Research Article

Comparison of Structural Architecture of HCV NS3 Genotype 1 versus Pakistani Genotype 3a

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1. Background

The hepatitis C virus (HCV) is a positive-sense, approximately 9.6 kilobase (kb) single-stranded RNA (ssRNA) which is translated into a polypeptide of over 3,000 amino acids long from a single open reading frame. HCV belongs to genus Hepacivirus of Flaviviridae family [1] and sole member of its family. Both the host and viral proteases process the HCV polyprotein into four structural proteins (core, E1, E2, and p7) and six nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [2].

HCV existed as quasispecies due to its better evolutionarily stable strategy. To date six genotypes [1–7] and more than 100 subtypes have been described [3]. These subtypes can exist as separate or in combination in an individual [4]. In Pakistan more than 10 million people are living with HCV, with high morbidity and mortality and most prevalent genotype of HCV was 3a [5]. HCV disease severity and its treatment response are greatly influenced by its nature [6]. Peg-interferon and ribavirin have been used with highest positive response rates for the treatment of genotypes 2 and 3 chronic HCV infection in developed countries [7–9]. It is ever challenging to characterize HCV heterogeneity and its treatment in developing countries due to different aspects including immunologic stress, HCV relapse rate, insignificant epidemiological studies, therapeutic cost, and patient’s access to treatment of disease complications over time [10]. This comparative study is therefore designed to define the influence of genetic variation on the structural architecture of HCV NS3 GT3a, that is, which was previously studied extensively by cloning of NS3-GT3a protease from local population [11].

NS3 protein has two separate independent functional domains. A serine protease domain localized to the 181 residues of N-terminal, which acts to cleave HCV polyprotein and RNA/DNA helicase composed of 456 amino acids in C-terminal. Helicase portion unwinds duplex RNAs formed during the viral RNA replication in a 3'→5' direction [12]. Inquisitively, the HCV helicase unwinds DNA more efficiently than RNA [13] even though the absence of any DNA intermediates in the HCV virulence cycle.

Several crystal structures of HCV NS3 including PDB IDs 3OBB [14], 1CU1 [15], ZZJO [16], 1AIV [17], IH01 [18], 3KQn [19], 2F5S [20], and 8OHM [21] and mechanistic studies [12, 21–27] helped to predict the ATP derived energy coupled helicase activity but all of the studies exclusively focused on...
genotype 1a or 1b of HCV. However, no molecular architecture of full length HCV GT3a NS3 has been elucidated. Little is known about the impact of residue variations on the HCV GT3a NS3 activities.

Objectives. The NS3 was chosen for this study because it has a clear biological relevance and can be relatively modeled and characterized easily by using the predetermined crystal structures of HCV genotype 1 NS3. This study described the characterization of HCV NS3 GT3a in parallel to genotypes 1a and 1b NS3. The goal is to understand the structural architecture of HCV genome variability and to further define the conserved targets for antiviral agents.

