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DETECTION OF ACUTE AND RECENT HIV INFECTION BY PROVIRAL DNA PCR ASSAY

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ABSTRACT

The objective of this study was to standardize an HIV Proviral DNA PCR from newly infected HIV-1 individuals. Thirty five consenting, HIV-1 seropositive adults were enrolled in this study. Proviral DNA was extracted by QIAmp® DNA Mini Kit (QIAGEN, Germany) method. Gene testing was performed using Hot Start Polymerase chain reaction (PCR) to avoid non specific amplification. After amplification, the expected size of amplicon (130bp) confirmed by using 2% agarose gel electrophoresis. As a result, we standardized a specific and sensitive Hot Start PCR-based technique for newly infected HIV-1 individuals. Our finding suggests that HIV Proviral DNA PCR, which is a very sensitive tool to detect very early infection of HIV.

KEYWORDS: ***HIV-1, ProviralDNA, Hot Start PCR, Gag gene, Agarose gel electrophoresis.***

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INTRODUCTION

Human immunodeficiency virus (HIV) type 1 Proviral DNA could be a useful marker for exploring viral reservoirs and monitoring antiretroviral treatment, particularly when HIV-1 RNA is undetectable in plasma.[1]A provirus is a virus genome that has integrated itself into the DNA of a host cell. One kind of virus that can become a provirus is a retrovirus. Integration can result in a latent infection or a productive infection. In a productive infection, the provirus is transcribed into messenger RNA, which directly produces new virus, which in turn will infect other cells.[2] when a retrovirus invades a cell; the RNA of the retrovirus is transcribed into proviral DNA by reverse transcriptase, and then inserted into the host genome by an integrase. [3]Briefly, HIV genome consists of two identical copies of single stranded RNA molecules, is about 9 kilo bases in length, contains 9 open reading frames and is characterized by the presence of structural genes gag, pol and env and a complex combination of other regulatory/accessory genes. The gag gene encodes the structural proteins of the core (p24, p7 and p6) and matrix (p17).[4] The HIV-1 Gag protein is the major structural protein required for virus assembly. It is synthesized as a polyprotein in the cytosol of an infected cell, and contains four functional segments; MATRIX (MA), CAPSID (CA), NUCLEOCAPSID (NC), and p6.[5] Gag is often referred to as an "assembly machine" because expression of Gag alone is sufficient to produce budding virus-like particles (VLP's), due to multimerization of roughly 2000 Gag molecules per virion. Viral particles can be classified as immature (pre-budding and non-infectious), and mature (post-budding and infectious), and this exchange is mediated by the HIV-1 protease.[6]

MATERIALS AND METHODS

Study population and Proviral DNA isolation

HIV-1 Blood plasma Samples collected from different distinct sites in Andhra Pradesh. A total of 35 newly infected individuals with HIV-1 were enrolled into the study. The U.S. Centers for Disease Control and Prevention (CDC) advises that 97 percent of people develop detectable antibodies within the first three months after exposure. HIV viral DNA was extracted from plasma samples using QIAmp® DNA Mini Kit (QIAGEN, Germany) according to the manufacture instructions. The purified DNA was used for amplification by using PCR method.

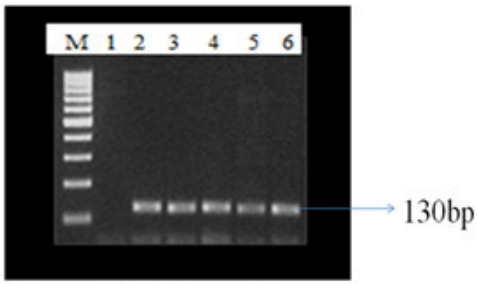
Proviral Amplification and detection

The PCR assay was performed using isolated DNA sample in a total reaction volume of 25µl. In this study, we develop a Hot Start PCR assay for Gag region of HIV-1, to avoid a non specific amplification. Amplification was performed in a thermal cycler with 25 µl reaction mixture and the optimized amplification profile are 95˚c for 5min followed by 35cycles of 95˚c for 30 sec, 55˚c for 1 min and 68˚c for 1 min. Final extension step at 68˚c for 7 min. Thirty five amplification cycles were performed using Gag forward 5'-ACA TCA AGC AGC CAT GCA AAT-3'- Gag Reverse 5'-CTA TGT CAC TTC CCC TTG GTT CTC T-3' and the amplicon size was a 130bp. After amplification, PCR products included 100bp ladder was loaded in 2% Agarose gel electrophoresis and the ethidium bromide stained PCR products were visualized under UV light.

RESULTS

The total Proviral DNA was isolated by QIAmp® DNA Mini Kit method from the infected blood samples. The viral load quantification was not done in this study. A total of Thirty five blood sample was collected from the different district Hospital, Hyderabad. The isolated DNA was electrophori zed in 1% Agarose gel. The DNA was purified by the spin column method. Analysis of the complete sequence of the Gag gene found at NCBI enabled the selection of two new PCR primers (forward and reverse). Sequences within the Gag gene were aligned, and the most highly conserved regions were selected to design primers for PCR. Specific primers were designed for the GAG gene using the sequences available in NCBI GenBank using Primer 3 Software. The predicted primers were validated in wet lab. The primers could yield an amplicon of the expected size 130bp.The PCR product was electrophori zed and visualized by 2% agarose gel. (Fig.1)

Figure 1
Agarose Gel Electrophoresis showing the 130bp of HIV-1, Gag gene.



