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# CD38-Expression-on-CD8-Cells and Its Influence-on-Development-of-Tuberculosis-in-HIV-

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## ABSTRACT

HIV *is* a virus spread through certain body fluids that attacks the body's immune system, specifically the CD4 cells, often called T cells. Over time, *HIV* can destroy so many of these cells that the body can't fight off infections and disease. These special cells help the immune system fight off infections.

HIV weakens the immune system, increasing the risk of TB in people with HIV. Infection with both HIV and TB is called HIV/TB coinfection. Latent TB is more likely to advance to TB disease in people with HIV than in people without HIV. TB disease ,CD38 expression on CD8+ cells seems to correlate well with HIV viral-loads, while the expression levels are thought to be low in patients with tuberculosis. This study aimed at determining the levels of CD38 expression in HIV+ individuals who develop tuberculosis. Expression levels of CD8 and CD38 were analysed in peripheral blood collected from HIV (73), TB (32), HIV-TB (31) and healthy controls (20). The percentage of CD8+/CD38+ cells significantly increased during the first few years of seropositivity and decreased during 5 - 6 years. A decline in the expression of CD38, especially on CD8+ cells in a HIV+ individual within first 2 years of seropositivity, may be indicative of susceptibility to tuberculosis. This observation was reiterated when two patients developed TB during follow-up. CD38 on CD8 cells could perhaps be useful as an early biomarker for tuberculosis in HIV-positive individuals.

## . INTRODUCTION

Right from the stage of infection with HIV, most of the individuals exhibit perturbation of T cells and their subsets, leading to their exacerbation in the absolute number with time. These immune cell turbulences along with variation in their function lead to progressive immunodeficiency [1] resulting in an increased risk of opportunistic infections, such as tuberculosis. HIV infection is well recognized with highly variable disease progression rates between individuals and are categorized as rapid, intermediate and long-term non-progressors [2]. Lympho proliferative response to HIV-specific antigen is well established in long-term non-progressors than those with more rapid progression [2]. It is well-known that CD4+ cells decline during infection with HIV and the numbers reduce further with disease progression [3]. Reduction in the number of peripheral CD4+ T cells in patients with active tuberculosis also and restoration to normal counts after successful chemotherapy has been reported earlier [4]. The influence of CD8+ cells on progression of HIV disease is a concern, as cytotoxic T lymphocytes (CTLs) are responsible for specific cellular immune response [2]. Evidences of HIV infection in humans and nonhuman primate models indicate the role of CD8+ T cells in controlling or limiting HIV-1 replication [5]. Studies revealed that HIV-1 specific CD8+ cells are associated with nonprogressive HIV-1 infection [6].

It is more or less accepted now that CD38 expression on the T cell subsets, specifically on CD8+ cells could be a useful tool to evaluate the state of cellular activation in HIV infection and also to predict the progression of the disease [3,7]. Rodrigues et al. reported that expression of CD38 on CD8 cells was high in active pulmonary tuberculosis [4]. On the other hand, Viegas et al. [8] reported that absence of CD38 rendered mice more susceptible to mycobacterial infection. HIV infection is known to aggravate susceptibility to tuberculosis by several folds. However, it is not clear how the CD8+/CD38+ expression levels vary in HIV-TB co-infection and whether it could be useful to predict susceptibility to tuberculosis in individuals with HIV infection. This study aimed at investigating variation in CD8+/CD38+ expression levels in individuals positive to HIV at different time frames and its role in early diagnosis of TB co-infection.

### AIM

This report is made in order to estimate CD38 expression on CD8+ cells—Its influence on development of tuberculosis in HIV positive individuals

