Analysis of Envelope 1 (E1) and Hyper Variable Region-1 (HVR-1) Encoding Sequences from Indian Isolates of Hepatitis C Virus

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Hepatitis C Virus (HCV) is a positive strand RNA virus and it affects ~170 million people worldwide. Diagnosis and treatment of HCV infection has become a challenging task owing to the incidence of large-scale mutations in the RNA genome. The N-terminus of 27 amino acids (aa) of the structural protein E2 is the most heterogeneous region, designated as the first Hyper Variable Region (HVR1). In this investigation, a total of 21 HVR1 sequences of Indian HCV isolates was cloned and sequenced. The nucleotide sequence of E1 region upstream of HVR1 was analyzed for subtyping of Indian isolates. Analyses of HVR1 disclosed large-scale alterations in nucleotides leading to extensive aa substitutions within the HVR1. The extent and nature of variability observed in the HCV genotype 1 was random and subtype independent. Even though various aa alterations were observed in the HVR1 region, the potential antigenic sites could be specifically identified in the region between 10th and 24th aa. The most commonly found HVR coding sequence was fused to the E1 structural protein encoding sequence (E1E2-100), and E1 and E1E2-100 fusion proteins were expressed in the E. coli system. Western blot analysis showed positive signals for E1 with four patients' sera, while sera of all the six patients revealed positive signals with E1E2-100, indicating an enhanced immuno-reactivity of the fusion protein as compared to E1.

**Keywords:** Hepatitis C Virus, Indian isolates of HCV, Hyper Variable Region 1 (HVR1), Envelope 1 protein (E1), E1E2-100 fusion protein

**Introduction**

Hepatitis C Virus (HCV), an enveloped RNA virus, belonging to Flaviviridae family (Robertson et al., 1998; and Choo et al., 1989) is the major causative agent of non-A, non-B hepatitis (Kuo et al., 1989). The HCV genome is a positive-sense RNA of ~9.6 kb encoding a single polyprotein of approximately 3,033 amino acids (aa) that generate 10 viral gene products (C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) after cleavage by viral and cellular proteases (Purcell, 1997; and Reed and Rice, 2000). HCV infects ~170 million people worldwide (CDC, 1998; and WHO, 1998) and more than
80% of the affected individuals become chronically infected. Persistent viral infections often develop into liver cirrhosis and hepatocellular carcinoma (Kiyośawa et al., 1990; Ohkoshi et al., 1990; Saito et al., 1990; Alter et al., 1992; and Muller, 1996). The approved therapy for HCV infection is a combination of interferon and ribavirin (Poynard et al., 1998; Wietzkkeberaua, 2001; and Arase et al., 2003) and proved effective for only 20-30% of the patients. In view of the high mutability of HCV genome, the development of an effective vaccine still remains a challenging task.

The N-terminus end of E2 is the most heterogeneous region of all the proteins, designated as the first Hyper Variable Region (HVR1) of HCV and is the principal neutralization determinant (Ogata et al., 1991; and Weiner et al., 1992). Zibert et al. (1997) found that an early appearance of antibodies directed to HVR1 is associated with acute self-limiting infection of HCV, while the persistence of HVR1 antibodies is associated with chronic HCV infection. Antibodies against HVR1 were shown to block adsorption of HCV to susceptible cells in vitro (Shimizu et al., 1996; and Zhou et al., 2002). Antibodies directed against HVR1 were found to protect chimpanzees against HCV infection after in vitro neutralization (Farci et al., 1994 and 1996). Antibodies raised against a specific HVR1 also showed reaction with HVR1 from other HCV isolates (Hattori et al., 1998). These results indicate that E2 containing HVR1 has the potential as a vaccine for prevention of HCV infection.

