Evaluation of Levels of p24 Antigen in HIV/AIDS Cases and Correlation with CD4 T Cell Counts

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Abstract

CD4 T cell count and p24 antigen concentration were determined in blood collected from 149 HIV infected patients. HIV infection status was confirmed as per the National HIV testing policy. p24 antigen quantification was done by heat-mediated immune complex dissociation and tyramide signal amplification-boosted ELISA, which has a diagnostic sensitivity similar to that of polymerase chain reaction (PCR). CD4 cells were estimated on FACSCount. Correlation of CD4 T cells and levels of p24 antigen revealed p24 antigen level to be a significant predictor of CD4 T cell decline. CD4 T cell counts at baseline showed that the superiority of p24 measurement was more pronounced at lower levels of CD4 cells (< 200 cells/µl). p24 antigen level may be of interest as a simple and inexpensive predictive marker of disease progression. p24 antigen level correlated well with that of CD4 lymphocyte count (< 50 CD4 cells r = -0.626, p = < 0.001 and 50 - 200 CD4 cells, r = -0.531, p = 0.016). The lowest level of p24 antigen measurable was 1,905 fg/ml in a patient with CD4 cell count of 222 cells/µl. p24 antigen could be estimated even in patients on ART with stably suppressed viraemia.

Key words: p24 antigen, CD4 T cells, CD8 T cells and HIV.

Introduction

Viral load measurement has become an integral part – and almost indispensable – in the management of patients infected with the human immunodeficiency virus (HIV). This parameter is used to predict progression of HIV disease, assess response to anti-retroviral treatment (ART), and to predict drug failure. However, the viral load assay is not cost-effective for the countries with limited resources like India. The p24 antigen is a major internal structural protein of the human immunodeficiency virus. HIV appears in serum at high level during the primary HIV infection and in the stages of AIDS and advanced AIDS1-3. p24 antigen is an excellent marker of HIV expression, replication level, and disease activity, that can be used in the same fields of application as HIV plasma RNA is used4-6.

In fact, p24 antigen may emerge as an important, and relatively cheaper marker comparable to viral load, which can be used in place of viral load and be of value in the resource-poor countries.

Recently it was shown that heating of plasma or serum sample caused irreversible dissociation of immune complexes leading to improved detection of p24 antigen in HIV infected European individuals. Presence of p24 antigen-antibody complexes lowers the sensitivity for detection of p24 antigen test7. This problem has been observed to be particularly prominent in HIV infected African individuals8. p24 antigen quantification involving heat-mediated immune complex dissociation and tyramide signal amplification-boosted ELISA gave diagnostic sensitivity similar to that of the RNA quantification by commercial polymerase chain reaction (PCR) kit9. The signal amplification boosted HIV-1 protein 24 (p24) antigen level in heat denatured plasma was comparable with HIV-1 RNA level in predicting CD4 lymphocyte decline and survival among persons with the advanced HIV disease10 and was an effective tool for monitoring response to anti-retroviral therapy. The predictive power of heat denatured p24 antigen level is reported to be comparable with that of CD4 lymphocyte count and/or HIV 1 RNA level, given the low cost11, the test could be of particular benefit in countries with the highest burden of HIV infection and limited resources.

Hence, a cross sectional study was undertaken to evaluate the role of p24 antigen quantification in HIV infected individuals and to find out whether there is any correlation between CD4 T lymphocyte count and p24 antigen level by performing p24 antigen assay using the heat denatured plasma samples.

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Materials and methods

Subject population

Two-hundred and thirty-three persons with various risk groups referred from various hospitals in Delhi during the period 2002 - 2004 were included in the study. Blood samples were collected and HIV diagnosis was confirmed by detection of HIV antibodies as per NACO guidelines. Out of these, 149 samples were confirmed HIV positive by Western blot assay. These HIV positive individuals on uniform anti-retroviral therapy (first line regimen as recommended by NACO) were divided into three groups: 1) with peripheral CD4 count < 50; 2) 50 - 200 cell/µl; and 3) with CD4 count above 200 cells/µl.

Seventeen (n = 17) samples giving discordant serological results on HIV testing were included for p24 antigen assay. In addition to that, 67 HIV serologically negative samples from cases suffering from infections like hepatitis, tuberculosis, malaria, and leishmaniasis (n = 67) were also included in the study.

