six months on cART.

Conclusion: In conclusion, the importance of CD4$^+$ T cell counts recovery indicate the central part of immune improvement phase in the defense mechanism against HIV virus.

Keywords: CD4$^+$ T cells, HIV Infected Individual, Combine Antiretroviral Therapy, functional differentiation, Ahmadu Bello University, Zaria.

INTRODUCTION

The CD4$^+$ T cells are central effectors of anti-HIV immunity and immunotherapy by the regulation of CD4$^+$ HIV and NK cells (Doitsh *et al*., 2010).

Reduction and loss of T cell function underlies the inability to control HIV growth and forms the basis for immune-restorative therapies (Takata *et al*., 2023). Since CD8 T cells and NK cells are the endpoint effectors of HIV cell killing, most studies focus on their mechanisms of dysfunction (Sun *et al*., 2017). However, increasing evidence indicates the importance of $CD4^+$ T helper (Th) cells in controlling HIV and increasing efficacy of immunotherapies, suggesting the need to better understand the increase or decrease of $CD4^+$ T cells count in individuals for period of time in response to HIV (Id *et al*., 2022) . Importantly, $CD4^+$ T cells and CD8 T cells possess different sensitivity toward immune checkpoint blockade and, as a result, likely are mediated by different regulatory mechanisms (McCune, 2002). Following activation, naive $CD4^+$ T cells differentiate into specific subsets, based on signals from the macrophage and other immune cells in response to HIV virus. The $CD4^+$ T cells response is guided to control individual types of infection, which may also be misguided that will lead to ineffective HIV control (Geldenhuys *et al*., 2018). In chronic viral infections, even though initially activated properly, the increased and ongoing antigen stimulation and inflammation attenuate $CD4^+$ T cell function, twisting immune responses, disease control, and response to immunotherapy (Aly, 2012; Bozhanova *et al*., 2022). Mechanistically, the continuing T cell receptor (TCR) stimulus (Peng *et al*., 2022), increase type I interferon (IFN-I) signaling(Jefferies, 2019), and upregulated suppressive molecules (e.g., IL-10, PD1:L1) prompted by chronic virus replication drive the loss of CD8 T cell helping $CD4^+$ Th1 cells (Read *et al*., 2019) and induce their trans-differentiation into B cell helping $CD4^+$ T follicular helper (Tfh) cells, leading to the progressive loss of virus-specific CD8 T cells (Curtis, 2005).

Increase antigenic stimulus and numerous of the inflammatory and suppressive states observed in chronic viral infections can also occur within the phagolysosomes action (Sim *et al*., 2020). Thus, alterations described in chronic infection may also impact antiviral immune responses. Studies using mouse models to understand antiviral $CD4^+$ T cell function have primarily focused on bulk $CD4^+$ T cells (i.e., not antigen specific), and generally rely on in vitro activation of HIV virus, which, although informative, but do not address how $CD4^+$ T cells are activated and development from HIV initiation(Chen *et al*., 2019). Furthermore, for understandable reasons, clinical studies characterize total $CD4^+$ T cells in HIV patients without known antigen specificity. Thus, there is limited understanding of how $CD4^+$ T cells count in individuals with HIV infection. Recently, the relationship between $CD4^+$ T cell glycolysis and their functions, showed the activation, proliferation, and survival rate of $CD4^+$ T cells increase with increases in their glycolysis level(Liu *et al*., 2023), suggesting that HIV-specific T cells can be driven to regulatory phenotypes promoting tumor progression. However, this study is associative and, thus, it is important to define $CD4^+$ T cell count in naïve HIV infected individuals and at six months on treatment.

Herein, we demonstrate the $CD4^+$ T cell counts in HIV-infected treatment naive participants at baseline and after six (6) months on treatment and apparently healthy normal control in Ahmadu Bello University Teaching Hospital Zaria. Longitudinal assessment for $CD4^+$ cell counts response is fundamentally aspect to address the issues associated with the poor clinical consequence including immunologic failures among HIV-positive patients on cART. The ease of this system allows the analysis of the responding $CD4^+$ T cells in individuals in order to monitor the disease progress while testing therapeutic. As expected, significant increase of $CD4^+$ counts were observed in all the participants. Thus, our approach showed the $CD4^+$ T cell count recovery in HIV-infected subjects on cART attending ABUTH.