2. Methods

The sequence of NS3 protein belonged to Pakistani strain of HCV genotype 3a previously reported (PK-NCVI/Pk3a NS3) with accession number (FJ839678) and NZL1 (NC_009824) was obtained from online database of Genbank maintained by NCBI. Only the sequence of PK-NCVI/Pk3a NS3 (FJ839678) was submitted to position-specific iterative BLAST [28] at NCBI to obtain homologous 3-dimensional (3D) structures of proteins deposited in the protein data bank (PDB). The further analysis was done based on highest sequence identity and smallest distance from PK-NCVI/Pk3a as potential templates with comparative modeling, including PDB Ids 3KQN [19] (X-ray structure of hepatitis C virus NS3 helicase in complex with ssRNA), 3O8B [14] (Crystal structure of HCV NS3 protein), and 1CU1 [15]. Sequences of crystal structures having PDB ids 3O8B [14], 1CU1 [15], 2ZJO [16], 1A1V [17], 1HEI [18], 2F55 [20], and 8OHM [21] were the other entries for NS3 protein in PDB and studied for protein-protein comparison. Multiple sequence analysis was carried out following an established protocol using ClustalW and T-Coffee along with rapid scanning and correction of multiple sequence alignments [31, 32]. The resulting multiple alignments were visualized and annotated using the sequence alignment protocol in CLC sequence viewer (CLCBIO, Aarhus, Denmark). The homology model for consensus sequence was produced using MODELLER [33, 34]. The different structural models were produced and clustered into groups by using NMRCLUST [35] based on the root mean square distance (RMSD) between the corresponding residues in their structures. Best model was selected using the results of NMRCLUST, ERRAT score, and MODELLER. Inspection of the final structural model indicates that some major mutations are present in the helicase and non-functional regions of PK-NCVI/Pk3a except for mutations of Lys224Asn in motif I (Walker motif: G207SKGSTK), Ia (Y223KVLTNPSVA), II (Walker B: D290ECH), III (V319LATATPP), IV (L365FCHSSKKK), V (A410TDALMTGTGDFF), and VI (V456SRSQRRGRTGR) were conserved in all HCV NS3 proteins of different genotypes. Structural architecture of these motives presented that those motives make a border to ATP binding cleft and project some residues into the nucleic acid binding site. The residues of these motives transform the chemical energy derived from ATP hydrolysis into a mechanical force necessary for helicase movements for the disruption of ssRNA or RNA base pairs. Analogous IM interaction pattern of those domains was seen in all of the crystal studies and comparative model of reported NS3 except for mutations of Lys224Asn in motif Ia and Tyr418Phe in motif V (Supplementary Figure 1 available online at http://dx.doi.org/10.1155/2014/749254). Results implicated that mutation in motif Ia was strengthening its confirmation while motif V was loosely bound.

The role of most of the key conserved residues including Ala204, Thr234, Lys210 Ser231, Ser370, Thr411, Thr266, Tyr267, Met288, Thr269, Asp290, Lys291, Cys292, His293, Thr322, His369, Glu493, Tyr392, Thr501, Arg39, Val432, Phe438, Phe444, Thr450, Glu460, Arg461, Arg462, Gly463, Arg464, Thr465, Gly466, and Arg467 of HCV genotype 1a NS3, using site directed mutagenesis, has already been investigated. The comparison of these residues along with phylogenetic analysis in order to identify percentage identity of reported NS3 GT3a (NZL1) with our target sequence PK-NCVI/Pk3a NS3 by comparative modeling. The target sequence and crystallographic analysis of PDB Id. 3O8B and 1CU1 shared the phylogenetic branch of target sequence as compared to other crystallographic studies (Figure 1(a)). Phylogenetic analysis was further confirmed by the multiple alignment percentage identity of 76.06% among their residues by using ClustalW and T-Coffee along with rapid scanning and correction of multiple sequence alignments (Figure 1(b)). The resulting multiple alignments were visualized and annotated using the sequence alignment protocol in CLC sequence viewer. It was found that most of the sequence variations were divided among all of the domains but N-terminal and middle portion of the reported NS3 protein were comparatively rich in variations (Figure 2). NS3-NS4A is composed of six subdomains (Figure 3(a)). The helicase, usually referred to as α-helical subdomain, consists of two structurally related β-α-β subdomains and a third subdomain of three short β strands and seven helices. The protease domain is folded into dual barrel fold, like other members of chymotrypsin serine protease family. The 13-residue NS4A proved to be a protease activation domain and is considered to be the sixth subdomain (Figure 3(a)). By using NMRCLUST, sixty models were produced and clustered into groups. The best structural model was selected using the results of NMRCLUST, ERRAT score, and MODELLER. Inspection of the final structural model indicates that some major mutations are present in the helicase and non-functional regions of PK-NCVI/Pk3a which presents some new non-covalent intermolecular (IM) interactions alongside of conserved sequence (Figure 3(b)). Major domains including motifs I (Walker motif: G207SKGSTK), Ia (Y223KVLTNPSVA), II (Walker B: D290ECH), III (V319LATATPP), IV (L365FCHSSKKK), V (A410TDALMTGTGDFF), and VI (V456SRSQRRGRTGR) were conserved in all HCV NS3 proteins of different genotypes. Structural architecture of these motives presented that those motives make a border to ATP binding cleft and project some residues into the nucleic acid binding site. The residues of these motives transform the chemical energy derived from ATP hydrolysis into a mechanical force necessary for helicase movements for the disruption of ssRNA or RNA base pairs. Analogous IM interaction pattern of those domains was seen in all of the crystal studies and comparative model of reported NS3 except for mutations of Lys224Asn in motif Ia and Tyr418Phe in motif V (Supplementary Figure 1 available online at http://dx.doi.org/10.1155/2014/749254). Results implicated that mutation in motif Ia was strengthening its confirmation while motif V was loosely bound.

