Un-corrected Proof

Characterization of the Immunodominant Regions within gp41 of *env* Gene of HIV in Pakistan

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Summary: Objective of the present study was to characterize the immunodominant sequences of gp41 in HIV strain in Pakistani population so that vaccine may be prepared based upon these regions. A total of 25 specimens were collected from HIV patients of different areas of Pakistan. The viral RNA was isolated using QIAamp MinElute® Spin Kit manufactured by Quiagen, California, USA. RT-PCR was done for amplification of the required region and confirmed by gel electrophoresis. The nucleotides of the required regions were sequenced using Big Dye terminator.
Less than 20% of the specimens had mutations in immunodominant regions, so it may be concluded that immunodominant regions of gp41 in HIV strain in Pakistani population are conservatives and vaccine based upon these regions may prove active immunization against the disease.

Introduction

Immunodominant regions are subunits of the antigenic determinant that are most easily recognized by the immune system and thus most influence the specificity of the induced antibody. The envelope glycoprotein of HIV is synthesized as a polypeptide precursor gp160. gp160 is cleaved post translationally and generate two non-covalently associated subunits, gp120 and gp41 (Hunter and Swanstrom, 1990; Hong *et al*., 1999). Main function of gp120 is recognition of the target cell and gp41 involves the fusion of virus with human cells (Wilkinson, 1996; Dimitrov, 1997; Kwong *et al*., 1998; Rizzuto *et al*., 1998). Moreover more then 99% of the HIV infected individuals produce antibodies against immunodominant regions (IDR) within the gp41; comprising of CTL epitope (aa 591 to 602; AVERYLKDQQLL), cysteine loop (aa 607 to 613; CSGKLIC), and ectodomain region (aa 671 to 676; ELDKWA) (Busch *et al.*, 1995; Horal *et al.*, 1991; Hunter 1997, Muster *et al.*, 1993; Oldstone *et al.*,1991)..

Objective of the present study was to analyze mutations in immunodominant sequences of gp41 in HIV strain in Pakistani population so that vaccine may be prepared based upon these regions. For this purpose, we sequenced the immunodominant regions of gp41 in HIV strain in Pakistani population.

Results and Discussion

To determine the sequence variability within the immunodominant regions of the gp41 protein, Cytotoxic T- lymphocyte (CTL) epitope (aa 591 to 602), Cysteine Loop (aa 607 to 613) and Ectodomain (aa 671 to 676) sequences derived from the 25 HIV infected individuals were aligned (Table 1). The immunodominant regions were much conserved as 84 % of the specimens had the same sequences. 4% of the specimens had variation in CTL epitope resulting in amino acid shift from K^{597} to Q. 8% of the specimens had variation in cysteine loop resulting in amino acid shift from K^{610} to R. 4% of the specimens had variation in cysteine loop resulting in amino acid shift from L^{611} to H.

It was very interesting that all the result for the detection of all the specimens from all the strips were positive.

Table-1: Amino Acid Sequence of immunodominant regions of gp41 in HIV strain in Pakistani population.

Number of Specimens	CTL epitope	Cysteine Loop	Ectodomain Region
21	LAVERYLKDQQLL	CSGKLIC	ELDKWA
2	LAVERYLKDQQLL	CSGRLIC	ELDKWA
1	LALERYLKDQQLL	CSGKHIC	ELDKWA
1	LAVERYLQDQQLL	CSGKLIC	ELDKWA

The study was conducted to determine the sequence analysis of immunodominant regions (IDRs) within the gp41 of env gene of HIV. The purpose of the study was to identify the mutations in immunodominant regions that can potentially alter antibody binding and the delineation of sequence variability in the CTL (Cytotoxic T-Lymphocyte), Cysteine Loop and Ectodomain.

There were certain variations observed in the immunodominant regions (IDRs) and these mutations resulted in the shifting of amino acids which finally leads to have the alter protein products. Antibody detection is directly affected by genetic diversity within the immunodominant regions (IDRs) of structural proteins.

In this study mutations were observed in Cytotoxic T- lymphocyte epitope resulting in K^{597} to Q substitution. This substitution was also observed by Jonathan Dorn *et al.*, (2000). Another mutation, K^{597} to R substitution was observed by Johnson *et al.*, (1992), but absent in our study.

Horal *et al.*, (1991) demonstrated that the CxxxxxxC domain constituting the cysteine loop was conserved in all specimens, regardless of specimen genotype and geographic origin, further confirming the functional importance of this conserved epitope. But K^{610} to R and L^{611} to H substitutions of cysteine loop were observed in our study and are in concordance with the experiment conducted by Jonathan Dorn *et al.*, (2000).