Envelope proteins of HCV (E1 and E2) were predicted to be type I membrane glycoproteins, and generally believed to constitute components of virion membrane (Grakoui et al., 1993; and Selby et al., 1993). Earlier studies have revealed that both E1 and E2 are involved in the receptor binding and membrane fusion, promoting HCV entry into target cells (Lagging et al., 1998; Garcia et al., 2002; and Triyatni et al., 2002). Vaccination of chimpanzees with E1 or E2 glycoprotein resulted in limited but measurable protection against homologous virus challenge (Choo et al., 1994). Therefore E1 and E2 have become two major targets in HCV vaccine research. However, evaluation of HCV envelope-protein-based vaccines requires an effective method for antigen detection and an inexpensive production of large quantities of antigens. E1 glycoprotein expressed in mammalian cell systems would best reflect properties of E1 protein present on HCV virion; however, low yields, besides difficulty in purification and scaling up, make such expression systems unsuitable for large-scale applications (Choo et al., 1994). Bacterial expression systems, compared to eukaryotic systems, usually offer higher yields of recombinant proteins at considerably lower cost (Terpe, 2006; and Hewitt and Mc Donnell, 2004).

In view of the above, the sequence analyses of HVR1 were carried out as a part of molecular epidemiological studies to evaluate the extent of variability in the Indian isolates of HCV. Further, E1 and fusion protein E1E2-100 were expressed in E. coli, and partially purified antigens were detected with HCV-infected Indian patients’ sera to evaluate the potentiality of these molecules as components of improved detection and in designing a vaccine specific to this geographical region.
Materials and Methods

Serum Collection and RNA Isolation

Serum samples collected from 100 Indian patients tested positive for anti-HCV were used for the analysis of HVR1 of HCV. RNA was isolated using single step method (Chomczynski and Sacchi, 1987). RNA extraction buffer (500 µL) containing 4 M guanidium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sacrosyl, 0.1 M 2-mercaptoethanol was added to 100 µL of serum at 4 °C. 50 µL of 2M Sodium acetate, 500 µL water saturated phenol and 200 µL chloroform:isoamyl alcohol (49:1) were added to the serum GITC mixture. Aqueous phase was collected after centrifugation at 10,000 rpm for 10 min. The RNA was precipitated by the addition of 2.5 volumes of isopropanol and incubated at −70 °C for 1 h. The RNA pellet was washed with 70% ethanol and resuspended in 20 µL of sterile distilled water.

c-DNA Synthesis

About 1 µg (10 µL) of total RNA, 1 µL of 10 µM gene specific reverse primer and 2 µL of sterile deionized water were taken for the first strand c-DNA synthesis in a 0.2 mL PCR tube. The contents were mixed and the tube was spun briefly. The sample was incubated at 70 °C for 5 min for removing the secondary structures of RNA and cooled on ice for 2 min to anneal the primer. 4 µL of 5X first strand synthesis buffer, 2 µL of 10 mM dNTP and 1 µL (200 units) of MMLV reverse transcriptase devoid of RNaseH activity were added to the tube and mixed by pipetting gently for final reaction volume of 20 µL. The tubes were incubated at 42 °C for 1 h. The termination of the first strand reaction was achieved by incubating at 70 °C for 10 min.

Amplification and Cloning of HVR1

Multiple sequence alignment of HVR1 from different isolates was used to generate primers for conserved regions flanking HVR1. PCR was performed using 5’CAT CGA ATG GCT TGG GAC ATG ATG 3’ as forward and 5’GGC AGT CCT GTT GAT GTG CCA 3’ as reverse primer. The PCR reaction was set by adding 8 µL of synthesized c-DNA, 31.5 µL of sterile MilliQ water, 5 µL of 10X PCR buffer containing 1.5 mM MgCl₂, 1.5 µL of 10 mM dNTP mix, 1.5 µL of each PCR primer (10 µM) and 1 µL of Taq polymerase for a 50 µL final reaction volume. The reaction contents were mixed well and kept in thermal cycler for amplification of desired product with program of initial denaturation 95 °C/2 min, and 40 cycles of 94 °C/30 sec, 52 °C/30 sec, 72 °C/45 sec with a final extension step of 72 °C/5 min. The PCR products were resolved on 1.5% agarose gel. The gel purified fragments were cloned in pGEM-T easy vector (Promega) and subjected to automated sequencing.