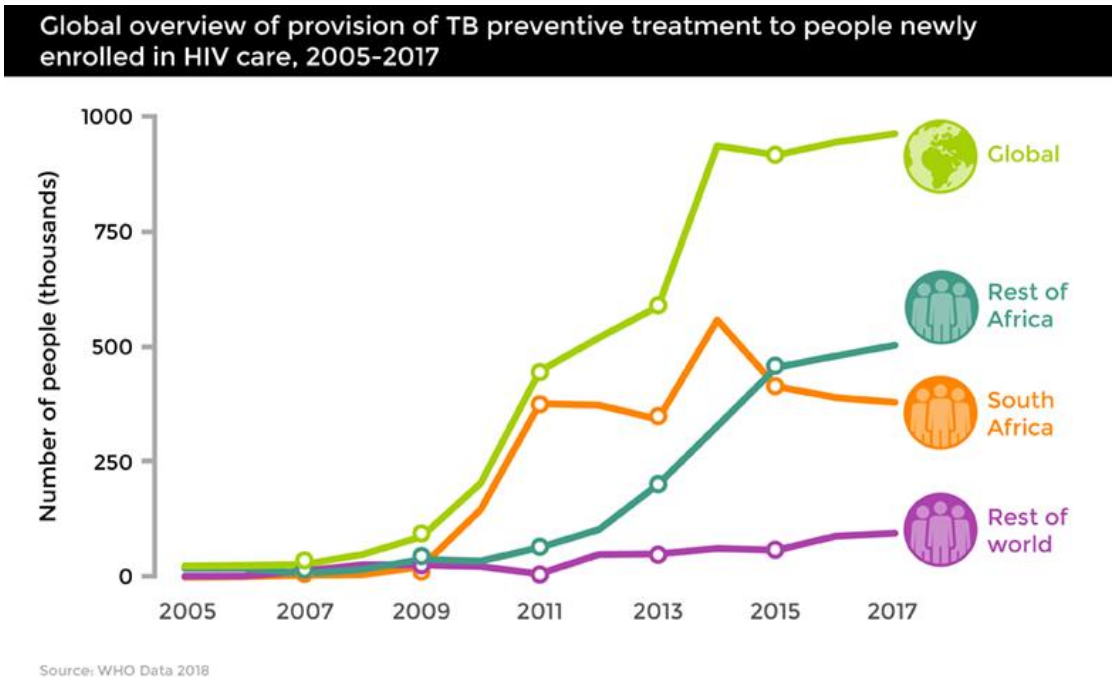
## MATERIALS AND METHODS

In this study subjects attending clinics of LEPRA India and Mahavir Hospital were included and categorized as: 1) HIV+ group (73) (HIV): HIV-1 infected individuals tested at ICTC (integrated counselling and testing center) who were asymptomatic, treatment-naive and had no history of tuberculosis; the subjects were further divided as per the time-lag after being diagnosed as seropositive up to 10 years; 2) Active tuberculosis group (32) (TB): HIV-1 seronegative patients with recently diagnosed active pulmonary or extra-pulmonary tuberculosis, based on clinical picture, sputum-positivity and radiological involvement suggestive of pulmonary tuberculosis and FNAC or biopsy from the infected sites in case of extra pulmonary TB; 3) HIV-TB co-infected group (31) (HT): Confirmed with HIV-1 and active tuberculosis disease; 4) Healthy control group (20) (CTRL): Asymptomatic volunteers HIV-seronegative with no history of TB. The peripheral blood cells were stained using a three colour panel of the fluorescence tagged monoclonal antibodies for: CD8 PerCP, CD4 FITC and CD38 APC (BD Biosciences, San Jose, CA). Briefly, 100 μL of EDTA treated blood was incubated at room temperature with a combination of monoclonal antibodies for 15 minutes in the dark, and then treated with haemolysis buffer of 1X dilution for further 15 minutes. Cells were washed and resuspended in sheath fluid for cytometric analysis. The subpopulation was analyzed as a function of the percentage of cells expressing CD4 and CD8 on the lymphocytes. The percentage surface expression of the CD38 molecule on CD8 T-lymphocytes was determined after establishing the quadrants in unstained samples. Thirty two samples from HIV+ patients were outsourced to VIMTA Labs and Tapadia Diagnostic Centre (Hyderabad) for the Quantitative/Viral Load assay using Real Time PCR method with a lower detection limit of 70 copies/mL.

