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Characterization of HR Region of *gp41* of HIV

M. IMRAN QADIR[1], SALMAN AKBAR MALIK†, TEHZEEB-UL-NISA, NADIA TABASSUM‡, SAJID ALI¶ AND LUBNA NISAR†

College of Pharmacy, Government College University, Faisalabad, Pakistan
†Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan
‡Department of Chemistry, COMSAT Institute of Information Technology, Abbotabad, Pakistan
¶Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan
[1]Corresponding author's e-mail: mrimranqadir@hotmail.com

ABSTRACT

The present study was conducted to assess HIV infection by reverse transcriptase polymerase chain reaction (RT-PCR) by using primers based on sequence of *gp41*, which is a more conservative region than the previously used regions. The study was carried out on 200 individuals: 100 normal (HIV seronegative) individuals, and 100 patients who were HIV seropositive. The HR region of *gp41* gene of HIV was characterized. So it may be concluded that "characterization of this region with the said primers" may be used for the diagnosis of AIDS. © 2010 Friends Science Publishers

Key Words: HIV; Heptad repeat (HR) regions; Characterization; Diagnosis; AIDS

INTRODUCTION

For years the test for diagnosis of AIDS was the enzyme-linked immunosorbent assay (ELISA) that looked for antibodies (Abou El-Hawa *et al*., 2006; Kamal *et al*., 2006) to the virus in the blood sample. If this test was positive, then AIDS was confirmed by another test called the Western blot, which checks for the presence of HIV proteins. It is performed, because non-HIV antibodies may cause a false-positive result on ELISA. These tests may take up to one week. Reverse-transcriptase polymerase chain reaction (RT-PCR) is a method that amplifies viral nucleic acid to allow for its detection in patient specimens (Shahnejat-Bushehri *et al*., 2006). It is a particularly specific and sensitive test, which can pick up very small numbers of viral particles. Advantage of reverse-transcriptase polymerase chain reaction (RT-PCR) for diagnosis of AIDS is that it takes time less than 12 h. Further more this method can detect minute quantities of the virus in blood. It is also useful in the diagnosis of HIV infection in babies born to infected mothers. Babies will carry maternal antibody up to approximately 15 months of age and therefore the antibody test is not a reliable indicator of infection in these children.

Different primers may be used for molecular diagnosis of AIDS by RT-PCR. Most of the previously used primers are based on the sequence of *gp120* and *gag*. HIV differs from many other viruses that it has a very high mutation rate: approximately 3×10^{-5} per nucleotide base per cycle of replication (Robertson *et al*., 1995). *gp41* is the most conservative region and thus its Heptad Repeat (HR) regions have been targeted for many of new drugs (Qadir & Malik, 2009), an evidence for its conservation. Thus characterization of this region may prove as effective diagnostic region due to its conservation.

Wild *et al*. (1994) tried to find out the function of Heptad Repeat regions (HR1 & HR2) of envelope of HIV and concluded that they have a critical role in the fusion of HIV membrane with human CD4 cells. Protein dissection studies confirmed the formation of a soluble, alpha-helical core consisting of a trimer of antiparallel dimmers on interaction of two heptad-repeat regions (Lu *et al*., 1995; Lu & Kim, 1997). Biophysical studies suggest that three N-terminal helices form an interior, parallel-coiled- coil trimer, while three C-terminal helices pack in the reverse direction into three hydrophobic grooves on the surface of this coiled coil (Lu *et al*., 1995). Crystallographic analysis of the *gp41* ectodomain core confirmed that it folds into a six-helix bundle (Chan *et al*., 1997; Tan *et al*., 1997; Weissenhorn *et al*., 1997). The formation of this six-stranded helical bundle induces a hairpin structure that brings the viral and cell membranes into proximity for fusion (Chan *et al*., 1997; Weissenhorn *et al*., 1997). Mutations in *gp41* can destabilize the fusion-active core structure and reduce the ability of the envelope glycoprotein to mediate membrane fusion (Cao *et al*., 1993; Ji *et al*., 1999; Ji *et al*., 2000; Weng *et al*., 2000). So for the effective membrane fusion and ultimately HIV infection, *gp41* must be conserved. The aim of the present study was to characterize Heptad Repeat region of *gp41* of HIV and to describe its use as diagnosis of AIDS.