The role of most of the key conserved residues including Ala204, Thr234, Lys210 Ser231, Ser370, Thr411, Thr266, Tyr267, Met288, Thr269, Asp290, Lys291, Cys292, His293, Thr322, His369, Glu493, Tyr392, Thr501, Arg39, Val432, Phe438, Phe444, Thr450, Glu460, Arg461, Arg462, Gly463, Arg464, Thr465, Gly466, and Arg467 of HCV genotype 1a NS3, using site directed mutagenesis, has already been investigated. The comparison of these residues along with
their interactions in modeled and template protein has been depicted in Table 1.

Some residues were seen to make different interaction pattern with their neighbors especially in the presence of ssRNA, ATP and solvent molecules. In the crystallographic studies of helicase interaction with ssRNA PDB Id, 3KQN Gln460 interacts with Thr324 and Arg464 but in the model of PK-NCVI/Pk3a and other studies without ATP and ssRNA, this interaction was seen with His293 and Arg464. Some amino acids like Glu291, Cys292, His293, and Thr324 were evaluated to be on surface and create a hydrogen bond with water used as solvent during crystallographic studies which was absent in the model of PK-NCVI/Pk3a. Some of the residues, which are not part of the functional regions of protein and nonconserved in PK-NCVI/Pk3a NS3, were actually involved in the structure stability. These residues are Arg9Gln, Leu332Pro, Leu354Ile, Ile605Val, and Ser622Cys [11]. The comparison of the IM interactions among nonconserved residues of PK-NCVI/Pk3a NS3 and of HCV NS3 of other genotypes is listed in Table 2.

Figure 1: Sequence comparisons of the HCV NS3 genotypes. (a) Neighbour-joining tree based on phylogenetic analysis showing the relationships of HCV NS3 GT3a with GT1a/1b (b). Diagonal represents the comparison of percentage identities among crystal structure of HCV NS3 with Pk3a HCV NS3 and HCV NS3 of NZL1 strain. Dark colored values showed a higher percentage identity among cross matched sequence.

|     | 1CU1 | 3KQN | 3O8B | 2F55 | 8OHM | 2ZJO | 1A1V | 1HEI | NZL1 | Pk3a HCV NS3 |
|-----|------|------|------|------|------|------|------|------|------|-------------|
| 1   | 67.51| 67.31| 66.40| 71.95| 68.98| 67.19| 75.77| 76.06| 86.95| 91.02       |
| 2   | 67.72| 96.34| 95.42| 88.63| 88.06| 90.69| 54.16| 54.31| 54.89| 56.35       |
| 3   | 67.72| 66.40| 71.95| 68.98| 67.19| 75.77| 76.06|       |      |             |
| 4   | 94.94| 87.37| 86.57| 89.36| 53.58| 53.58|       |      |      |             |
| 5   | 86.95| 86.37| 89.14| 52.85| 53.43|       |      |      |      |             |
| 6   | 91.02| 88.63| 56.20| 56.35|       |      |      |      |      |             |
| 7   | 95.31| 56.35|       |      |      |      |      |      |      |             |
| 8   | 54.89| 54.89|       |      |      |      |      |      |      |             |
| 9   | 97.04|       |       |      |      |      |      |      |      |             |
| 10  |       |       |       |      |      |      |      |      |      |             |