E^{671} to A, L^{672} to F and A^{676} to S substitutions within the ectodomain of gp41 were not seen in our study but Xu *et al.*, (1991) and Jonathan Dorn *et al.*, (2000) observed these substitutions.

As shown earlier in another study by Horal *et al.*, (1991), Oldstone *et al.*, (1991) and Xu *et al.*, (1991), the sequence variation within CTL and cysteine loop can lead to escape from immune detection, indicating that sequence variation within HIV may contribute to the evolution of viral variants that are no longer recognized by the host immune response. This may lead to the failure of the HIV detection tests based on antibodies. Specifically, the cysteine loop, containing an intermolecular disulfide bond, represents the most immunogenic region of gp41 (Hunter, 1997) suggesting that the disulfide bridge is necessary to maintain the three-dimensional structure required for antibody recognition (Xu *et al.*, 1991).

As in our study, most of the specimens had same sequence of immunodominant regions, so it may be concluded that immunodominant regions of gp41 in HIV strain in Pakistani population are conservatives and vaccine based upon these regions may prove active immunization against the disease.

Experimental

Blood samples were collected from 25 HIV patients from different areas of Pakistan. Plasma samples were tested using the antibodies-based diagnostic strips “One-Step HIV Test” manufactured by World of Health Biotech Co., Ltd**.** Beijing, China.Viral RNA was extracted from plasma samples using QIAmp viral RNA kit (Quiagen, Valencia, Califrnia, USA). Reverse transcription-polymerase chain reaction (RT-PCR) was then performed using primers designed to amplify a 589 bp region within gp41 that includes all the required immunodominant regions. 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). The purified nested PCR products from the gp41-amplified region were used for automated sequencing with a BigDye terminator cycle-sequencing ready reaction kit (PE Applied Biosystems, Foster City, Califrnia, USA) according to the manufacturer's protocol.

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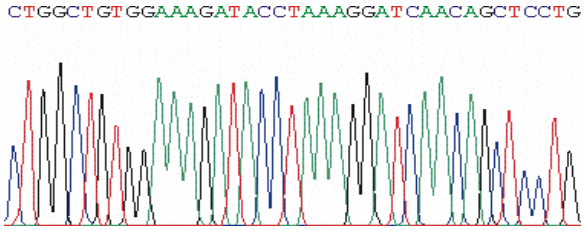

Fig. 1: Chromatogram showing the common nucleotide sequence of CTL of gp41of *env* gene of HIV of Specimen 1.

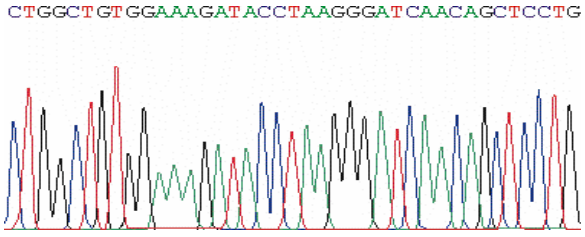

Fig. 2: Chromatogram generated showing the Nucleotide sequence of CTL of gp41of *env* gene of HIV with mutation from Adenine (A) to Guanine (G) resulting in amino acid shift from Lysine (K) to Glutamine (Q).

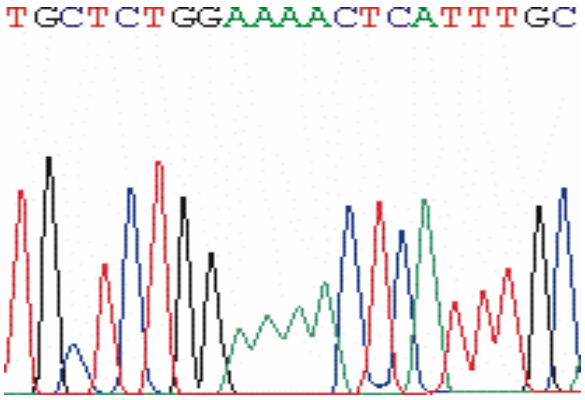

Fig. 3: Chromatogram showing the common nucleotide sequence of Cysteine Loop of gp41 of *env* gene of HIV of Specimen 1.