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MATERIALS AND METHODS

The study was carried out on 200 individuals: 100 HIV seronegative individuals and 100 patients, who were HIV seropositive. HIV blood samples were collected from the main special clinic by National AIDS Control Program, (Govt. of Pakistan) suited in Pakistan Institute of Medical Sciences, Islamabad, Pakistan and National Institute of Health, Islamabad, Pakistan, where the AIDS patients from different areas of Pakistan are registered for their treatment. HIV seronegative individuals were selected randomly.

QIAamp MinElute® Spin Kit was used to isolate viral RNA from blood samples of HIV patients. Reverse transcription-polymerase chain reaction (RT-PCR) was then performed using primers designed to amplify a 589 bp region within *gp41* that includes both HR1 and HR2 domains. The outside primers were L5′*gp41* (TTCAGACCTGGAGGAGGAGATA) and L3′Env (GGTGGTAGCTGAAGAGGCACAGG). First-round amplification was performed using 50 pmole each of L5′*gp41* and L3′Env. PCR conditions were 2 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 50°C and 1 min at 72°C, with an extension of 5 min at 72°C. Second-round amplification was performed using the same protocol as above, but with primers L5′HR1 (AGAAGAGTGGTGCAGAGAGAAAA) and L3′HR2 (GGTGAGTATCCCTGCCTAACTCT). These primers are used for research purpose only.

The isolated DNA was evaluated by agarose gel electrophoresis after staining ethidium bromide and exposure to UV light. 200 bp DNA Step Ladder prepared by Promega Corporation USA was used to determine the size of unknown DNA sample and was loaded in wells in the right side of the gel. This ladder is recommended for sizing and approximate quantification of PCR products. The first band of the ladder on the lower side of the gel contains 200bp, the second 400bp, third 600bp, fourth 800pb and the last band contains 1000bp. The bands were identified using Bio Rad analyzer, which made faint band visible under UV light. The size of bands was estimated by referring to DNA ladder. The protocol was also run for HIV seronegative individuals.

RESULTS

Test for HIV: Presence of HIV in the patients and absence in the seronegative individuals was confirmed by Rapid Anti-HIV Test™ One Step Method, manufactured by InTec Products, Inc, Xiamen.

Quantification and purity of RNA: Table II shows the concentration and purity of the RNA in HIV samples. It was observed that concentration of viral RNA in all HIV samples ranged from about 0.242 to about 0.996 µg/µL. The average concentration of viral RNA was 0.68562 µg/µL with SD ± 0.188. Obviously there is very little variation in concentration of the RNA present in the samples.

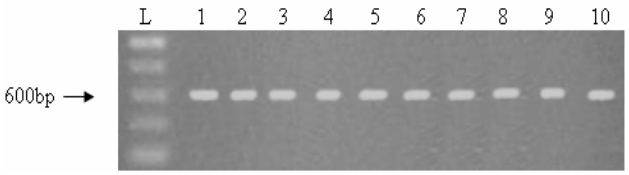
Table I: Nucleotide sequences of primers used for Characterization of HR region of *gp41* of HIV

Primer	Nucleotide Sequence
L5′gp41	TTCAGACCTGGAGGAGGAGATA
L3′Env	GGTGGTAGCTGAAGAGGCACAGG
L5′HR1	AGAAGAGTGGTGCAGAGAGAAAA
L3′HR2	GGTGAGTATCCCTGCCTAACTCT

Table II: Average Concentration (± SD) of RNA samples and their purity ratio in HIV samples

Absorbance at 260 nm	Absorbance at 280 nm	Concentration of RNA (µg/µL)	*A*260/*A*280 ratio
0.34280±0.094	0.18867±0.053	0.68562±0.188	1.82±0.15

