Comparison and Correlation of CD\textsuperscript{4}T Cells Count with Viral Load Prior to and after Initiating HAART in HIV Iraqi Patients

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Abstracts

Background: HIV that cause of AIDS worldwide distributed and the CD\textsuperscript{4} positive T cells consider chief target cells of HIV. Elimination of HIV is the final goal of HIV treatment, but is rarely achieved.

Objective: As CD\textsuperscript{4}T cells count using flow cytometry recently available, we investigated whether CD\textsuperscript{4}T cells count can substitute for HIV plasma viral load RNA quantification in treatment monitoring through comparison the change in CD\textsuperscript{4} positive T cells count in patients with HIV previous to & afterward initiating “highly/active/antiviral/therapy”=‘HAART’ & correlation of CD\textsuperscript{4} T cells counts with viral load prior to and after initiating HAART in HIV Iraqi patients.

Method: Within this study, 25 HIV patients were prospectively analyzed. Patients have been treated with Bilateral/Didanosine, combination (Efavirenz, Emtricitabine, and Tenofovir Disoproxil Fumarate), in addition to Quadruple combination (Elvitegravir,cobicistat,emtricitabine,tenofovir alafenamide (TAF). Quantitative CD\textsuperscript{4}T cells count were determined with flowcytometry. HIV plasma viral load RNA were determined with RT-PCR at given time points.

Results: We find weak negative non-significant correlation (R=−0.267, p≈ 0. 197 & R=−0.161, p≈0. 441) among CD\textsuperscript{4}positive T cells counts & ‘plasma viral load of HIV RNA’in both treated and untreated patients respectively. More importantly, there was significant concordance between an increase or decrease of CD\textsuperscript{4}T cells counts with HIV RNA plasma viral load prior and after initiating of treatment. However, the curve and increase of CD\textsuperscript{4}T cells count enabled prediction of eventual of viral clearance.

Conclusions: Quantitative CD\textsuperscript{4}T cells count cannot substitute for HIV RNA plasma viral load quantification during assessment of antiviral therapy: However, the increase of CD\textsuperscript{4} T cells count does predict eventual HIV RNA plasma clearance. A 2 log 10 increase to above 200 IU/ml is associated with a high likelihood of HIV plasma RNA clearance.

Keywords: Plasma viral load of HIV, CD4 positive T cell counts, Pearson correlation.

Introduction

HIV that cause of AIDS worldwide distributed and the CD\textsuperscript{4}positive T lymphocytes consider the chief target cells of HIV. The reduction of CD4 positive T lymphocytes establishes a significant assurance of AIDS (Becerra et al., 2016\textsuperscript{(1)}). Monitoring of successful or failure of HAART against HIV mainly through RT-PCR (Stevens et al., 2010\textsuperscript{(2)}). High number of HIV plasma viral loads mean destruction high number of CD\textsuperscript{4}T cells and increase of opportunistic infection. Treatment with highly active antiviral therapy (HAART) may decrease of HIV plasma viral loads and increase of CD\textsuperscript{4} T cells \textsuperscript{(3)}.
Previous study showed that in many viral diseases viral load correlates with viral proteins in the serum; for example, a relationship was demonstrated between levels of HIV viral load and p24 (4). Likewise, a strong correlation has been reported between serum CMV DNA viral loads and cytomegalovirus (CMV) pp65-positive cells (5). Authors previously described a good correlation between levels of hepatitis C virus (HCV) core antigen determined by the Trak-C assay and HCV RNA viral load (6-8). Accordingly, few studies have looked into the character of CD4 positive T lymphocytes quantification relation to treatment response and its prediction(9,10). In light of the fact that quantitative assays for CD4 T cells have recently become available through using of flowcytometry, the aim of this study was to investigate the correlation and concordant increase and decrease of CD4 positive T lymphocytes relation to “plasma viral load of HIV RNA” in patients before & after therapy with “highly/active/antiviral/therapy” “HAART” that include Bilateral Didanosine, Triple combination (Efavirenz, Emtricitabine, and Tenofovir Disoproxil Fumarate) and Quadruple combination (Elvitegravir, cobicistat, emtricitabine, tenofovir alafenamide (TAF).