Sequence Analysis

ClustalW and NJ plot (swift.embl-heidelberg.de), respectively, were used for multiple sequence alignment and construction of the phylogenetic tree. EMBOSS(http://liv.bmc.uu.se/cgi-bin/emboss/antigenic) was employed for antigenic site prediction. The sequence
upstream to the isolated HVR-1 consisting of 70 nucleotides was used in genotype analysis by comparing with the reference sequence for genotype prediction based on E1 region.

Amplification and Cloning of E1 and E1E2-100
c-DNA clone of envelope coding sequence of the Indian isolate, AY051292 was employed as a template for amplification of E1 and E1E2-100. The sequence containing N-terminal 100 aa of E2 fused to E1 was designated as E1E2-100. A common forward primer 5’ATT GAA TTC TGT CGA AGT GCG CAA CTC T 3’ and two reverse primers, 5’ATA AGC TTT GCA TCG CCA GCG AAG A 3’ and 5’ ATT AAG CTT CAC GAT ACC GCA CGG GCG T 3’ were employed in PCR for the amplification of E1 and E1E2-100 respectively, with reaction setup and conditions similar to the amplification of HVR1. The PCR amplified fragments were double digested with EcoRI and HindIII for ligation at respective sites of pET21b (Sambrook et al., 1989).

Expression of E1 and E1E2-100
BL21(DE3) competent cells of *E. coli* were transformed with recombinant plasmid (pET21b-E1 or pET21b-E1E2-100) and grown on LB plate containing ampicillin (70 µg/mL). A single colony was inoculated in 10 mL of LB medium containing 70 µg/mL ampicillin and grown overnight in a 50 mL flask at 37 °C. 100 mL of pre-warmed media containing antibiotic was inoculated with 1 mL overnight grown culture and incubated at 37 °C with vigorous shaking till OD600 reached 0.6. After reaching 0.6 OD 1 mM IPTG was added to the culture for expression of recombinant proteins. Prior to induction, 1 mL aliquot of culture was collected; cells were pelleted and stored at –20 °C for further analysis. The samples were collected after 3 h and 6 h of IPTG induction.

Preparation of Inclusion Bodies
The induced culture (100 mL) of Bl21 (DE3) containing pET21b-E1 or pET21b-E1E2-100 was centrifuged at 10,000 g for 15 min to pellet the cells. The supernatant was discarded and pellet was resuspended in 10 mL of phosphate buffer (50 mM NaH₂PO₄, pH 8.0, 300 mM NaCl). The pellet was washed thrice with phosphate buffer containing 0.1% triton to remove cell debris. Partially purified inclusion bodies of E1 or E1E2-100 protein were dissolved in 2 mL of phosphate buffer containing 0.5% sodium lauryl sarcosine.

Detection of E1 and E1E2-100
The partially purified inclusion bodies of E1 and E1E2-100 proteins were subjected to western blot analysis. Western blots were carried out (Towbin et al., 1979) using semi-dry graphite transfer apparatus of Amersham. Anti-His antibody was used to detect the presence of His tag in the expressed envelope proteins. A total of six HCV infected patients’ sera samples were employed in the western blot analysis for
detection of E1 and E1E2-100. Anti-Human-IgG raised in rabbit conjugated with alkaline phosphatase (ALP) was used as a secondary antibody. The blot was developed using NBT/BCIP in ALP reaction buffer till the appearance of clear bands. The reaction was stopped by washing the blot thrice with tris buffered saline (TBS) with 0.1% Tween 20.