Fig. 1: Electropherogram of ethidium bromide stained 2% agarose gel showing the required bands with length of approximately 600 bp obtained after RT-PCR of RNA from sample 1 to 10, 200 bp DNA Step Ladder by Promega was used as reference



Likewise, these samples were also tested for purity of viral RNA isolated from HIV samples by measuring absorbance at 260 nm and 280 nm. The ratio of the absorbance at 260 nm to absorbance at 280 nm (A_{260}/A_{280}) provides purity of RNA with respect to contaminants that absorb UV light, such as protein. The purity ratio of all samples tested fell in range of 1.21-2.08, which is practically almost within the universally accepted ratio of 1.6-2.0 for PCR amplification. The average purity ratio of RNA was 1.82 with SD ± 0.15.

Characterization of the RT-PCR amplified products: Fig. 1 shows one of the results of RT-PCR amplified products after running on 2% agarose gel using 3 µg viral RNA. Ladder was loaded in a well designated as L on agarose gel and samples in lanes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10.

Seronegative individuals: Concentration of viral RNA in all seronegative individuals was nearly to zero. There were no bands by RT-PCR of samples from seronegative individuals, even after using 25 µL of the product obtained from QIAamp MinElute® viral RNA isolation kit.

DISCUSSION

Quantification of isolated viral RNA was carried out to calculate its amount used for RT-PCR amplification. It has been observed that all HIV samples had about 0.242 to about 0.996 µg RNA per µL that was further used for

cDNA synthesis. These concentrations were further used to determine the amount of viral RNA required for cDNA synthesis. When 1 µg RNA was used for cDNA synthesis and RT-PCR was performed, no bands of samples were observed. This result inferred that the amount of viral RNA was insufficient for synthesizing cDNA. When cDNA was synthesized using 2 µg RNA and RT-PCR was carried out, faint bands of the samples were observed on agarose gel, which is indicative of the inadequate amount of viral RNA. However addition of 3 µg RNA for cDNA synthesis gave clear bands of samples after RT-PCR indicating successful amplification of cDNA. Thus 3 µg is a minimum amount of template RNA that gave excellent results for PCR amplification.

Similarly, purity ratio A_{260}/A_{280} was determined spectrophotometrically. All samples tested had values ranging from 1.21-2.08 with an average value of 1.82 and SD value ± 0.15, which is practically within the universally accepted ratio of 1.6-2.0 for PCR amplification (www.protocol-online.org). This clearly indicates that RNA isolated from HIV samples was pure and free of any contamination like proteins. Viral RNA isolated, was converted to cDNA and amplified by RT-PCR. This process of RT-PCR for amplification of cDNA was found out to be sensitive, precise and specific and convenient (Shahnejat-Bushehri *et al.*, 2006).

The amplified products were run on the agarose gel. Clear bands of HR region were seen in all lanes. All bands traveled same distance on agarose gel. This distance was almost 600 bp in relation to DNA ladder. To confirm the reproducibility of primers used for cDNA amplification and authenticity of results same experiment was repeated using fresh HIV samples and similar results were obtained. Chances of any error were minimized by repeating the experiment with same set of primers and PCR conditions.

Our study also provides information regarding the size of HR region. Hanna *et al*. (2002) also reported heptad repeat of 589 bp using same primers which is practically the same as ours. The amplified products were obtained in all samples indicating that there were no significant mutations in primer binding sites. However Xu *et al*. (2005) reported 486 bp length of HR region. This is due to the fact that he used different set of primers for amplification.

The HR region of *gp41* gene of HIV was characterized. As mutations occur in HIV at a very high rate and there would be a possibility that too mutations would be present in this region so that the primer would not be able to attach the complementary sequence. As a raw estimate of mutations in the said region we may conclude that there were not drastic enough mutations to prevent the attachment of their nucleotides with the primer used. So, it may be concluded that "characterization of this region with the said primers" may be used for the diagnosis of AIDS.

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