

# Relationship between Interleukin 12 Levels and Suppressed CD4 Counts in HIV Patients

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**Background and study aim:** Interleukin 12 (IL-12) increases T cell proliferation, elevates natural killer (NK) and cytotoxic T cell activity, and induces the production of interferon gamma (IFN- $\gamma$ ). The aim of this study was to assess the potential relation of serum interleukin 12 levels in the suppression of CD4+ T-cell count in HIV patient in spite of low viral load after Highly Active Anti-Retroviral Therapy (HAART).

**Patients and Methods:** Thirty seropositive HIV male patients were selected with low viral load after HAART. They were divided into two groups according to their immunological response. The first group included 15 male patients with low CD4 counts. The second group included 15 male patients with high CD4 counts. All patients were investigated for complete blood count (CBC), liver function test (LFT), kidney profile (KP), estimation of the levels of TNF- $\alpha$ , IFN- $\gamma$ , IL-10, IL-12.

**Results:** Serum levels of IL-12 were significantly higher in Group II than in Group I (mean $\pm$ SD IL-12 levels of 11.91 $\pm$ 2.8 versus 6.9 $\pm$ 2.9,  $p < 0.05$ ). The serum levels of IL-12 were positive correlated with CD4 counts ( $r = 0.514$ ;  $p < 0.05$ ). Similarly, a positive correlation between IL-12 levels and IFN- $\gamma$  levels were noted ( $r = 0.602$ ,  $p < 0.01$ ). No significant correlations were observed between IL-12 levels and viral loads and also, no correlation between serum levels of IL-12 and serum levels of IL-10 and TNF- $\alpha$ .

**Conclusion:** Reduction of the levels of IL-12 in immunologic non-responders HIV-infected patients may play a role in impairment of immunological recovery following HAART. Although, we found that IL12 production was correlated with IFN- $\gamma$ , the mechanism by which the reduced production of IL-12 in immunologic non-responders HIV-1-infected patients remains poorly understood.

## INTRODUCTION

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that results in *acquired immunodeficiency syndrome* (AIDS) [1]. HIV primarily infects vital cells in the immune system of human such as helper T cells (CD4+ T cells), dendritic cells and macrophages. Cell-mediated immunity is impaired, and the body becomes progressively more susceptible to opportunistic infections When CD4+ T cell numbers decline below a critical level [2].

Highly Active Anti-Retroviral Therapy (HAART) keeps the levels of HIV in the body at a low level, so that the

immune system is able to recover and work effectively. HAART in HIV-1-infected patients has a broad spectrum of clinical outcomes. In the majority of patients, CD4+ T-cells increase over time and the plasma viral load becomes undetectable. However, in a number of subjects, a discrepancy between CD4+ T-cell recovery and plasma viral load is noted. CD4+ T-cell count can increase despite persistently detectable plasma viral load (virologic non-responders) which occurs in 7–15% of the patients [3], or conversely, CD4+ T-cell numbers do not rise despite plasma viral load suppression (immunologic non-responders) [4]. It is noted that 7%-20% of patients receiving

Long-term HAART are "immunologic non-responders," [5], i.e. patients who fail to achieve a CD4+ T cell count above 200 cells/ $\mu$ l at 6, 12, 18, 24 months of HAART [5].

Interleukin (IL) 12 was initially described as a cytotoxic lymphocyte maturation factor and a NK cell stimulatory factor [6]. It was identified by G. Trinchieri et al. [7], as a heterodimer consisted of p35 and p40 subunits, which, when combined together form the bioactive IL-12p70 [8]. This cytokine increases T cell proliferation, elevates natural killer (NK) and cytotoxic T cell activity, and induces the production of interferon gamma (IFN- $\gamma$ ) [6,9]. In the murine model, administration of IL-12 up-regulates NK cell activity, elevates the serum IFN- $\gamma$  level, and causes a shift toward a T-helper 1 (Th1) response to specific pathogens and antigens [6]. Many studies demonstrated that the macrophages, monocytoïd lineage and myeloid dendritic cells are the primary and the physiological sources of IL-12 in response to a large variety of infectious agents [9]. It was reported that there is an impaired of IL-12 production in HIV-infected patients, and addition of exogenous IL-12 in vitro can restore HIV-specific cell-mediated immune responses in HIV-positive persons [10].

