

# A Retrospective Analysis of Discordant CD4 and Viral Load Responses in HIV Patients on Anti-Retroviral Therapy

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**Abstract-** In industrialized countries 15%-30% of patients receiving long-term highly active antiretroviral therapy have discordant responses with a poor CD4 count response despite full suppression of HIV replication, 6 months to 2 years after starting therapy<sup>(1, 2)</sup>. There are few data representing the scenario of such discordant cases in developing countries like India.<sup>(1)</sup> The present study was aimed to find the rate of discordant responses among HIV patients attending a tertiary care centre in Navi Mumbai.

**Methodology:** The CD4 counts and viral loads of 142 treatment naïve HIV 1 patients who were put on anti retroviral treatment were carried out by Immunocount assay and Real Time PCR respectively. The counts obtained every three months during January 2009 to December 2011 were analysed and mean CD4 counts and viral load values calculated.

**Results:** On the basis of the mean values, the patients were categorized into four groups based on different permutations and combinations of the low or high CD4 counts and viral loads. Only 30.95 % of individuals showed an improvement in CD4 counts and reduction in viral load while 35.71% showed a discordant response. The paired 't' test was applied using SPSS 17 software; all the 4 categories gave a p value of < 0.05 showing statistical significance of the data.

**Conclusion:** HIV positive patients on ART may show discordance in the CD4 and viral load counts. This should be kept in mind by clinicians while monitoring the progression of HIV disease.

**Index Terms-** HIV naïve patients, Discordant CD4 and viral load count, anti retroviral treatment.

## I. INTRODUCTION

Antiretroviral therapy (ART) is the recommended treatment for HIV infection. ART involves taking a combination (regimen) of three or more anti-HIV medications daily.

- Although anti-HIV medications can't cure HIV, people with HIV are enjoying healthy lives and living longer thanks to ART. The introduction of ART into clinical practice has led to dramatic reductions in morbidity and in mortality associated with infection with the human immunodeficiency virus (HIV).
- Viral load and CD4 counts are two important parameters used to monitor the progression of HIV /AIDS and make critical decisions regarding antiretroviral therapy.

- The initiation of HAART generally leads to a rapid reduction in HIV-1 RNA plasma levels and to an increase in peripheral CD4+ cell counts.<sup>1-3</sup> However, some patients experience a 'discordant response', whereby the HIV-1 RNA plasma level is suppressed but the CD4+ cell count response is blunted. Other patients exhibit a different pattern of discordant response, characterized by a sustained CD4+ cell count response, despite persistent viraemia.
- In industrialized countries, discordant responses have been reported to occur in 20–30% of patients 6 months to 2 years after starting therapy. Studies have shown that 15%-30% of patients receiving long-term highly active antiretroviral therapy (HAART) have discordant responses with a poor CD4 count response despite full suppression of HIV replication.<sup>(1,2)</sup> However there are few data representing the scenario of such discordant cases in developing countries like India.<sup>(1)</sup> The present study was aimed to find the rate of discordant responses among HIV patients attending a tertiary care centre in Navi Mumbai.

## II. RESEARCH ELABORATIONS

### Aims and objectives:

- To carry out a three years retrospective analysis of the CD 4 count and viral load in patients with HIV/AIDS.
- To detect the rate of discordant immunological and virological response among these patients.

## III. METHODOLOGY

### **I. Selection of the Data and study participants:**

- We included the data of one hundred and forty two treatment naïve patients who were above eighteen years of age with baseline CD4 cell counts of 200 cells/ml, who visited the tertiary care center from the year January 2009 to December 2011. Among the patients, twenty two were female and sixteen were male.
- Patients who failed to follow-up, died or if they had no plasma HIV RNA testing performed for 1 year were excluded from the study.

- Patients, put on anti retroviral drug therapy were asked to fill a drug request form that compiles information on applicant's address, past HIV specific drug history, previous CD4 and viral load counts, clinical history if any. The patients were counseled before and after collection of the blood for CD 4 counts and viral load test.
- HAART was defined as treatment with three antiretroviral drugs, including two nucleoside reverse-transcriptase inhibitors plus a non-nucleoside reverse-transcriptase inhibitor or a protease inhibitor.

#### IV. TEST PROCEDURES FOR CD4 AND VIRAL LOAD TESTS

The counts were obtained every three months during January 2009 to December 2011.

##### a. **CD 4 counts:**

The patient's blood samples were analysed for CD4/CD8 count by antigen-antibody slide reaction, a method which makes use of combination of antibodies such as monoclonal antibodies against CD4 & CD8, peroxidase anti peroxidase complex tagged rabbit anti mouse. In brief, the WBC's are obtained by histopaque (Sigma Aldrich) method and coated on a printed slide. Fixation is done by 25 % gluteraldehyde, followed by treatment with mixture of antibodies. Slides are then subjected to a mix of substrate and chromogen (1.25 ml: 1.5ml) and then counterstained with hematoxylin. Finally the cells are counted microscopically.