MATERIALS AND METHODS

Study participants and data collection

This study (which comprises of intervention, control and no randomization) assessed the $CD4^+$ cell counts among HIV-infected treatment naive participants at baseline and at six months on Combine Antiretroviral Therapy (cART) as well as in apparently healthy Control individuals from the age of ≥18 to ≤50 years. A purposive non-probability sampling technique was used for HIV-infected treatment naive participants at Nasara Treatment and Care Center (NTCC). Blood samples were collected from aged-matched comparatively similar, apparently healthy blood donors from the Donor Bay of the Haematology Department, ABUTH Zaria as Controls. The study was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans (Ballantyne and Eriksson, 2019). Written informed consent was sought and obtained from each participant prior to enrollment into the study as all participants were adults. The participants were adequately informed of their right to choose to or not to participate or withdraw at any point so wished.

ETHICAL APPROVAL

The Ethical approval/certificate dated 4th March, 2022; ABUTH Ethics Committee assigned number: ABUTHZ/HREC/A12/2022 with reference number (NHREC/10/12/2015; D-U-N-S NUMBER:954524802) was obtained from the Health Research Ethics Committee (HREC) of ABUTH, Zaria Nigeria before commencement of sample collection. For ethical reasons, participants' initials were de-identified and coded.

Consent

Written informed consent was sought and obtained from each participant prior to enrollment into the study as all participants were adults. The participants were adequately informed of their right to choose to or not to participate or withdraw at any point so wished.

Data analysis

Thirty-eight (38) naive HIV positive study participants at Nasara Treatment and care Centre (NTCC) in Ahmadu Bello University Teaching Hospital (ABUTH) Zaria were recruited for this study alongside ten (10) comparatively similar for age in apparently healthy donors as controls.

Five millilitres (5ml) of venous blood sample were collected from each participant that is HIV infected treatment naive and at six months on treatment in EDTA bottle. Twenty (20) µL of whole blood were stained with the "CD4 Count Kit-Dry" (Partec CyFlow Counter; Germany) for the determination of $CD4^+$ T cell count. Twenty (20) µL of whole blood from the patients were stained with twenty (20) µL of $CD4^+$ monoclonal antibody. This was mixed gently and incubated for 15 minutes at room temperature (protected from sunlight). Eight hundred (800) µL of no lyse buffer was then added and the tube was plugged in and the measurement started immediately and analysed. All samples were performed in triplicate and results were printed out (Olisah *et al*., 2015) .

RESULTS

Data managements and analysis: The data were edited using Microsoft excel, missing values and outliers were identified and recorded appropriately. Normality test, descriptive test and tests of significance were conducted using Graph Pad Prism version 8.0.1. In Figure 1, the $CD4^+$ T cell counts at baseline, in treated HIV-infected participants after six months and Controls. Using Friedman test showed a significant difference with p-value 0.0001. The median and interquartile range (IQR) of $CD4^+$ T cells of HIV-infected treatment naive participants at baseline, at six months and on apparently healthy controls were 199 (92.75-402.8), 379.5 (265.5-569.8), and 1316 (1082-1480) cell/µl respectively.

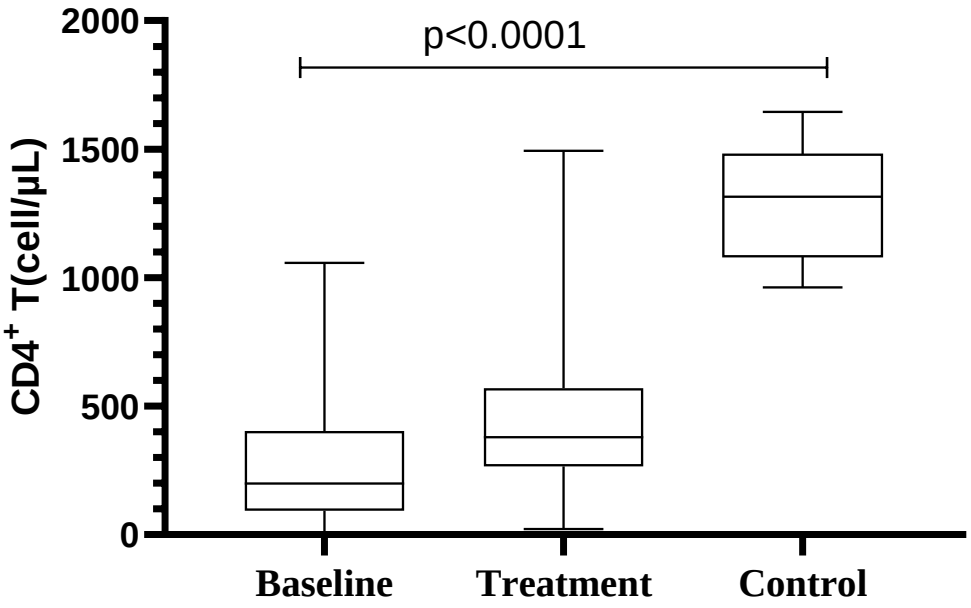

Figure 1: $CD4^+$ T cell count in HIV-infected treatment naive at baseline, on treatment and apparently healthy controls using Friedman test.

