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Brief Communication

Effect of *Withania somnifer* on CD38 expression on CD8+ T lymphocytes among patients of HIV infection

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ABSTRACT

CD38 on CD8 + T cells is considered a reliable marker of HIV disease progression. *Withania somnifer*, a traditional ayurvedic medicine, has Th1 immunomodulatory properties. PBMCs from 38 HIV patients were exposed to *Withania somnifer* root extract at standardized concentration. An overall decline in the percentage of CD38 expressing CD8 + T lymphocytes was observed, though the statistical significance was varied with different categories of HIV patients. *Withania somnifer* could have promising impact on HIV disease and therefore warrants a further study on larger parameters.

CD38 is primarily considered as an activation marker of the immune cells, though it is associated with multiple functions [1]. Expression of CD38 on CD8+ T lymphocytes is a reliable marker of disease progression in HIV infected individuals [2,3]. This marker can be seen on as high as 95% of CD8+ T lymphocytes during AIDS condition prior to initiation of anti-retroviral therapy. CD38 gradually declines with time as anti-retroviral therapy is initiated. An increase in CD38 expression on CD8 T lymphocytes after 6 months of anti-retroviral therapy is linked to virological failure. Co-infection can affect CD38 expression on CD4 T cells but not on CD8 T cells [3]. Since dramatic reduction of CD4 cells in HIV infection is the result of abortive infection and pyroptosis of cells caused by HIV (in absence of productive infection or release of virus in plasma) [4], CD38 expression on CD8 T cells could prove to be a better marker in HIV disease.

Withania somnifer, commonly known as ashwagandha in India, is an ayurvedic supplement to treat numerious disorders linked to immune system [5]. Apart from India, its usage is also documented in China, Africa and native America. *Withania somnifera* prevents myelosuppression, increases WBC count and body weight in mice models [6]. *W. somnifera* causes Th1 polarization by significantly enhancing proliferation and differentiation of T lymphocytes, improving their ability to secrete IL-2 and IFN-γ, moderately down regulating IL-4 and B cell switch over to secrete IgG2a [7]. Immunomodulatory role of *Withania somnifera* root powder is demonstrated in experimental induced inflammation [8]. Consumption of its root extracts with whole milk twice daily resulted in significant increased CD4+ CD3+ T cells after 96 h [9]. A double blind placebo control study on patients of pulmonary tuberculosis (a common co-infection in HIV patients) showed that *Withania somnifera* as an adjuvant to DOTS for 8 weeks resulted in higher sputum conversion along with higher CD4 & CD8 count [10].

There is scarcity of information about the effect of *Withania somnifera* on HIV infected patients, particularly on an important HIV prognostic marker; CD38 expression on CD8 T lymphocytes. For this purpose, we extracted peripheral blood mononuclear cells from 38 patients which were reactive for HIV-1 gp120 and HIV-1 gp41 antibodies. 10^5 cells in 100 µL of phosphate buffer saline (PBS) were subjected to 1 µl of aqueous supernatant obtained from the root extract of *Withania somnifer* (40 µg/ml) and incubated for two hours at 37 °C. This was followed by addition of 1 ml of phosphate buffer saline (to dilute or nullify conc. of *Withania somnifer*) and incubation for another 1 h. The cells were centrifuged; supernatant discarded, resuspended in 100 µl of PBS and stained with anti CD3, CD8 and CD38 monoclonal antibodies for 30 min. The cells were acquired on BD FACS Calibur flow cytometer. Acquisition of cellular events was done on Cell Quest Pro and analysis on Flow Jo.

The analysis of cells on FlowJo revealed that CD3+CD8+ cells treated with aqueous supernatant of *Withania somnifer* had decline in CD38 expression (Fig. 1). The decline ranged from 1.18% to 37.26% in comparison to their respective untreated cells and the mean ± SD of the decline was 15.27% ± 11.11%. The paired *t*-test for the percentage of CD38 expressing CD8+ T cells in treated and untreated conditions had p value as < 0.0001. Ten patients that were having immunological failure (persistent CD4 cell count < 100 cells/µl) after > 6 months of Anti-Retroviral Therapy (ART) were included among the thirty eight selected HIV patients and these cases were our priority. The decline in CD38 on CD3+ CD8+ cells following exposure to *Withania somnifer* in