Results

Characterization of HVR1 of HCV

A total of 100 sera samples, tested positive for anti-HCV antibodies, were employed for the analysis of HVR1. Out of these samples, only 21 yielded an amplified product of ~340 bp when RT PCR was carried out using primers for the conserved regions flanking HVR1. Amplified fragments of HVR1 were cloned in pGEMT-Easy vector and were subjected to sequencing. Multiple alignment of nucleotide sequences of HVR1 from 21 isolates, in comparison to the Indian isolate AY051292, disclosed marked variations in 2hvr, 3hvr 8hvr, 20hvr and 21hvr, while 4hvr, 11hvr, 17hvr, 18hvr and 19hvr showed moderate nucleotide substitutions. Conversely, 11 sequences, viz., 1hvr, 5hvr, 6hvr, 7hvr, 9hvr, 10hvr, 12hvr, 13hvr, 14hvr, 15hvr and 16hvr, showed a little variation (Figure 1). Among various isolates, HVR1-nucleotide-sequence of 8hvr—compared to the reference sequence AY051292—exhibited maximum variation (44.4%), followed by 21hvr (28.4%), 20hvr (23.4%), 2hvr (20.1%) and 3hvr (18.5%). The base sequence has been relatively conserved in the regions flanking HVR1; and the region representing E2 was more variable than E1. However, 5hvr and 16hvr showed single nucleotide changes in the flanking regions. Genotyping using phylogenetic analysis of E1 (upstream of HVR1) sequences of various Indian isolates revealed that all the analyzed sequences belong to the genotype 1. Further, 8hvr could be classified as subtype 1a, while 17hvr showed homology to 1b. However, the remaining 19 HVR1 sequences were assigned to the subtype 1c (Figure 2).

The deduced aa sequence of HVR1, when subjected to multiple sequence alignment, revealed extensive aa variations in the central region (Figure 1). However, aa glycine at 6th, 7th and 23rd positions was found conserved. On the other hand, 19hvr is the lone exception wherein glycine at 7th position is replaced by alanine. A critical analysis of the variability in 8hvr showed that changes in the nucleotide sequence flanking HVR1 failed to alter the coded aa, while base substitutions within HVR1 changed the respective aa (Figure 1). Homology between HVR1 of the Indian isolates and representative sequences along with the predicted antigenic sites are presented in Table 1. Despite various alterations in the HVR1 region, potential antigenic sites could be observed in the region between 10th and 24th aa of HVR1 in all the sequences analyzed.
Figure 1: Multiple Sequence Alignment of Nucleotides and Deduced Amino Acid Sequences of HVR1 of Indian HCV Isolates

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Figure 1 (cont.)

Clustal Consensus

|       | Y | V | S | N | H | V | K | A | R | K | L | V | L | L | C | A | G | U | D | A | T | Q |
| 90    |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 100   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 110   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 120   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 130   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 140   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 150   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |
| 160   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |

[AY051292]
VTGTAAGRNYALASLTSTGSPSNIQL
11hrv:
VTGTAAGRNYALASLTSTGSPSNIQL
[3hrv:]
TTGGAAATNNSLHLSGSPSNIQL

[4hrv:]
YTGGAAATTNNAHNLHLSGSPSNIQL

[5hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[6hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[7hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[8hrv:]
TCAGGGGGG....TTGGCACT.....GCT...GCT...GCT...GCT...

[9hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[10hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[11hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[12hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[13hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[14hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[15hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[16hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[17hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[18hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[19hrv:]
VTGTAAGRNYALASLTSTGSPSNIQL

[20hrv:]
RTGGAAATTNNAYNMTLRSLSSPGPS

Analysis of Envelope 1 (E1) and Hyper Variable Region-1 (HVR-1) Encoding Sequences from Indian Isolates of Hepatitis C Virus.
Figure 1 (cont.)

Clustal Consensus

Note: Dotted lines indicate no change in nucleotide sequence when compared with reference sequence AY051292 and dashed lines indicate lack of base information. The deduced aa sequence is represented below the respective nucleotide sequence. The continuous stretch of aa in bold and red indicate the HVR1 sequence.
Detection of Antibodies for HCV Envelope Proteins (E1 and E1E2-100) in Indian Patients

A clone consisting of c-DNA for HCV envelope region of the Indian isolate (AY051292) was selected as a template for PCR amplification leading to the formation of amplified products of ~580bp and ~910bp of E1 and E1E2-100, respectively. These PCR products were cloned at EcoRI and HindIII sites of pET21b. Sequencing of pET21b-E1 and pET21b-E1E2-100 showed the presence of E1 and E1E2-100 in frame to ATG of pET21b.