##### b. **RNA extraction:**

RNA was extracted according to the kit manufacturer's instructions (Qiagen, Hilden, Germany) using spin columns and a series of wash and elution buffers.

##### c. **Real time PCR:**

- Measurement of HIV 1 blood plasma level was performed by Real time Polymerase chain reaction using the Taqman principle.
- Real time PCR reactions were performed using, Roche Lightcycler master mix. Each reaction consisted of 1X Roche Lightcycler master mix, internal control HIV specific primer pairs and fluorescently-tagged hydrolysis probes (5' reporter dye and downstream quencher dye) in a total reaction volume of 25  $\mu$ l.
- Primers and probe target the gag gene of HIV 1
- Reactions were performed using a Roche Lightcycler 480 thermal cycler utilizing a 96 well plate.
- Thermal cycle conditions
  - Initial holding step of 50<sup>0</sup>C/15mins.
  - Second hold at 95<sup>0</sup>C/10 minutes.
  - Denaturation step at 95<sup>0</sup>C / 15seconds.
  - Annealing step at 55<sup>0</sup>C/20 seconds.
  - Extension step at 72<sup>0</sup>C/15 seconds
  - Followed by 45 cycles
  - Fluorescence was detected after every 72<sup>0</sup>C extension incubation in FAM and JOE channel.
  - For standard curves, real time PCR was performed on a 10-fold dilution

series of kit specific positive controls ranging from 10<sup>1</sup> to 10<sup>7</sup>copies/mL.

- Quantitation of PCR products was performed using Roche Lightcycler 480 software. Color compensation was turned on in all assays to subtract bleed through between adjacent channels used to detect specific fluorescent tags.
- Analytic range of the assay is 48 - 10,000,000 HIV-1 RNA copies/mL.

##### d. **Statistical analysis**

The paired't' test was applied on the data using SPSS 17 software.

#### V. RESULTS

- The mean CD 4 count and viral load of the patients were calculated.
- On the basis of the mean values, the patients were categorized into four groups based on different permutations and combinations of various immunological and virological responses.
- Only 30.95 % of individuals showed an improvement in CD4 counts and reduction in viral load while 35.71% showed a discordant response, while 28.57% patients did not show any improvement immunologically and virologically.
- Other studies on this subject have reported a range of results. Piketty et.al found equal rates (10.5%) of immunological only and virological only responses. Another study from Canada reported 11.7% and 15.4% out of 1547 subjects showed immunological and virological only responses respectively. In a study conducted in France, out of 2236 patients 19% and 17.3% patients exhibited immunological and virological only responses respectively. In all the above studies, the definition of discordant responses varied considerably.
- There was no association between the sex of the patient and the discordance observed. However the patients belonging to category 4 with suppression of viral load and a blunted CD4 response were of older age group (> 50). This can be attributed to the host characteristic (old age), as immune restoration depends upon the thymus activity which decreases with age.<sup>(3,4)</sup>
- The paired't' test was applied on the data using SPSS 17 software; all the 4 categories gave a p value of < 0.05 showing statistical significance of the data.

## VI. CONCLUSION

- HIV positive patients on HAART may show discordance in the CD4 and viral load counts.
- Immunological and virological responses can be an important factor in determining the survival rate of the patients. This should be kept in mind by clinicians while monitoring the progression of HIV disease.
- Little is known about the pathogenesis of discordant responses, which seems to depend on the interaction of a multitude of viral, host and treatment-related factors.<sup>(1)</sup>
- Further research is required to understand the risk factors and pathogenesis, prognosis and clinical management associated with the discordant treatment responses despite its relative frequency.
- There is a need to develop a standardised and universally accepted definition for discordant response which will help in defining the therapy responses having clinical significance for the patients and designing trials of possible therapeutic interventions.

## REFERENCES

- [1] Mauro S and Suely HT (2006) Discordant immunological and virological responses to antiretroviral therapy. *Journal of Antimicrobial Chemotherapy* 58:506–510.

- [2] Gazzola L, Tincati C, Bellistri GM, Monforte A, Marchetti G (2009) The absence of CD4 T cell count recovery despite receipt of virologically suppressive highly active antiretroviral therapy: clinical risk, immunological gaps and therapeutic options. *Clin Infect Dis* 48:328-337
- [3] Nicastrì E, Chiesi A, Angeletti C et al (2005) Clinical outcome after 4 years follow-up of HIV-seropositive subjects with incomplete virologic or immunologic response to HAART. *J Med Virol* 76: 153–60.
- [4] Moore DM, Hogg RS, Yip B et al (2005) Discordant immunologic and virologic responses to highly active antiretroviral therapy are associated with increased mortality and poor adherence to therapy. *J Acquir Immune Defic Syndr* 40: 288–93.

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