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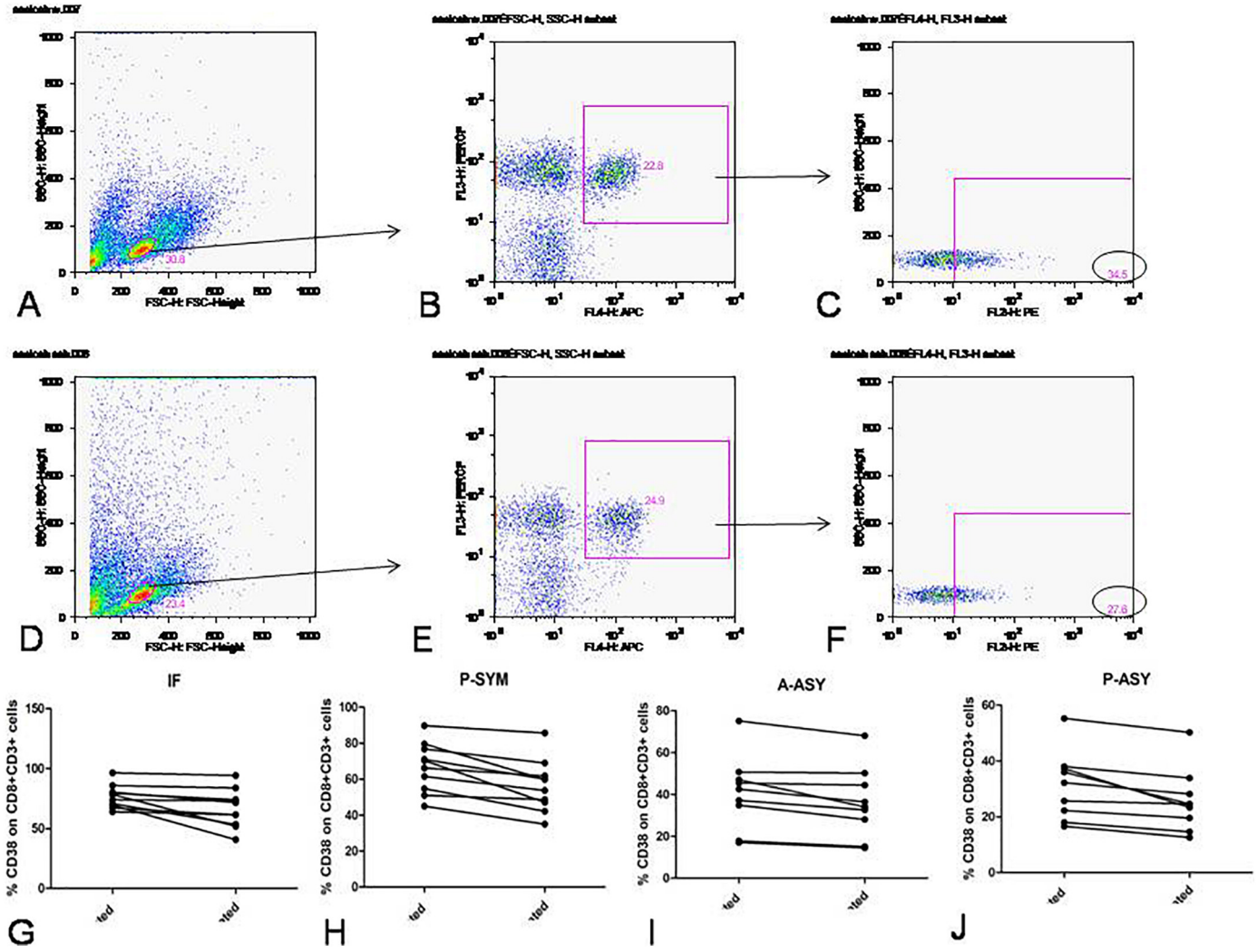

Fig. 1. Diagrammatic representation of gating for lymphocytes (A), CD3-PerCP and CD8-APC positive lymphocytes (B) and CD38-PE expressing CD3 + CD8 + lymphocytes (C) in an HIV infected patient. Note the decline in CD38-PE expressing CD3 + CD8 + lymphocytes (F) in comparison to (C) after exposure to *Withania somnifer*. Also note the decline of CD38-PE expressing CD3 + CD8 + lymphocytes with *Withania somnifer* in patients of immunological failure (IF) after ART (G), symptomatic and AIDS indicator patients (P-SYM) prior to ART (H), Asymptomatic patients after ART (A-ASY) (I) and prior to ART (P-ASY) (J).

this group of patients, however, was less significant in comparison to other HIV patient groups (p value- 0.01 vs. < 0.0001; immunological failure HIV group vs. other HIV groups). The mean ± SD of CD38 expressing CD8 + cells prior to the exposure to *Withania somnifer* were 76.6% ± 9.7% and after exposure were 66.7% ± 15.9% in patients of immunological failure with percentage of decline as 13.4% ± 14.4%.

The rest HIV patients included in this small study were ten ART naïve symptomatic and AIDS indicator cases (Mean CD4 cell count/μl = 135 ± 122); nine asymptomatic cases after > 6 months of ART (Mean CD4 cell count/μl = 704 ± 166) and nine ART naïve asymptomatic cases (Mean CD4 cell count/μl = 699 ± 229). The percentage of CD38 expressing CD8 + T cells in these groups of HIV patients were 66.6% ± 13.8%, 40.9% ± 17.6% and 31.2% ± 12.1% respectively. After exposure to *Withania somnifer* in test conditions, CD38 expressing CD8 + T cells were 56.3% ± 14.4%, 35.9% ± 16.8% and 25.7% ± 11.2% respectively. The decline in CD38 expressing CD8 + T cells following exposure were 15.6% ± 9.7%, 13.2% ± 8.2% and 18.9% ± 11.6% respectively. The decline of CD38 expressing CD8 + T cells was significant in all three groups (p value by paired t-test- 0.001, 0.005 and 0.004 respectively).

The results showed that there is decline in CD38 expression on cytotoxic T lymphocytes on treatment with *Withania somnifer*, though the results may vary significantly during different clinical conditions of HIV patients. The reason behind the decline is difficult to predict and therefore, a further study on a larger cellular parameters is highly desired. *Withania somnifer* should also be tested against reverse transcriptase based HIV quantitative assays to explore a direct evidence of action on the virus. There is a strong possibility that supplementing root extract of *Withania somnifer* with anti-retroviral therapy will be beneficial in improving overall cellular immunity of HIV infected patients. A significant population in African and south Asian countries are managing opportunistic infections in HIV by traditional medicine which is largely overlooked.

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