Method

Routine databaseself-control experimental study were conducted for symptomatic HIV-1 infected patients during a period between 5October 2018 and 1 May 2019 in central public health laboratory. The mean age of HIV, patients were (34.50±11.45 years). Quantitative CD4 positive T lymphocytes countusing flowcytometry & “plasma viral load of HIV RNA” viaRT-PCR had been measured at particular time points prior therapy initiation of for all patients. Of 25 HIV patients, seven patients have been treated with Bilateral/Didanosine, 12 patients have been treated with Triple combination (Efavirenz, Emtricitabine, and Tenofovir Disoproxil Fumarate) and 6 patients have been treated with Quadruple combination (Elvitegravir, cobicistat, emtricitabine, tenofovir alafenamide (TAF))(n=6). The antiviral therapies were performed within Institutional Reviewer Board (IRB) approved treatment protocols. Anbar medical College approved the protocol. All patients provided informed consent for participation in the study.

HIVRNA plasma viral loads quantification:
HIVRNA plasma viral loads quantification was performed within the routine clinical case using RT-PCR at given time points prior and after to initiation of therapy for all patients according to manufacturer criteria (Abbott laboratories, North Chicago, Illinois, USA).

Quantitative CD4 levels: Quantitative CD4 T cells countwere determined with flowcytometry at given time points prior and after to initiation of therapy for all patients as descried by manufacturer company.

Statistical Examination: Statistical investigation had been done though using the “SPSS/software 24.0”. Correlations between CD4 positive T lymphocytes count & “plasma viral loads of HIV RNA”prior & after therapy were calculated according to Pearson correlation. Difference in the efficacy of antiviral therapies and between HIV RNA plasma viral before and after treatment and between CD4T cells count before and after treatment was tested by Wilcoxon signed rank test. Difference in the concordance results was tested by chi-square test.

Results

Correlation of CD4positive T lymphocytes count & “plasma/viral/loads of HIV/RNA” were negatively correlated to 25 HIV infected patients. A weak negative correlation amongstCD4positive T lymphocytes countwith ‘plasma/viral/ load of HIV/RNA’ after treatment as determined by Pearson correlation(R=−0.161; p-values 0. 441) (Figure 1B).

To exclude that this weak negative correlation is a treatment effect, we next study only those patients who had a sample available before therapy. CD4positive T lymphocytes count & “plasma/viral/loads of HIV/RNA” had been correlated with 25 HIV patients prior to initiation of therapy (R=−0.267; p-values 0. 0. 197) (Figure 1A). In these treatment-naïve patients, there was a weak correlation of “ plasma/viral/load of HIV/RNA” with CD4positive T cells count(Figure 1A).

Plasma CD4positive T lymphocytes count versus ’ “plasma viral load of HIV/RNA” “before treatment showed that mean of CD4positive T cells & “plasma viral load of HIV/RNA” “was 321.56 (IU/ml,566148.33 (copy/ml) respectively, whereas mean of CD4 T cells count increased to 652.08 (IU/ml) and mean of HIV RNA plasma viral loads decrease to 288957.7273 (copy/ml) after treatment. Corr. Coef (r) between CD4positive T lymphocytes count & “plasma/viral/loads of HIV/RNA” prior & after treatment with HAART were -0.267 (P=0. 197),-0.161 (P=0. 441) respectively using Pearson’s correlation coefficients (r)
Concordance of increasing and decreasing of CD$^+$ T lymphocytes count (Figure 1) in relation to HIV RNA plasma viral loads changes.