(a) Neighbour-joining tree based on phylogenetic analysis showing the relationships of HCV NS3 GT3a with GT1a/1b. (b) Diagonal represents the comparison of percentage identities among crystal structure of HCV NS3 with Pk3a HCV NS3 and HCV NS3 of NZL1 strain. Dark colored values showed a higher percentage identity among cross matched sequence.
Figure 2: Multiple sequence alignment of different reported NS3 structures and target sequences of PK-NCV1/Pk3a NS3. Motifs I-VI are marked by boxes on the first sequence and labeled, respectively. The conserved sequences are shown as dots.
The catalytic triad located in a cleft between two subdomains (or barrels), with His57 and Asp81 in the N-terminal subdomain and Ser139 in the C-terminal, has the similar behavior of IM interactions with their environment as shown in genotype 1a/1b. Along with other amino acids involved in making an active site of ATP molecules, Tyr241 and Thr419 sandwich the ADP adenine base (Figure 4). We found the mutation of Tyr241Phe which can affect the ATP orientation and its effect on the helicase activity although the electrostatics of site produce changed environment. Main-chain nitrogen (from Gly207 to Thr212) and side-chain atoms (Lys210 and Thr212) were
Table 1: The intermolecular interactions shared between NS3 of HCV genotypes 1 and 3.

| Residue | Interactions shared among model and templates (bonding → atom of residue) |
|---------|-------------------------------------------------------------------------|
| Ala204  | HB → NZ of Lys210                                                        |
| Lys210  | HB → O of Ala204                                                         |
|         | HB → O of Pro205                                                         |
| Ser231  | HB → OG1 of Thr235                                                      |
|         | HB → O of Thr235                                                        |
| Thr266  | HB → OH of Tyr284                                                       |
| Tyr267  | HB → O of Ser211                                                        |
| Ile288  | HYD → CB of Val319                                                      |
| Asp290  | HB → OG of Ser211                                                      |
|         | HB → OH of Tyr270                                                      |
| His293  | HB → OG1 of Thr322                                                     |
| His369  | HB → OH of Tyr350                                                      |
| Tyr392  | HB → OD2 of Asp375                                                     |
| Phe438  | HYD → CB of Phe444                                                     |
|         | HYD → CE2 of Phe531                                                     |
|         | HYD → CE of Met623                                                      |
|         | HYD → CZ2 of Trp532                                                     |
|         | HYD → CD1 of Leu627                                                    |
| Glu460  | HB → NE of Arg464                                                      |
| Arg461  | HB → OD1 of Asp410                                                     |
|         | HB → OD1 of Asp425                                                     |
| Arg462  | HB → O of Val331                                                       |
|         | HB → OE2 of Glu338                                                     |
| Gly463  | HB → NH1 of Arg467                                                     |
| Arg464  | HB → O of Tyr418                                                        |
|         | HB → O of Gly420                                                        |
| Thr465  | HB → O of Phe422                                                        |
|         | HB → O of Asp423                                                        |
|         | HB → OG1 of Thr465                                                     |
| Gly466  | HB → ND2 of Asn335                                                     |
| Arg467  | HB → O of Thr419                                                        |

HB = hydrogen bond, Hb = hydrogen bond, CB = carbon atom with number, O = oxygen in hydroxyl group, OG = oxygen of amino acid with some number, that is, G, OG1 = oxygen with number; that is, Gl, OH = oxygen with distinct number, that is, H, CE = carbon atom of amino acid with some number, that is, E, CE2 = carbon atom with some number, that is, E2, CDI = carbon atom with some distinct number, that is, D1, NE = nitrogen atom with number, that is, H1, HYD = hydrophobic bond.

conserved and showed similar interactions in the model of PK-NCV/Pk3a NS3. Previous mutagenesis and arginine methylation studies have already confirmed the importance

Table 2: List of nonconserved residues in HCV NS3 genotype 3a structure in comparison with other HCV NS3 genotypes. Bold residues were seen shared among templates and models.