Therefore, this study was conducted to assess the potential relation of serum interleukin 12 levels in the suppression of CD4+ T-cell count in HIV patient in spite of low viral load after Highly Active Anti-Retroviral Therapy.

## PATIENTS AND METHODS

This study was conducted between November 2014 and April 2016, at the Infectious Disease Hospital (IDH), Kuwait. In this study, the patients were diagnosed as seropositive HIV and two years prior to the study were started on HAART in the form of combination of three or more anti-HIV medications from at least two different classes [non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), or protease inhibitors (PIs)]. All patients received HAART regimen according to individual needs. Of 148 patients who were on HAART for more than two years, 118 were excluded due to any opportunistic infections, co-infection with viral hepatitis, advanced age, advanced stage of the disease, diabetic state, female sex and history of previous treatment interruption. Thirty patients were selected with low viral load after HAART and divided into two

groups. The first group (group I) included 15 male patients with low CD4 count. The second group (group II) included 15 male patients with high CD4 count. All patients were subjected to full history taking, thorough clinical examination, body mass index (BMI) (kg/m<sup>2</sup>), complete blood count (CBC), liver function test (LFT), kidney profile (KP), fasting blood sugar, estimation of the levels of TNF- $\alpha$ , IFN- $\gamma$ , IL-10, IL-12 (Beckman Coulter, France) using enzyme-linked immunosorbent assay (ELISA).

### Statistical analysis:

The statistical package for social sciences (SPSS) version 8.0 software was used for analysis the data. Quantitative data were represented as mean  $\pm$  standard deviation (SD) and the independent t-test was used to evaluate the significance of differences between mean values of the study variables. Quantitative data were represented as number & percentage (%) and the significance of differences between proportions was performed using the Chi-square test. Pearson correlation coefficient was used to measure the correlation between the studied parameters. P value is considered significant when it is less than 0.05.

## RESULTS

Thirty seropositive HIV male patients with low viral counts after HAART were selected for this study; they were divided into two groups according to their immunological response to HAART. Group I consisted of patients with low CD4 counts, while Group II comprised of patients with high CD4 counts.

The age and BMI of patients in both groups were compared; no significant differences in their means between the groups were observed (as shown in table I). There was highly significant difference as regard virological suppression between the studied groups (as shown in table I). IL-12 levels were significantly higher in Group II than in Group I (mean IL-12 levels of 11.91 versus 6.9,  $p < 0.05$ ).

A positive correlation between IL-12 levels and CD4 counts ( $r = 0.514$ ;  $p < 0.05$ ) were observed (Table II). Similarly, a positive correlation between IL-12 levels and IFN- $\gamma$  levels were noted ( $r = 0.602$ ,  $p < 0.01$ ).

As far as serum cytokine levels are concerned, we found significantly higher levels of the pro-inflammatory cytokine TNF- $\alpha$  in Group I; the

TNF- $\alpha$ /IL-10 ratio is also higher in Group I as compared to Group II, which is suggestive of a stronger pro-inflammatory bias in Group I. Furthermore, there was a significant negative correlation between the TNF- $\alpha$  and the CD4

level (Table II). No significant correlations were observed between IL-12 levels and viral loads and also, no correlation between serum levels of IL-12 and serum levels of IL-10 and TNF- $\alpha$ . (Table II).