CD4/CD8 T lymphocytes enumeration was done by flow cytometry. In brief, a standard flow cytometry method with lysed whole blood and a panel of two combinations of fluorescein isothiocyanate and phycoerythrin conjugated monoclonal antibody reagents obtained from a single manufacturer (Becton-Dickinson, San Jose, California) were used to determine the expression of each antigen or antigen combination following the instructions of the manufacturer12. Data acquisition was performed on configured FACS Count. p24 antigen level was estimated by ELISA method (according to the manufacturer's protocol). The kits were obtained from Perkin Elmer Life Sciences, Wallac Oy, Finland Turkey. Briefly, the immune disruption of plasma samples was done by using the 1:6 sample dilution on a dissociation buffer followed by boiling for 5 minutes at 100°C. Samples were cooled and transferred to micro plate wells that are coated with a highly specific mouse monoclonal antibody to capture both free HIV-1 p24 and that which has been released upon disruption of immune complexes in the plasma samples. The captured antigen is complexed with biotinylated polyclonal antibodies to HIV 1 p24 antigen followed by streptavidin +HRP conjugate step. Signal is detected after incubation with OPD which produces a yellow colour that is directly proportional to the amount of HIV-1 p24 antigen captured. Absorbance was analysed on ELISA reader at 492 nm wavelength.

Correlation between CD4 T cells and p24 antigen level could be done in 75 cases.

Statistical analysis: Proportions were analysed by Spearman's correlation.

Results

A total of 233 samples were included in the study. 52.5% were males and 47.5% were females. Mean age of patients studied was 35 ± 1. Evaluation of performance characteristics in terms of sensitivity and detection limit of p24 viral antigen was done in comparison to CD4 T cell counts.

Out of 17 discordant samples, two samples were found to be positive by p24 antigen detection system with an antigen level of 12,080.0 fg/ml and 24,550 fg/ml, respectively.

Out of 149 Western blot confirmed serologically positive cases of HIV, p24 antigen was detected in 143 cases. Sensitivity was found to be 96% and specificity was found to be 97.1%. Lower limit for p24 antigen detected was 1,905 fg/ml in a patient with CD4 T cell count of 222 cells/µl and highest limit of p24 antigen detected was 16,62,000 fg/ml in a patient with CD4 cell count of 194 cells/µl. In the HIV positive group with CD4 T cell count of < 50 correlation between CD4T cells and p24 antigen level was found to be –0.626 and p value was found to be < 0.001 (Fig. 1). Whereas, in the group with CD4 T cell count ranging between 50 - 200 cells/µl the correlation with p24 antigen levels was found to be –0.531 and p values 0.016 (Fig. 2). Correlation of CD4 T cell values ranging > 200 and p24 antigen levels was found to be 0.141 There was no significant correlation between CD4 T cell counts and p24 antigen levels at counts ranging > 200 cells/µl (Fig. 3).

Table I gives the mean and standard deviations of CD4 T cells and p24 antigen levels in the three HIV positive groups with variable ranges of CD4 T cells. Table II gives the correlation between the mean CD4 T cell values and the mean p24 antigen levels in the three groups studied.
Table I: The mean of amount of p24 antigen detected at various levels of CD4 cells.

<table>
<thead>
<tr>
<th>CD4 cells &lt; 50/mm</th>
<th>N</th>
<th>Mean ±</th>
<th>Standard deviation ±</th>
</tr>
</thead>
<tbody>
<tr>
<td>p24 antigen detected</td>
<td>25</td>
<td>± 24.24</td>
<td>± 12.98</td>
</tr>
<tr>
<td>CD4 50 - 200 cells/mm</td>
<td>20</td>
<td>± 141.40</td>
<td>± 32.96</td>
</tr>
<tr>
<td>p24 antigen detected</td>
<td>20</td>
<td>± 4,05,565.45 fg/ml ± 8,93,038.16 fg/ml</td>
<td></td>
</tr>
<tr>
<td>CD4 &gt; 200 cells/mm</td>
<td>25</td>
<td>± 321.88</td>
<td>± 104.85</td>
</tr>
<tr>
<td>p24 antigen detected</td>
<td>25</td>
<td>± 9,619.16 fg/ml ± 17,646.19 fg/ml</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td></td>
<td></td>
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</tbody>
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Table II: Correlation coefficient.

| CD4 cells < 50/mm and p24 antigen level (Figure 1) | Correlation = -0.626 | p - value < 0.001 |
| CD4 cells 50 - 200/mm and p24 antigen level (Figure 2) | Correlation = -0.531 | p - value = .016 |
| CD4 cells > 200/mm and p24 antigen level (Figure 3) | Correlation = +0.141 | p - value = .500 |

Discussion

We found a single step procedure for immuno-complex dissociation that does not require chemical reagents easy to perform and feasible. The test can easily be performed by laboratories undertaking ELISA tests for HIV diagnosis.