This analysis revealed that before treatment, the minimum "plasma/viral/loads of HIV/RNA" & CD$^+$ T cells count were 77685 copy/ml, 110 IU/
ml respectively and the maximum "plasma/viral/loads of HIV/RNA" copy/ml and CD4positive T cells count were 989866 copy/ml, 780 IU/ml respectively (the mean of "plasma/viral/loads of HIV/RNA" 566148.33 copy/ml & CD4positive T lymphocytes count 321.56 IU/ml), whereas after treatment the minimum "plasma/viral/loads of HIV/RNA" copy/ml and CD4positive T lymphocytes T cells counts were 32142.00 copy/ml, 179 IU/ml respectively & Maximum "plasma/viral/loads of HIV/RNA" copy/ml and CD4positive T lymphocytes T cells count were 910880.00 copy/ml, 1200 IU/ml respectively (the mean of "plasma/viral/loads of HIV/RNA" 288957.7273 copy/ml & CD4 T cells count 652.08 IU/ml).

This analysis revealed that no significant difference for "plasma/viral/loads of HIV/RNA" & CD4positive T lymphocytes count before and after treatment (P=0.197, P=0.441 respectively) (Table 1). This is weak negative correlation, through CD4positive T lymphocytes counts decreasing associated with increasing of "plasma viral load of HIV/RNA" before treatment & CD4positive T lymphocytes count increasing associated with decreasing of "plasma viral load of HIV RNA" after treatment as shown in (Table1).

This study showed that statistically major change between "plasma viral load of HIV RNA" before & after treatment (P=0.001) and also there was statistically significant difference between CD4positive T cells count before and after treatment via Wilcoxon test(P=0.001) (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>P.value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma viral before treatment</td>
<td>25</td>
<td>77685</td>
<td>989866</td>
<td>566148.33</td>
<td>0.001</td>
</tr>
<tr>
<td>Plasma viral after treatment</td>
<td>25</td>
<td>32142.00</td>
<td>910880.00</td>
<td>288957.7273</td>
<td>0.001</td>
</tr>
<tr>
<td>CD4 T cells counts Before treatment</td>
<td>25</td>
<td>110</td>
<td>780</td>
<td>321.56</td>
<td></td>
</tr>
<tr>
<td>CD4 T cells counts After treatment</td>
<td></td>
<td>179</td>
<td>1200</td>
<td>652.08</td>
<td></td>
</tr>
</tbody>
</table>

This study showed that concordance between increasing of CD4positive T lymphocytes count & decrease of "plasma/viral/loads of HIV/RNA" before and after treatment with different antiviral therapy as shown in Table 2. However, this study was not extended to analysis the different strengths of different treatments, as too few patients at a scattered time points could be included prohibiting a real analysis(Table 2).

<table>
<thead>
<tr>
<th>Treatment type (No.)</th>
<th>CD4 T cells counts before treatment</th>
<th>CD4 T cells counts after treatment</th>
<th>HIV RNA plasma viral loads before treatment</th>
<th>HIV RNA plasma viral loads after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral/Didanosine (7)</td>
<td>244.00</td>
<td>569.71</td>
<td>675862.52</td>
<td>439544.2468</td>
</tr>
<tr>
<td>P. value before and after treatment</td>
<td>P=0.000</td>
<td>P=0.001</td>
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<tr>
<td>Triple combination(12)</td>
<td>353.08</td>
<td>609.50</td>
<td>535688.75</td>
<td>273481.7879</td>
</tr>
<tr>
<td>P. value before and after treatment</td>
<td>P=0.000</td>
<td>P=0.001</td>
<td></td>
<td></td>
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<tr>
<td>Quadruple combination(6)</td>
<td>349.00</td>
<td>833.33</td>
<td>499067.61</td>
<td>144225.3333</td>
</tr>
<tr>
<td>P. value before and after treatment</td>
<td>P=0.000</td>
<td>P=0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (25)</td>
<td>321.56</td>
<td>652.08</td>
<td>566148.33</td>
<td>288957.7273</td>
</tr>
<tr>
<td>P. value before and after treatment</td>
<td>0.459</td>
<td>0.226</td>
<td>0.375</td>
<td>0.079</td>
</tr>
</tbody>
</table>
Analyzing patients with an increase of CD\textsuperscript{4} T cells counts to > 200 IU/ml when analyzing all patients who achieved an CD\textsuperscript{4} T cells counts > 200 IU/ml (Figure 2), the graphical HIV RNA plasma viral loads near from negative organization suggests that there are three patients likely to eventually become HIV RNA plasma viral loads negative on the basis of a continuous sleep decline of their CD\textsuperscript{4} T cells counts. Because of delay between performing this study and preparation for publication, we were able to proven this hypothesis.