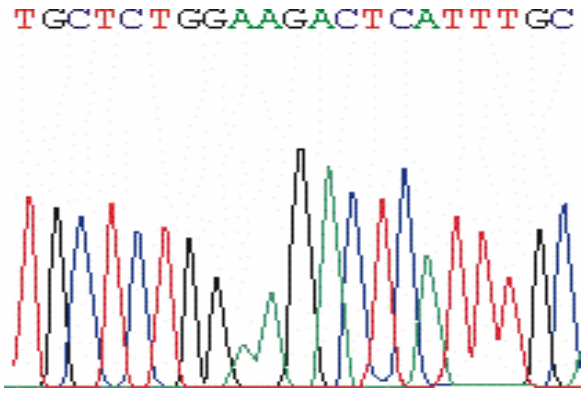

Fig. 4: Chromatogram showing the Nucleotide Sequence of Cysteine Loop of gp41 of *env* gene of HIV with mutation from Adenine (A) to Guanine (G) resulting in amino acid shift from Lysine (K) Arginine (R).

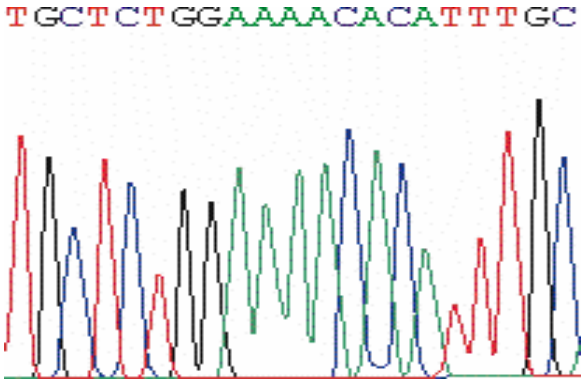

Fig. 5: Chromatogram showing the Nucleotide Sequence of Cysteine Loop of gp41 of *env* gene of HIV with mutation from Thymine (T) to Adenine (A) resulting in amino acid shift from Leucine (L) to Histidine (H).

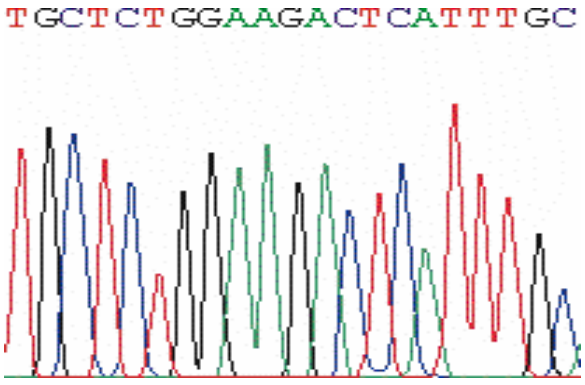

Fig. 6: Chromatogram showing the Nucleotide Sequence of Cysteine Loop of gp41 of *env* gene of HIV with mutation from Adenine (A) to Guanine (G) resulting in amino acid shift from Lysine (K) Arginine (R).

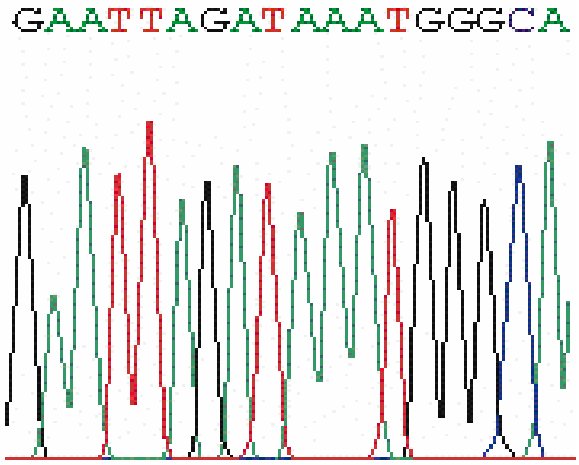

Fig. 7: Chromatogram showing the common nucleotide sequence of ectodomain of gp41of *env* gene of HIV

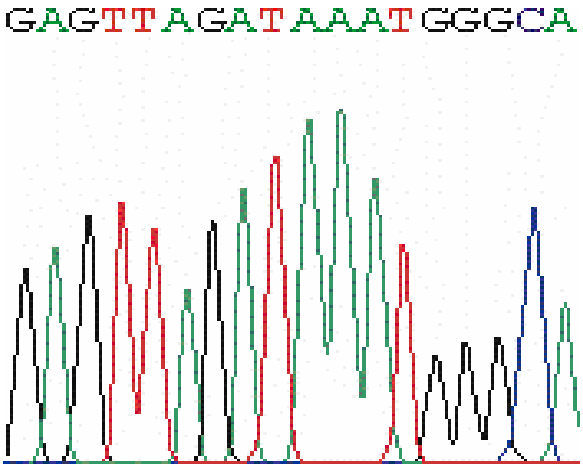

Fig. 8: Chromatogram showing the nucleotide sequence of ectodomain of gp41of *env* gene of HIV showing silent mutation.