Note: ◁Indicates the sequences having maximum variation.
Samples induced with IPTG when resolved on 12% SDS PAGE showed the expression of E1 and E1E2-100 proteins having molecular weights of ~20 kD and ~30 kD, respectively; while these bands were absent in the uninduced sample (Figure 3). Expression levels of E1 protein were higher than that of E1E2-100. Both the proteins were expressed in the insoluble fraction. Expressed E1E2-100 protein was unstable and degraded upon induction for more than 3 h. Partially purified inclusion bodies of E1 and E1E2-100 in western blots could yield positive signals with anti-His as primary antibody (Figure 4a). Western blot analysis of purified E1 showed positive signals at ~20 kD in four out of six HCV RNA positive sera (Figure 4b). However, all the six sera showed positive signals at ~30 kD when western blot analysis was carried out with E1E2-100 (Figure 4b).

**Discussion**

The present investigation mainly deals with the analyses of HVR1 sequences from Indian isolates of HCV. These isolates have been assigned to specific genotype based
Figure 3: Expression of E1 and E1E2-100 Proteins

Lane 1. Uninduced pET21b-E1/BL21(D3)
Lane 2. Induced pET21b-E1/BL21(D3)
Lane 3. Uninduced pET21b-E1E2-100/BL21(D3)
Lane 4. Induced pET21b-E1E2-100/BL21(D3)
Lane M. Protein Marker (kD)

Figure 4: Western Blot Analysis of E1 and E1E2-100 Proteins

A. Western blot using anti-His antibody
   Lane 1. E1 protein
   Lane 2. E1E2-100 protein
   Lane 3. Induced pET21b/BL21(DE3) lysate

B. Western blots of E1 and E1E2-100 developed with different patients’ sera
   Lane 1. E1
   Lane 2. E1E2-100
on the sequence information of E1. This study further shows the potentiality of E1E2-100 fusion protein for an improved detection of HCV infection. PCR amplification of HVR1 employing primers for conserved flanking regions exhibited an amplification product of ~340 bp in only 21 specimens. Low sensitivity (21%) of amplification observed may be attributed to low viral copies present in the original specimen, degradation of viral RNA, and high degree of variation in the nucleotide sequence despite conserved aa present in this region. The primers employed in this study were designed based on the representative nucleotide sequence but not as the degenerate sequence for conserved aa sequence. The sequence analysis of flanking regions of HVR1 (E1 and E2) of various isolates revealed changes in the nucleotide sequence which, however, failed to alter the coded aa, thereby supporting the importance of employing degenerate primers for effective amplification. Earlier studies for optimization of primers in PCR-based variability analysis revealed that a certain level of primer degeneration compatible with specific product amplification would be desirable (Fan et al., 2001; and Bracho et al., 2004).

Multiple alignment of HVR1 nucleotides of the various isolates disclosed that the flanking regions of HVR1 (E1 and E2) were relatively conserved compared to HVR1 coding region (Figure 1). Because of its conserved nature, sequence data of E1 were employed for HCV genotyping (Bukh et al., 1993; and Corbet et al., 2003). Phylogenetic analysis of E1 sequences indicated genotype 1 as the most prevalent type (Figure 2). As compared to the reference sequence AY051292 belonging to subtype 1c, isolate 8hvr of subtype 1a exhibited maximum variability in HVR1, followed by isolates 21hvr, 20hvr, 2hvr and 3hvr of subtype 1c and 17hvr of subtype 1b; whereas minimal variation was observed in other isolates of subtype 1c, suggesting that the extent of variability generated in HVR1 was subtype independent in genotype 1. However, HVR1 sequences, viz., 1hvr, 5hvr, 6hvr, 7hvr, 9hvr, 10hvr, 12hvr, 13hvr, 14hvr, 15hvr and 16hvr, showed minimal variation in comparison to other isolates. The observed sequence stability may be attributed to weak positive selection pressure or collection of samples prior to elicitation of the humoral and cellular immune response. Similar results of sequence stability were reported from chimpanzees challenged with a well-characterized strain of HCV (H77) obtained via plasmapheresis from a patient with acute HCV infection (Ray et al., 2000).

