Journal of Rashid Latif Medical College 1 (2022) 13-15

Journal of Rashid Latif Medical College

journal homepage: www.jrlmc.org

Original Article

HR regions of *env* gene in HIV: PCR Diagnostic targets for AIDS

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KEY WORDS:

AIDS, diagnosis, PCR, HR regions

ABSTRACT

Objective: Two heptad repeat regions are present at envelop of HIV. It is assumed that this region plays an important role in the entry of viral genome into human cell. Present study was conducted for isolation and characterization of the HR region of *env* gene in samples of HIV taken from Pakistani patients. **Methods:** QIAamp MinElute® Spin Kit was used to isolate viral RNA and RT-PCR was used to amplify the specific regions from synthesized cDNA; and gel electrophoresis was used to characterize it. **Results:** It was observed that on comparison with marker DNA, all samples of HR regions have almost 500 base pairs identifying HR regions. **Conclusion:** In this study the identification of HR regions shows that occurrence of mutations in HR regions are not enough strong to prevent their attachment with the primer used.

Introduction

Two heptad repeat regions are present at envelop of HIV. It is assumed that this region plays an important role in the entry of viral genome into human cell. Carl *et al*., (1994) tried to find out the function of Heptad Repeat regions (HR1 and HR2) of envelope of HIV and concluded that HR regions of HIV envelope have a critical role in the fusion of HIV membrane with human CD4 cells[1] Studies on Protein separation confirmed the formation of a soluble, alpha-helical core comprising of a trimmer of antiparallel dimmers on interaction of two heptad-repeat regions. Biophysical studies suggest that three N-terminal helices form an interior, parallel-coiled-coil trimmer, while three C-terminal helices pack in the inverse direction into three hydrophobic grooves on the surface of this coiled coil.[2] Crystallographic analysis of the gp41 ectodomain core confirmed that it folds into a six-helix bundle.[3,4] The six-stranded helical bundle forms and induces a hairpin structure that brings the viral and cell membranes into zone for fusion.[4,5] The vital role of interhelical packing interactions between the N- and C-terminal regions of the gp41 ectodomain provides an opportunity for antiviral intervention.[6] For example, peptides corresponding to the C-terminal heptad repeat region of gp41, termed C peptides, are capable of inhibiting entry of HIV-1 at nanomolar concentrations.[2,7] In humans, antiviral activity is shown by one such peptide, T20.[8] Biochemical and structural studies strongly suggest that C peptides act in a dominant-negative manner by binding to the N-terminal coiled-coil region of gp41 in its prehairpin transitional state, so interfering with its transition to the fusion-active six-helix bundle structure.[2,4,9] Similarly, derivations of peptides from the N-terminal heptad-repeat region of gp41 (called N peptides) are thought to block HIV-1 fusion by binding to the C-helix region of the gp41 intermediate.[2,10] N peptides may show its activity by intercalating into the N-helix coiled-coil structure.[11]

Rapid mutations are shown by HIV. Fusion-active gp41 core structure can destabilize due to mutations in gp41 that alter the interhelical packing interactions and reduce the ability of the envelope glycoprotein to

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mediate membrane fusion.[13-14] The fusion of HIV with its targeted membrane may affect due to so many mutations in these regions. Therefore, nature needs the presence of minimum mutations in these regions to promote viral fusion with human cell membrane.

Aims and Objectives

The objective of our study was isolation and characterization of Heptad Repeat regions of *env* gene of HIV in Pakistani strains and to predict the extent of mutations in the said regions, so that this region may be used for diagnostic purpose.

Materials and Methods

Blood samples were collected from 100 HIV patients from Pakistan. These patients were registered in AIDS Control Program of Pakistan by WHO. To isolate viral RNA from blood samples of HIV patients QIAamp MinElute® Spin Kit was used. cDNA was synthesized using sequence specific primers. RT-PCR amplification of cDNA was carried out. Different amounts of viral RNA were used to synthesize cDNA to determine the minimum amount of RNA required for successful PCR amplification.

A 506-bp region of gp41 covering both the HR1 and HR2 domains will be amplified by reverse transcription-PCR (RT-PCR). The following primer pairs will be used for sequencing in a nested PCR: primers gp50F1 (AAAAATTGAACCACTAGGAG TAGCACCCAC) and gp41R1 (AACGACAAAGGTGAGTATCCCTGCCTAA) as the outer primers and primers gp40F1 (TCTTAGGAGCAGCAGGAAGCACTATGGG) and gp48R2 (TCCTACTATCATTATGAATATTTTTATATA) as the inner primers.