In an HIV infected individual there is an initial peak of viral replication which is followed by a period during which little virus and/or p24 antigen is present in plasma (clinical latency stage). It has been reported that p24 antigen level was a significant predictor of CD4 T cell count and was superior or equivalent to plasma viral RNA level.

CD4 count at baseline showed that the superiority of p24 - antigen measurement was more pronounced at lower levels of CD4 cells (< 200 cells/µl) as shown by a negative correlation between the two parameters. In our study we also found as expected that the p24 antigen level decreases with increase in CD4 lymphocyte count.

Low levels of p24 antigen may not always be due to immune complex formation but may reflect an effective host immune response which is initially successful in restricting/ controlling the viral replication. There is an inverse relationship between p24 antigen and the CD4 lymphocyte count particularly when the CD4 counts range from < 50 cells to < 200 cells/µl of blood. Increase in free antigen level usually occurs, only when there is a decline in p24 antibody and a drop in the count of CD4 T lymphocytes either early during infection, stage of AIDS and/or in cases of failure of ART.

Viral protein like p24 antigen could be at least as good a marker of HIV disease activity as plasma viral load, provided that it is measured with assays having sufficient sensitivity and accuracy. The assay used has been found to have a sensitivity of 96% and specificity of 97.1%. The p24 antigen assay is inferior to RT-PCR in detecting HIV infection. The p24 antigen detection assay used in the present study has been reported to have sensitivity and specificity similar to RT-PCR for detecting infection in early and late stages of HIV infection when the HIV viral load is expected to be high.

The results of the study show that the p24 antigen quantification may be suitable for monitoring anti-retroviral treatment in both adults and children, particularly in resource-poor countries like India. p24 antigen was measurable even in patients with stably suppressed viraemia, and its concentration correlated negatively with less than 200 CD4 T cells concentration, and positively with the concentrations of activated CD8 T cell subsets.
Since the half-life of virus particles is only a few hours\(^{14}\), if not minutes\(^{15}\), a large part of trapped virus will be degraded and viral RNA will be digested. In agreement with this, the viral RNA load in the acute seroconversion phase is high in lymphoid tissues, but low in plasma. So p24 antigen marker is extensively used for diagnostic purpose in the early infection phase rather than as an indicator of the viral load.

p24 antigen concentrations are similarly high in patients exhibiting 100, 200 or 300 CD4 T cells/µl. While patients with 50 CD4 cells/µl or below exhibit slightly lower antigen concentrations\(^{16}\). Our study agrees with the above findings, i.e., p24 antigen with CD4 T cell count < 50 correlation was found to be –0. 626, and in patients with CD4 cell counts ranging between 50 - 200 cells/µl the correlation was found to be 0.531.

It is likely that the destruction of the follicular dendritic cell network, which is typically present in advanced HIV disease, leads to a decreased retention and destruction of HIV particles, and more virus reaches the peripheral blood\(^{17}\). A reduced virus production in the final stage, as suggested by the decreasing concentrations of p24 antigen in plasma would be in keeping with the total destruction of the CD4 T cells. Inverse correlation shows that total p24 antigen increases when the host immune response is depressed.

The level of heat denatured p24 antigen thus predicted subsequent clinical progression in early stage of HIV infection, and closely correlated with CD4 T lymphocyte counts\(^{18}\). p24 antigen may be detectable transiently during primary HIV infection and again after years of HIV infection with clinical or laboratory evidence of immunodeficiency\(^{19}\). Free antigen may be detected during the period before sero-conversion and during the late symptomatic phase of HIV disease. We have observed that the level of p24 Ag by ICD assay is likely to be high when the CD4 lymphocyte level is low.

The ability of heat denatured p24 antigen level to predict HIV disease progression among adults with various risk groups and the use of the test to resolve the result of serologically discordant samples has not been assessed.
Conclusion

Heat dissociated (HD) p24 antigen is a sensitive, specific, and quantitative marker for HIV in adults. These properties together with the other advantages of ELISA such as simplicity and assay speed, the possibility of automation and effectively low cost render testing for HD-Ag an attractive alternative to the technically demanding and expensive PCR and expensive viral load assay. The systematic use of HD-Ag contributes to earlier diagnosis, identification of, and to better knowledge of quantitative viral aspects in the pathogenesis of AIDS. Appearance of ICD p24 Ag in an asymptomatic patient may occur because of a diminution in effectiveness of the immune response and may signify progression to the symptomatic phase of the disease.

Additional studies in a larger number of adults are important to further delineate the prognostic value of p24 assays. Evaluation of ICD p24 antigen level assay vis-à-vis viral load estimation will further emphasise the status of this assay for use in monitoring response to ART.

References