DISCUSSION

The increase of $CD4^+$ T cells is central effector of anti-HIV immunity and immunotherapy of HIV-infected individuals by the regulation of the immune cells recovery. $CD4^+$ T cells are rapidly primed and begin to divide following HIV infection. $CD4^+$ T cell proliferation is rapidly delay by a functional interplay of regulatory T cells and CTLA4 (Guo *et al*., 2023). Reduction and loss of T cell function underlies the inability to control HIV growth and forms the basis for immune-restorative therapies. We show here, that there is significant increase in $CD4^+$ cells count at six (6) months on HAART. The increase of $CD4^+$ cell counts were observed from naïve participants at baseline to six (6) months on HAART, and in apparently healthy controls. Almost all the participants were found to recover their $CD4^+$ cells count at six months. The result is in conformity with the results obtained in Zaria Northern Nigerian with median $CD4^+$ cells count at baseline in cohort study (Obiako O Reginald *et al*., 2012), (Akase *et al*., 2017) both at ABUTH; in a case report (Ogoina *et al*., 2010); and in a tertiary health center with a federal government supported AIDS treatment program (Ahmed *et al*., 2013). This study is also comparable to the study of Anude J Chuka *et al*. (2013), with $CD4^+$ baseline in the determination of immuno-virologic outcomes and discordance and their associations in HIV patients and this is also in accordance in six sub-Saharan African countries (Kroeze *et al*., 2018), in Northwest Ethiopia (Mulu *et al*., 2014), and in Africa, the median baseline $CD4^+$ cell counts reported (Asfaw *et al*., 2015). However, Kayigamba *et al*. (2016) which reported median $CD4^+$ cell counts at baseline on a prospective cohort study in Rwanda on discordant treatment responses on HAART and also by Barasa Gelba *et al*. (2020) in Ethiopia a retrospective cross-sectional study showed contrary at ART initiation.

In this study we demonstrated the rapid increase in $CD4^+$ cells count at six (6) months on treatment by mean value (an increase of +180 cells/µl from baseline) yield impressive value. This is in agreement with findings in Nigeria at twelve (12) months on treatment (Anude J Chuka *et al*., 2013); according to Fiseha *et al*. (2022) the patients had an increase in $CD4^+$ cells count. Moreover, Kaufmann *et al*. (2005) who worked on the Determinants, and Clinical Relevance of $CD4^+$ T cell recovery in HIV with the median $CD4^+$ T cell count increased from baseline to 5 years. The huge increase may be as the result of years on ART initiation. Also, the study done on immunological recovery, failure, and factors associated with $CD4^+$ T-cells progression in Northern Ethiopia (Desta *et al*., 2019) with high mean $CD4^+$ T cell count. Nonetheless, a study conducted in Liberia revealed a higher median $CD4^+$ count (Loubet *et al*., 2015) . This increase may be due to long duration on treatment of patient and the research method (retrospective). In Conflicting result by Anude J Chuka *et al*. (2013) Immuno-virologic outcomes and immunovirological discordance among adults alive and on anti-retroviral therapy at 12 months in Nigeria, and the study conducted in the capital city of Addis Ababa, with low $CD4^+$ counts (Teshome & Tefera, 2015). This variation may be due to the differences in the minimum standards of $CD4^+$ count to initiate ART treatment, awareness and delayed initiation of ART.

CONCLUSION AND RECOMMENDATION

As we aimed to determine the $CD4^+$ T cell count recovery in HIV-infected subjects on cART attending ABUTH within six months. This analysis of $CD4^+$ T Cell Counts Among HIV Infected Individual has been remarkably highlighted the increases in six months on treatment of HIV infected individual. Moreover, the demographic, economic and clinical data employed for this study increase the chances of close monitoring of patients and regular follow up on patients by intensified patients' adherence support for repeat testers after suspected failure of the drug. Counselling should also focus on encouraging spouses of married patients to act as treatment supported for their partners.

Limitation

In this study, we analyze thirty-eight subject and ten controls. A large number of sample size should be recruited from various tertiary institution from all geopolitical zones in Nigeria. This will give a wider coverage and give more inside on patient management system and development.

Conflict of interest

There is no any conflict of interest about study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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