Table 1: Nucleotide sequences of primers used for characterization of HR regions of *env* gene of HIV

Primer	Nucleotide Sequence
gp50F1	AAAAATTGAACCACTAGGAG TAGCACCCAC
gp41R1	AACGACAAAGGTGAGTATCCCTGCCTAA
gp40F1	TCTTAGGAGCAGCAGGAAGCACTATGGG
gp48R2	TCCTACTATCATTATGAATATTTTTATATA

By using agarose gel electrophoresis the isolated DNA was assessed after staining ethidium bromide and provide UV light exposure. GeneRuler™ 50 bp DNA ladder was used for reference and was loaded in wells designated as L on agarose gel. The gels were loaded with ladder for detailed assessment among bands of different HIV samples. This ladder is recommended for sizing and approximate quantification of PCR products. The range of this ladder is from 50 bp to 1000 bp. The ladder is composed of thirteen chromatography-purified individual DNA fragments (in base pairs): 1000, 900, 800, 700, 600, 500, 400, 300, 250, 200, 150, 100, and 50. The ladder is dissolved in TE buffer.

Results and Discussion

Presence of Human Immunodeficiency Virus (HIV) in the patients was confirmed by ADVANCED QUALITY ™ One Step Test manufactured by Intec Products, Inc., Xiamen.

Table 1 shows the purity and concentration of the RNA in HIV samples. It was observed that concentration of viral RNA in all HIV samples ranges from about 0.7 µg/µl to about 0.8 µg/µl. There is very little difference observed in concentration of the RNA present in the samples.

Similarly, purity of viral RNA isolated from HIV samples were tested by measuring absorbance at 260 nm and 280 nm in these samples. The ratio of the absorbance at 260 nm to absorbance at 280 nm (A_{260}/A_{280}) provides purity of RNA with respect to impurities that absorb UV light, such as protein. The purity ratio of all samples tested fell in range of 1.79-2.08 which was practically within the universally accepted ratio of 1.6-2.0 for PCR amplification. The average purity ratio of RNA was 1.9 with SD ± 0.1.

Figure 1 shows results of RT-PCR amplification which was carried out when 4 µg volume of RNA was used to synthesize cDNA. Presence of clear bands of HIV samples in lanes 1, 2, 3, 4, 5, 6, and 7 indicate successful cDNA synthesis and identification of HR regions. Lane L shows DNA ladder of the known size.

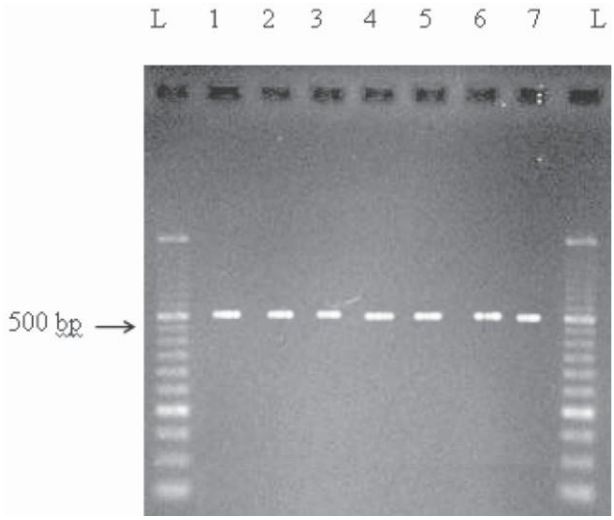

Figure 1: 2% agarose gel showing RT-PCR products of 4 µg RNA

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 500 bp in relation to DNA ladder.

Our study also provides information regarding the size of HR region. [15] and [16] reported heptad repeat of 600 bp. This is due to the fact that they used different set of primers for amplification.

As mutations occur in HIV at a very high rate and there would be a possibility that many mutations would be present in HR regions so that the primer would not be able to attach the complementary sequences. But our study shows that the mutations occurring in HR regions were not rigorous enough to prevent their attachment with the primer used. Therefore, HR regions of *env* gene in HIV may be used as PCR diagnostic targets for AIDS.

Acknowledgements

Authors are thankful to the Higher Education Commission, Islamabad, Pakistan for providing the financial support for the project.

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