Variations in the nucleotide sequence in the flanking region of HVR1 in 8hvr did not alter the aa sequence probably because of codon degeneracy. Large-scale changes in nucleotide sequence leading to aa substitutions within the HVR1 and their absence in the relatively conserved flanking region may be attributed to the selection pressure exerted by the host immune system, as the principal neutralization epitopes are localized in the HVR1 region (Weiner et al., 1992). Analyses for the presence of antigenic sites predicted the region between 10th and 24th aa endowed with high propensity (Table 1). Although distinct variations were observed for aa residues in the central region of HVR1, the localization of predicted antigenic sites is conserved in different HCV isolates analyzed. The conserved glycines at 6th, 7th and 23rd aa positions might facilitate formation of turns in the secondary structures where region between them
is in α helix or β sheet. As such, the aa sequence present between the conserved glycines might get exposed to the host immune pressure, contributing to the variability observed in this region. Penin et al. (2001) reported that the variation observed in HVR1 aa sequence, consequent to host immune pressure, did not affect its chemico-physical properties and conformation.

HVR1 sequence of different HCV isolates with reference sequences disclosed nucleotide homology of ~67% and protein identity ranging between 29% and 51% (Table 1). The distinctive nature of HVR1 observed in the present study warrants further investigations to gain insights into receptor and immune modulations caused by HVR1 in Indian patients. Long-term followup of these cases might enable determination of mutational and clearance rates of HCV. Data generated in this study indicate the feasibility of designing an HCV vaccine specific to this geographical region employing prevalent HVR1 sequence.

HCV envelope coding sequences, E1 and E1E2-100, of the Indian isolate were cloned in pET21b for expression in E. coli. The presence of antibodies in sera of four out of six patients (66.7%) for E1, observed in this study, was higher than those reported previously, in which infected sera were tested with recombinant E1 expressed in insect and bacterial cells (Ray et al., 1994; and Hussy et al., 1997). However, no positive signal was observed when an uninduced E. coli lysate was used, owing to the absence of cross-reacting antibodies in the patients' sera. Lack of positive signals for anti E1 in two patients may be attributed to the low levels or absence of antibodies to E1 in the sera. Whereas, the E1E2-100 fusion protein employed in western analysis revealed positive signals at ~30 kD in all the six sera, suggesting an improved immunoreactivity of E1E2-100 over E1. These results further indicate that the immune response against E2 might be elicited prior to E1. Earlier studies reported the appearance of anti-HVR1 antibodies prior to anti E1, and suggested that the earlier appearance of anti E2 might be responsible for the clearance of HCV (Cerino et al., 1997; Zibert et al., 1997; Meyer et al., 2002; and Zhang et al., 2003). Also, antibodies specific to HVR1 were found to protect against the re-infection of HCV (Farci et al., 1996; and Habersetzer et al., 1998).

The overall results amply suggest the feasibility of developing an improved diagnostic kit employing the fusion protein E1E2-100 for detection of early-stage HCV infections. Furthermore, E1E2-100 seems to have great potential as a therapeutic vaccine specific to the geographic region, as the fusion protein is derived from the most prevalent sequence identified from the HVR analysis.

**Conclusion**

Multiple alignment of nucleotide sequences of HVR1 from 21 HCV isolates, compared to the Indian isolate AY051292, disclosed high variations in five HVR sequences. Another five sequences showed a few nucleotide substitutions, while 11 sequences, by contrast, showed a little variation. These results suggest that the nature and extent of variability...
generated in HVR1 is random and subtype independent. Multiple alignment of deduced aa of HVR1 revealed large-scale changes in the nucleotide sequence leading to aa substitutions within HVR1. Absence of aa alterations in the relatively conserved flanking regions may be attributed to the selection pressure exerted by the host immune system. Despite numerous alterations noted in the HVR1 region, potential antigenic sites could be observed specifically in the region between 10th and 24th aa. Out of six anti-HCV positive sera analyzed, four showed positive signals with E1, while all the six sera revealed positive signals with E1E2-100, suggesting an improved immunoreactivity of the fusion protein over E1.

Acknowledgment: MSK is highly grateful to the DBT and CSIR, Government of India, New Delhi, for the award of fellowship. The authors extend their thanks to Prof. T Papi Reddy, former Head, Department of Genetics, Osmania University for his helpful suggestions in improving the manuscript.

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Reference # 66J-2010-08-01-01