**Table I:** Comparison between age, BMI, Interleukin 10, Interleukin 12, IFN- $\gamma$ , TNF $\alpha$ , CD4 count and viral load in studied groups.

|                      | Group I<br>(Target group) | Group II<br>(control group) | Significant<br>Difference (p) |
|----------------------|---------------------------|-----------------------------|-------------------------------|
|                      | n=15                      | n=15                        |                               |
| Age                  | 40.4 $\pm$ 1.4            | 39.4 $\pm$ 1.74             | 0.66 (NS)                     |
| BMI                  | 23.5 $\pm$ 0.39           | 24.02 $\pm$ 0.35            | 0.29 (NS)                     |
| FBS                  | 6.09 $\pm$ 0.18           | 5.66 $\pm$ 0.26             | 0.184 (NS)                    |
| CD4                  | 238.3 $\pm$ 26.17         | 691 $\pm$ 43.93             | <0.0001 (S)                   |
| Viral Load           | 309.1 $\pm$ 67.1          | 697.3 $\pm$ 128.06          | 0.014 (S)                     |
| IL-12                | 6.9 $\pm$ 2.9             | 11.91 $\pm$ 2.8             | 0.05 (S)                      |
| TNF- $\alpha$        | 11.14 $\pm$ 1.76          | 5.58 $\pm$ 0.45             | 0.045 (S)                     |
| IFN- $\gamma$        | 9.73 $\pm$ 1.31           | 13.27 $\pm$ 1.17            | 0.02                          |
| IL-10                | 10.23 $\pm$ 2.37          | 12.11 $\pm$ 3.08            | 0.89 (NS)                     |
| IFN/IL-10            | 1.07 $\pm$ 0.16           | 1.93 $\pm$ 0.29             | 0.81                          |
| TNF- $\alpha$ /IL-10 | 1.34 $\pm$ 0.23           | 0.79 $\pm$ 0.09             | 0.042 (S)                     |

**Table II:** Correlation between Interleukin 10, Interleukin 12, IFN- $\gamma$ , TNF $\alpha$ , CD4 count and viral load in studied groups.

|               | IL-12  |            | TNF- $\alpha$ |            |
|---------------|--------|------------|---------------|------------|
|               | r      | p          | r             | P          |
| CD4 count     | 0.514  | 0.05 (S)   | -0.423        | 0.021 (S)  |
| Viral Load    | 0.398  | 0.087 (NS) | -0.279        | 0.143 (NS) |
| IL-12         | 1.000  | 0          | -0.242        | 0.235 (NS) |
| IFN- $\gamma$ | 0.602  | 0.01 (S)   | 0.419         | 0.091 (NS) |
| IL-10         | -0.286 | 0.151 (NS) | 0.21          | 0.51 (NS)  |

## DISCUSSION

The advent of highly active antiretroviral treatment (HAART) has transformed HIV infection from an inevitably fatal disease and a death sentence, to a chronic condition marked by reduced morbidity and mortality [11]. In this study, we have attempted to identify a possible link between reduced serum IL-12 levels and failure of immunological response to HAART and to demonstrate a possible connection between serum levels of IL-12 and CD4 counts in HIV patients.

We have investigated serum IL-12 levels in a total of 30 HIV patients who were selected from 148 HIV patients and classified according to their response to HAART into two groups: group I (immunological non-responders) and group II (immunological responders). The results of current

study indicated that serum IL-12 levels were significantly elevated in group II when compared to those in group I.

An early immune dysfunction characterized by the gradual erosion of CD4+ T cell is one of the hallmarks of HIV infection since the CD4+ T cells are the primary target of virus infection. HIV infection causes a reduction in levels of CD4+ T cells by three main mechanisms: First, increased rates of apoptosis of infected cells; second, direct viral killing of infected cells; and third, killing of infected CD4+ T cells by CD8+ cytotoxic T lymphocytes that recognize infected cells [7].

Several studies of antigen-specific immune responses concluded that both CD4+ and CD8+ T cell responses were markedly enhanced ex vivo

by the addition of IL-12 [12]. The enhancement of CD4+ T cell antigen-specific responses was boosted further in HIV-infected patients by the demonstration that IL-12 inhibited apoptosis in this cell lineage [13].

