AN UNUSUAL SEROCONVERSION PROFILE IN A PREGNANT WOMAN INFECTED WITH THE HUMAN IMMUNODEFICIENCY VIRUS-1: NEED FOR USING LATER GENERATIONS HIV SCREENING ASSAYS

The first HIV-1 marker that appears in blood following infection is HIV-1 RNA and usually the load is in millions of copies/ml preceding seroconversion. A 24-year-old pregnant woman, gravida 2, parity 1 was tested for HIV as part of antenatal screening. Three samples were collected and tested from this individual over a period 70 days. The HIV-1 RNA level during seroconversion phase was very low contrary to the well understood natural history of HIV infection. The reactivity rate in the ELISA and the Western Blot profile showed a gradual increase over the 70 days with a weak reactivity in a second generation assay (detects IgG only) for the third sample. This case illustrates the uncertainties regarding the serological window period in HIV infection and the need to use at least a third generation assay in testing centres for early detection of HIV infection.

Key words: Acute HIV infection, HIV; India, seroconversion

There are several inadequacies described for the serological diagnosis of acute HIV infection. The better markers for the detection of HIV infection at this stage are the plasma HIV RNA and p24 antigen as the levels of these two markers are usually very high due to the higher replication of the virus during this period of infection. [1-4] Higher generations of HIV assays in a routine testing laboratory can aid in the diagnosis of acute HIV infection early in its course. The case reported here illustrates the nuances in the diagnosis of HIV infection for care providers as well as awareness of the unusual seroconversion profile in HIV infection.

Case Report

A 24-year-old pregnant woman, gravida 2, parity 1 was seen in the obstetrics and gynaecology outpatient facility in the month of January 2007. As part of the antenatal screening her blood sample was received in the virology department for HIV antibody testing. All serological tests for HIV antibody testing used were WHO/UNAIDS approved. This individual was married since four years and the first pregnancy and delivery was in the year 2004 at a tertiary care centre in south India. During the antenatal check-up related to her first pregnancy the HIV antibody status was negative. The first child was delivered by caesarean section and there was no history of blood transfusion or any other invasive procedures then or thereafter before the second pregnancy. Subsequently, she came for the antenatal check-up of the second pregnancy in January 2007 when she was again tested for HIV antibody. She was asymptomatic during this visit. The sample was found to be weakly reactive for HIV antibody/antigen by two different fourth generation assays and one third generation assay while the sample was negative by a second generation assay. The Western blot showed a weak reactivity to p24 antigen. Subsequently, we requested for two plasma samples from the individual for HIV RNA testing 30 days apart and 40 days after the first serum sample (Table). The HIV-1 viral RNA in the plasma samples were also estimated by two different assays. In the first assay, the viral load was estimated using real time
Table: The antibody reactivity of the three consecutive samples collected from the pregnant woman in various serological tests

<table>
<thead>
<tr>
<th>HIV sample ID with date</th>
<th>The *reactivity rate in screening (Fourth generation) assays#</th>
<th>Third generation assay (Rapid test) results##</th>
<th>Second generation assay (rapid) results##</th>
<th>Western Blot results##</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No.1 (Serum)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.02.07</td>
<td>3.27</td>
<td>Weak reactive</td>
<td>Negative</td>
<td>Weak reactivity to p24</td>
</tr>
<tr>
<td>Sample No.2 (Plasma)</td>
<td>05.04.07</td>
<td>7.42</td>
<td>Weak reactive</td>
<td>Reactivity to p17, p24, gp120, gp160 and Weak reactivity to p55, p66</td>
</tr>
<tr>
<td>Sample No.3 (Plasma)</td>
<td>03.05.07</td>
<td>10.03</td>
<td>Weak reactive</td>
<td>Reactivity to p17, p24, gp120, gp160 and Weak reactivity to, p31, p39, gp41 and p55</td>
</tr>
</tbody>
</table>

*Reactivity rate of > 1 is reactive for HIV; ‘Fourth generation assay I - Abbott Axsym (Abbott, USA); Fourth generation assay II-Genscreen plus Ag/Ab (BIORAD, USA); Third generation assay- Retrocheck (Qualopro, Goa, India); Second generation assay- Tridot (JMitra and Co. New Delhi, India); ##Western Blot assay - HIV Blot 2.2 (Genelabs, Singapore)