| Position of the residue | Crystal structures Residue | Interactions | Model of NS3 GT3a Residue | Interactions |
|------------------------|---------------------------|--------------|---------------------------|--------------|
| 219                    | ALA                       | —            | VAL225                    | ILE248, LEU265 |
| 224                    | LYS                       | ALA283, ASP285 | ASN                       | ASP285 |
| 286                    | ILE                       | LEU317, VAL319 | VAL                       | LEU317, VAL319 |
| 297                    | SER                       | GLU493       | ALA                       | —            |
| 315                    | ALA                       | —            | VAL                       | ILE285 |
| 354                    | ILE                       | ILE359, ILE366, ILE426 | LEU | LEU341, ILE426, PHE349, LEU358 |
| 358                    | THR                       | —            | LEU                       | LEU358 |
| 377                    | LEU                       | LEU381, VAL409 | ILE                       | LEU381 |
| 384                    | LEU                       | —            | MET                       | ILE345 |
| 407                    | ILE                       | —            | VAL                       | ILE357, ILE364, LEU379 |
| 430                    | THR                       | ARG461       | VAL                       | ARG461 |
| 433                    | THR                       | —            | GLU                       | ARG479 |
| 445                    | THR                       | —            | SER                       | SER439 |
| 477                    | THR                       | —            | SER                       | ASN429, ARG458 |
| 489                    | SER                       | —            | VAL                       | PHE557, VAL436, ILE446 |
| 500                    | ALA                       | LYS551       | SER                       | ASP496 |
| 505                    | THR                       | —            | GLN                       | THR509 |
| 510                    | SER                       | GLU533       | THR                       | —            |
| 530                    | GLU                       | —            | ASP                       | SER534 |
| 553                    | ALA                       | —            | GLN                       | LYS583 |
| 561                    | VAL                       | MET581, TRP582, LEU602 | THR | PHE557 |
| 605                    | VAL                       | VAL609       | ILE                       | VAL609, LEU598 |

of Lys210, Arg464, and Arg467 in the ATP binding site [38–41]. Glu460 and Ala323 along with three positively charged side chains of Lys210, Arg464, and Arg467 were conserved and showed the similar interactions with γ-phosphate of ATP.

3.2. Structural Comparison of HCV NS3 Protease Domain. Protease domain in HCV NS3 is covalently connected to helicase by a loop of residues including Pro182, Val183, Phe184, Thr185, and Asp186 which forms a solvent-exposed
strand in all of the crystal structures of HCV NS3. Due to the flexible nature of this loop, different residues of protease and helicase domains get involved in making intradomain interaction. Mutations at position 183 and 185 with Ser in this loop of PK-NCVI/Pk3a NS3 presented almost conserved residues for protease-helicase interaction.

The interaction site for the protease and helicase was also studied in different genotypes along with PK-NCVI/Pk3a NS3 (Figure 5). Interaction between helicase and protease normally involves Tyr56, Gly60, Ser61, Val78, and Asp79, Arg155 from the protease domain and Gly327, V329, Ser481, Pro482, Ser483, Gly484, Met485, Pro520, Gly521, Val522, Val524, and Gln526 from the side of helicase portion of NS3. These residues are conserved in all of the sequences except Val329 which is substituted with Ile329 in comparative model. The only conserved interactions seen in comparative model of NS3 for genotype 3a involved Tyr56, Ser61, Val78, and Asp79, Arg155, Gly327, Ser481, Gly521, Val522, and Gln526 (Figure 5) while rest of the IM contacts were not seen. This difference of interaction pattern between these two domains computationally confirms the orientation of protease domain with respect to helicase domain due to a flank motif connecting helicase and protease domain (Figure 5).

The residues of NS3 C-terminal 626–631 made an antiparallel β-sheet in extended conformation along the brink of protease involving His57, Lys136, Ser139, Arg155, Lys165, and Arg168 from protease domain and Asp625, Asp626, Glu628, Thr430, Thr449, Arg155, Lys136 and Ser139 were found in this interaction for the first time in our study and implicate some strong interaction of C-terminal with protease domain. Mutation of Val to Thr at position 630 showed conserved interaction pattern with protease domain. NS4a protein is involved in the completion of N-terminal protease of NS3 into barrel fold and the assembly of catalytic triad. Mutations of Ser7Ala, Gln9Arg, Gln28Val, and Gln30Thr were present at the borders of NS4a cofactor. Effects of these mutations were examined by running energy minimization protocol, which showed a different conformation for its folding. Similarly, mutations of Gln580Glu, Asp555Leu, Pro558Ser, Ala153Gln, Ala604Pro, Val256Asn, Thr449Arg, Thr430Val, Thr295Gln, Ser294Ala, Thr299Ser, and Ser297Ala were found to be present on either side of ssRNA pathway in helicase.