Despite the virological suppression is better in Group I than in group II, the CD4 counts in Group II are higher than in group I (Table I). In addition, we found a clear positive correlation between serum IL-12 levels and CD4 count (Table II) which supports the notion that the high levels of IL-12 in group II has an important role in improving CD4 counts in HIV patients.

IL-12 is a central inducer of the Th1 response and cell-mediated immunity by inducing differentiation and proliferation of Th1-type cells and also, by stimulating the production of IFN- $\gamma$  from T cells and NK cells [8]. IFN- $\gamma$  is the primary cytokine that defines Th1 cells: Th1 cells secrete IFN- $\gamma$ , which in turn differentiate CD4+ cells (Th0 cells) to Th1 cells, representing a positive feedback loop, while suppressing Th2 cell differentiation [8]. The current study showed that group I of HIV patients with low CD4 count had significantly lower concentration levels of IFN- $\gamma$  ( $p=0.02$ ) compared to group II of HIV patients with high CD4 count. A clear positive correlation was noted between IL12 levels and IFN- $\gamma$  levels.

In this study, group II had slightly higher concentration levels of IL-10 compared to group I and no significant difference was noted between the two groups. Stylianou et al. [14] observed significantly higher circulating IL-10 levels in HIV-infected patients; the same study demonstrated a significant fall in concentration levels of IL-10 during HAART and observed that HAART had an effect on IL-10 levels. Also, the same study found that the HIV patients on HAART had slightly higher IL-10 levels ( $p=0.008$ ) compared to HIV negative patient [14]. Jane et al., also demonstrated that the HIV patients on HAART had significantly low concentration levels of IL-10 ( $p=0.001$ ) compared to treatment naïve HIV patients [15].

In the current study, we could not find a correlation between IL-12 and IL-10 in the studied groups. Some studies have found that HIV and/or its proteins increases expression of IL-10, an anti-inflammatory cytokine, leading to myeloid dendritic cells suppression [16,17]. High expression of IL-10 has been implicated in the suppression of IL-12 during HIV infection [18], though other studies have shown IL-12 levels to

be independent of IL-10 [19]. Consistent with these latter studies, we did not find evidence to support a role of IL-10 in suppression of IL-12 in group I.

An interesting feature of this study is the finding of a significant difference in the levels of the pro-inflammatory cytokine TNF $\alpha$  between the two groups (Table I). This matches what has earlier been reported by Resino S et al. who found higher levels of TNF- $\alpha$  at lower HIV viral loads [20]. Another report by Hestdal P et al., describes higher levels of serum TNF- $\alpha$  in HIV patients with lower CD4 counts [21]. This is in line with our observation of elevated TNF- $\alpha$  levels in patients with decreased CD4 counts. A plausible explanation for high TNF- $\alpha$  levels in patients with low CD4 counts is that the increased TNF- $\alpha$  may have been produced by other cells such as CD8+ T cells the levels of which may have been increased in these patients. The ratio of TNF- $\alpha$  to IL-10 is higher in group I. In other words the Th1/Th2 cytokine ratio is higher in this group, again suggestive of CD8+ T cell predominance in those subjects.

Finally, we can concluded that there was significant reduction in IL-12 levels in immunologic non-responders HIV patients which is not linked to the immunomodulatory effect of IL10 or TNF- $\alpha$ , on the other hand IL12 showed a statistically significant positive correlation with IFN- $\gamma$ . Therefore, one of the underlying mechanisms leading to a poor immune reconstitution despite good virological control following HAART may be driven primarily by reduction in the concentration levels of IL-12. Although, we found that IL12 production was correlated with IFN- $\gamma$ , the mechanism by which the reduced production of IL-12 in immunologic non-responders HIV-1-infected patients remains poorly understood.

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