Lane-M: 100bp marker,
Lane-1: Negative Control,
Lane-2: Positive Control,
Lane-3,4,5,6: Test Samples.

DISCUSSION

In this present work, an assay for HIV Proviral DNA PCR has been developed targeting the virus genome. Blood samples were received from hospital for disease diagnosis. We have tested the abilities of both Qiagen column and alkaline lysis method for the extraction of HIV DNA from blood, plasma samples and were coupled with PCR for HIV gag region. The Qiagen method was found to be the most suitable and has been used for future experiments. Virus has been detected in plasma anywhere from 4 to 11 days following infection with the PCR test. Antibody testing can sometimes be negative up to 6 weeks, even in someone that is infected. The main use of the PCR test is to detect very early infection. [7, 8] The hot start PCR is a modified form of PCR, which avoids a non-specific amplification of DNA by inactivating the taq polymerase at lower temperature and nonspecific primer annealing can occur due to these low temperatures in the conventional PCR, generating nonspecific products and lowering product yields.[9] The polymerase chain reaction(PCR) assay is an extremely sensitive technique for detecting nucleic acid sequences of infectious agents, including human immunodeficiency virus type 1(HIV-1). In addition, PCR assays can be performed in 1 day, as opposed to weeks for culturing. [10] Detection and quantification of circulating HIV in plasma or serum play an important role in diagnosing and monitoring HIV infection as well as assessing response to therapy. The most specific marker of HIV virus DNA indicates an ongoing reproduction of the virus and viral activity. [11] A latent infection results when the provirus is transcription ally silent rather than active it will be identified by Proviral DNA PCR method. [2] Early HIV infection is associated with high levels of HIV RNA and a corresponding high risk of viral transmission.[12, 13] To initiate antiretroviral therapy in early infection must balance these potential benefits with the potential risks of ART. It is reasonable to assume that a reduction in transmission risk would similarly occur by lowering and suppressing viral RNA level through ART in patients with early HIV infection. Early ART was also associated with lower HIV DNA and RNA cellular reservoir sizes.[14]

CONCLUSION

To conclude, we standardized a HIV Proviral DNA PCR, which is a very sensitive tool to detect very early infection of HIV. It is very specific for diagnosing HIV infection in infants born to HIV infected mothers and its result has to be interpreted with caution in vertical transmission of HIV.

REFERENCES

1. Nathalie De´Sire´, Quantification of Human Immunodeficiency Virus Type 1Proviral Load by a Taqman Real-Time PCR Assay. Journal of clinical microbiology, 39: 1303–1310, (2001).
2. Robert Belshaw, Long-term reinfection of the human genome by endogenous retroviruses. United States of America, 101: 4894-4899, (2004).
3. David C, HIV Entry and Its Inhibition Minireview. Cell, 93: 681–684, (1998).
4. Belasio EF, HIV virology and pathogenetic mechanisms of infection: a brief overview. Ann Ist Super Sanità 46: 5-14, (2010).
5. Garvey K.J., Nucleotide sequence and genome organization of biologically active proviruses of the bovine immunodeficiency-like virus. Virology, 175: 391-409, (1990).
6. Ganser-Pornillos BK, The structural biology of HIV assembly. Curr Opin Struct Biol, 18(2):203-17, (2008).
7. De Rossi A, Vertical transmission of HIV-1 lack of detectable virus in peripherial blood cells of infected children at birth. AIDS, 6:1117-20, (1992).
8. Krivine A, HIV replication during the first weeks of life. Lancet, 339:1187-89, (1992).
9. Primrose S B, Richard M, Twyman R W. Principles of gene manipulation. Wiley-Blackwell: p. 23, (2001)
10. Brooks Jackson J, β_2-microglobulin, HIV-1 p24 antibody and acid-dissociated HIV-1 p24 antigen levels: Predictive markers for vertical transmission of HIV-1 in pregnant Ugandan women. AIDS, 7(11):1475-1479, (1993).
11. Harald H K, Molecular detection of human immunodeficiency virus: where do we stand? Molekularer Nachweis des humanen Immunschwächevirus: Aktueller Stand, 29(1):44-49, (2005).
12. Wawer MJ, Rates of HIV-1 transmission per coital act, by stage of HIV-1 infection, in Rakai, Uganda: J Infect Dis, 191:1403, (2005).
13. Cohen MS, Acute HIV-1 Infection. N Engl J Med, 364:1943,(2011)
14. Ananworanich J, Early ART intervention restricts the seeding of the HIV reservoir in long-lived central memory CD4 T cells. Presented at the 20th Conference on Retroviruses and Opportunistic Infections. Atlanta, March 3-6, 2013. Abstract 47.