PCR, Rotor -Gene 3000 (Corbett Life Science, Sydney, Australia) with artus HIV-1 RG RT-PCR assay (Qiagen GmbH, Germany). The manufacturer’s instructions were strictly followed for the extraction of RNA and for reverse transcription PCR. The primers in this assay were designed to amplify a region in the LTR part of the genome. The sensitivity of the assay is established as 53 HIV-1 RNA copies/mL. The second assay for the HIV-1 RNA detection was an in-house real time PCR assay to amplify a target from the gag region standardized with the plasmid standards from National Institute for Biological Standards and Controls, UK. In the in-house assay, HIV-1 RNA was extracted using QIAamp elute virus spin assay (Qiagen, GmbH, Germany) and amplification done using 10 μL of the extract. The sensitivity of this real time PCR assay was assessed by the WHO first international standard for HIV-1 RNA 97/656 and found to be 1000 copies/mL.

Results

The virus load as per the commercial assay was < 53 copies/mL for the first plasma sample and 755 copies/mL for the second sample, while virus load by the in-house assay were 2166 copies/mL and 7617 copies/mL respectively for the two consecutive samples. As expected, the reactivity rate in the ELISA and the Western Blot profile showed a gradual increase over the 70 days. The antibody reactivity of the three samples collected from the individual in various serological tests is shown in the table. The figure show the increasing reactivity of the three samples in Western Blot (Gene Labs, Singapore) collected over a time period of 70 days. The individual was diagnosed to be HIV-1 infected when the sample was positive by a fourth, third generation serological assays and the HIV-1 viral load was 2166 copies/mL. It was reported positive at a time when the second generation assay was also weakly positive. The spouse’s sample was also found to be positive for HIV-1 by serology. Even after several counselling sessions he did not give history of any high risk behaviour except receiving multiple injections.

Discussion

It is now established that the first marker to appear in the blood following HIV infection is the viral RNA, usually at the end of second week. In newly infected individuals HIV RNA level will increase rapidly and predictably in serial samples.[1] The peak level of RNA in the blood is usually in the millions and precedes the antibody seroconversion because of the rapid dissemination of virus which happens during that period.[2,3] Fiebig et al, classified the primary infection of HIV in to seven stages based on the presence or absence of different plasma markers: stage 0 (eclipse period, characterized by
undetectable viral markers) stage I (detectable HIV RNA), stage II (HIV RNA + p24 antigenaemia), stage III (HIV RNA + p24 antigenaemia + IgM antibody), stage IV (HIV RNA + Western blot indeterminate), stage V (HIV RNA + Western blot positive without p31 band) and stage VI (HIV RNA + Western blot positive with p31 band).[4] Stage II through IV will have the highest plasma HIV-1 RNA level while stage V to VI the p24 antigen may or may not detectable. In contrast to this well described natural history of HIV infection, the viral load in the individual we are reporting on was very low during seroconversion. Low level HIV-1 viraemia during the very early period of primary HIV infection preceding ramp-up viraemia has been reported earlier as well.[5] There are uncertainties regarding the serological window period in HIV infection. A majority of the individuals develop detectable antibodies within eight weeks of infection. One of the reasons for the reduced level of HIV-1 RNA copies may be the reduced replication fitness of the infecting strain. There are several mutations/polymorphisms in the pol gene of HIV-1 like K65R, L74V, T215Y, G190S, and D30N that can reduce the replication fitness of the virus.[6] The pol sequence (nucleotide position 2199 to 3575) of the virus in this individual showed the infected strain to be subtype C. The sequence did not show any of the known mutations that reduce the replication fitness.

Hence, the exact reason for this unusual seroconversion profile may be assumed to be a host related phenomenon or other viral related factors like co-receptor usage. In this case we have used fourth generation EIA while the third generation and second generation assays were rapid tests. Though there could be a concern on the comparison of sensitivity of EIA and rapid tests, the reality is that rapid tests are the assays regularly available in the prevention of parent to child transmission (PPTCT)/ integrated counselling and testing centres (ICTC) in a developing country like India. Hence, our case illustrates the need to use at least a third generation assay in testing centres for early detection of HIV infection.

References

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