Performing a multiple regression analysis including baseline CD\textsuperscript{4} T cells counts baseline HIV RNA plasma viral loads in of the HIV patients and form of therapy revealed that an increase of CD\textsuperscript{4} T cells counts to > 200 IU/ml was the most relevant predictor of potential HIV RNA plasma viral loads clearance (P<0.001). A 2 log 10 increase to above 200 IU/ml is associated with a high likelihood of HIV plasma RNA clearance.

Figure 2: Increase in CD\textsuperscript{4} T cells count with decrease "plasma/viral/loads of HIV/RNA" after antiviral therapy over time

CD\textsuperscript{4}positive T cells count quantification can predict of HIV clearance: In the patients well to modern antiviral therapy, HIV RNA plasma viral loads will become undetectable at some point; however, it would be useful to be able to predict whether a patient might be eventually become with increasing CD\textsuperscript{4} T cells counts or whether an alternative treatment approach is required (Figure 3 A,B).

Figure 3 A,B indicate the potential strength of CD\textsuperscript{4} T cells counts in this regard: it enables further monitoring in in patients who have already become HIV RNA plasma viral loads negative. However, discriminating their CD\textsuperscript{4} T cells counts curves indicates that only one of these patients will probably clear of CD\textsuperscript{4} T cells counts in the long term (Figure 3, Figure 4).
Discussion

In this study, we demonstrate only a poor negative correlation for CD$^{+}$ T cells count & “plasma viral load of HIV RNA” could be shown. Poor negative correlation of “plasma viral load of HIV RNA” with CD$^{+}$ T cells count could result from the HIV can infect other cells like macrophage, monocyte, dendritic cell and some rectal lining cells that express or contain low level of CD$^{+}$ and core receptors (Brooks et al., 2010). This study was consistent with a recent paper that did report a correlation but the R-value was only
0.26\(^{(12)}\), which is much lower than the r-value of > or = 0.5 usually accepted as correlation.

A slightly higher R-value of 0.37 was observed in CD\(^{4}\)positive T cells positive HIV patients, where high CD\(^{4}\)positive T/cells count were also determined in HIV patients in the absence of detectable “HIV RNA plasma viral loads”\(^{(13)}\). Much higher correlations with R-values up to 0.79 were reported from America \(^{(14)}\). When we tested 25 HIV patients with the flowcytometry assay, their CD\(^{4}\)positive T/cells count negatively correlated with "HIV/RNA/plasma/viral/loads" after therapy (R-values of - 0.161; P =0.441).

More important than the correlation of CD\(^{4}\)T cells count with "HIV/RNA/plasma/viral/loads", is the concordance of decrease and increase of these parameters. In our analysis, good concordance was observed between vicissitudes in CD\(^{4}\)positive T cells count & “HIV/RNA/plasma/viral/loads”. Similarly, disconcordant fluctuation of CD\(^{4}\)positive count versus “HIV/RNA/plasma/viral/loads” described in/patients continuously responding to Quadruplecombination, patients developing resistance to Quadruplecombination\(^{(15)}\). As HIV RNA plasma viral loads mostly becomes undetectable during treatment with potent antivirals\(^{(15)}\), quantification of CD\(^{4}\)positive T cells count will be helpful to further monitor the therapeutic efficacy to antiviral therapies once “HIV RNA plasma viral loads” become negative, especially its aim is eventual HIV elimination. Our study shows for the first time the increaseof CD\(^{4}\)positive T cells count during therapy with different antivirals predicts HIV clearance. We demonstrate here that CD\(^{4}\)positive T cells kinetics can predict eventual HIV clearance, but also report that CD\(^{4}\)positive T quantification cannot substitute for “HIV RNA plasma viral loads” quantification.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