## RESULTS

CD8 expression was significantly high in HIV-TB and HIV groups when compared with control and TB groups. Whereas, TB group had similar expression to that of control. In Different Study Groups Expression of CD8+/38+ was highly significant in HIV-TB group ($p < 0.05$) when compared with control, HIV and TB groups. HIV group expressed high CD8+/ 38+ ($p < 0.05$) when compared with control and TB, whereas, Control and TB groups had similar expression. The viral load was found to be directly proportional to CD38 expression ,The % CD8+/38+ was significantly low in HIV+

individuals 5 - 6 years after diagnosis when compared to those with time-lag 1 - 2 years. Whereas, the expression levels remained almost similar at remaining time frames after diagnosis of HIV, Regarding Expression of IFN-γ In Different Study Groups IFN-γ expression was almost similar in HIV-TB, HIV and had no significant association when compared with control or TB groups. TB group expressed significantly low levels of IFN-γ in plasma when compared with controls ($p < 0.05$). No significant co-relation was found between the plasma IFN-γ levels and CD8+/38+ expression




## DISCUSSION

CD38 expression on CD8+ cells was high in patients with HIV-TB and HIV infection in this study. There are several reports on the increase in the number of CD4+ and CD8+ cells positive to CD38 during infection with HIV. HIV drives cell-activation and proliferation [10]. Since CD38 expression is antigen-driven, virally produced proteins could be the cause for the high expression, as also the host cytokines [11]. On the other hand, there is a gradual decrease of CD38 expression on CD8+ ,Majority of the CD8+ cells expressed CD38 in HIV+ individuals in this study. As the disease advances, increase in CD38 expression on CD8+ cells (89%) was also reported by Rodrigues et al. [3]. In case of intermediate progression as a consequence of chronic cell activation resulting in apoptosis causing cell-death may lead to decrease in the CD38 expression. The decline in CD38 expression a few years (i.e., 5 - 6 years) later, observed in this

study could be probably attributed to this phenomenon. It is thought that CD8+CD38+ proportions lose their prognostic significance over time at 5 years follow up [2].

Langford et al. reported that low frequencies of HIV specific CD8+ T-cells are associated with poor survival outcomes for experienced patients providing evidence for the significant role of CTL response [2]. Rodrigues et al. [3] reported a decrease in CD8+ cells in TB patients, while they were not altered in HIV+ patients. Biancotto et al. [13] reported that apoptosis leads to cell death of T-cells and dendritic cells, resulting in impairment in both innate and adaptive immune responses. Hence, lack of CD8+ cell proliferation or decrease in CTL cells may also be responsible for decrease in CD8+/38+ expression in HIV+ individuals at the time of TB diagnosis, an observation made in our study. On other hand co-infected patients have decreased proliferative responses to M. tb antigens with a reduced production of IFN-γ, compared to patients with TB and no HIV ,Viegas et al. [8] reported that absence of CD38 rendered mice more susceptible to mycobacterial infection probably due to the ineffective Th1 differentiation and polarization. In addition, absence of CD38 seems to compromise the maintenance of the granulomatous barrier, leading to dissemination and unrestrained growth of mycobacteria.Thus, CD38 may be involved in development of protective immune responses against mycobacteria [8]. The CD8+/38+ levels in two patients who enrolled in the study soon after testing positive to HIV, were lower (>30% decrease) when they presented with rapid progression of HIV and developed TB coinfection. As reported earlier TH1 cytokines such as interferon-γ promote strong cellular responses and early HIV viremic control [2]. Decline in the IFN-γ levels in this study during the follow up, indicated down regulation of the CMI in the patients probably leading to manifestation of tuberculosis within two years of HIV diagnosis. After infection with tuberculosis, the expression of CD38 in a HIV+ individual is enhanced. The expression levels in this study in HIV+ patients were presumably lower than that observed in the co-infected patients. Viegas et al., [8] reported decreased IFN-γ pro-

The increase in CD38 expression on CD8+ cells after the co-infection observed in this study, could be due to the HIV viral loads which increase after the manifestation of tuberculosis. It is known that there is an increase in the percentage of cells expressing CD38 in patients with active TB, returning to normal after therapy [4].

## CONCLUSION

CD38 expression on CD8+ cells is high in patients with HIV-TB and HIV infection this is maybe due to the effect of the virus in immune system homeostasis as it affect one of the most regulator cells in our immune system.

## FUTURE WORK

More studies and researches should be done regarding the HIV-TB coexistence considering the weakened immune system and the hazardous aspects of this infection among such patients.

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