3.3. Structural Comparison of HCV NS3 Helicase Domain. The model of PK-NCVI/Pk3a was superimposed to the structure of NS3 (PDB Id. 2F55) and the residues involved in the helicase active site were characterized. The proposed ssDNA entry site in one of the dimer shown in PDB ID 2F55 can be seen in (Figure 6(a)). The entry site for ssDNA had a substitution of Val256Asn in homology model of PK-NCVI/Pk3a (Figure 6(b)). Mutations of Gln580Glu, Asp555Leu, Pro558Ser, Ala153Gln, Ala604Pro, Val256Asn, Thr449Arg, Thr430Val, Thr295Gln, Ser294Ala, Thr299Ser, and Ser297Ala were found to be present on either side of the entry of ssDNA (Figure 7). The NTP binding site was located at the periphery of the NTPase domain. The G207SGKST and the D290ECH sequences were forming lining of active site. One of the two loops connecting the RNA binding domain and NTPase had an invariant T322AT. His293, Thr322, and Thr324 are thought to function as a triad in coupling the helicase activity and NTP hydrolysis. We found that the
neighbors of His293 (i.e., Ser294Ala and Thr295Gln) were non-conserved, which were affecting its flexibility to make hydrogen bonds, NTP hydrolysis, and the helicase activity (Figure 8). In the electrostatics computations for the ssDNA entry site, the probability of nonconserved residues in both genotypes presented a difference in the size of orifice for ssDNA/ssRNA entry (Figures 9 and 10). The probability for the small opening may result in a strong grip of helicase upon ssRNA which enhanced the helicase activity.

4. Discussion

The comparative structural modeling (CM) was used to predict the structure-function connection which remained undetectable at the sequences level. Several crystal structures of HCV NS3 and mechanistic studies helped to visualize helicase and protease activity but all of the studies exclusively focused on genotype 1a or 1b of HCV. Here, we used CM to investigate whether the PK-NCVI/Pk3a NS3 of Pakistani origin is employing some new key residues. The HCV NS3 protease along with 54 residues of NS4a protein is necessary to have a correct fold. In the absence of NS4a protein, NS3 protease is partially unfolded [42]. NS4a protein is involved in the completion of N-terminal protease of NS3 into barrel fold and the assembly of catalytic triad. Mutations of Ser7Ala, Gln9Arg, Gln28Val, and Glu30Thr were present at the borders of NS4a cofactor. The energy differences were also present and crystal structures of NS3 were at higher energy level as compared to the energy of PK-NCVI/Pk3a. Although, this procedure somewhat defines the folding pattern of NS4a in NS3 protease domain but further molecular dynamics simulations are needed which will provide some new insights about the folding of NS4a and its involvement in the stabilization of NS3 protease domain. Similarly, mutations of Gln580Glu, Asp555Leu, Pro558Ser, Ala153Gln, Ala604Pro, Val256Asn, Thr449Arg, Thr430Val, Thr295Gln, Ser294Ala, Thr299Ser, and Ser297Ala were found to be present on either side of ssRNA pathway in helicase. The effect of these mutations will be confirmed and validated by running QM-MM computational techniques in the further enhancement of these studies. Crystallographic studies (PDB Id: 2F55) have already confirmed the role of NS3 dimer formation and a long stretch of ssDNA passing through this dimer [20]. The NTP binding site was located at the periphery of the NTPase domain. The G2075GKST and the D290ECH sequences were forming lining of active site. The NTPase
had an invariant T322AT. His293, Thr322, and Thr324 are thought to function as a triad in coupling the helicase activity and NTP hydrolysis. These nonconserved amino acids in the helicase portion may be involved in the strong binding of ssDNA/ssRNA, which may enhance the helicase efficiency of enzyme. The strong grip of helicase might be involved in the rate of ssDNA/ssRNA unwinding. Involvement of nonconserved residues is necessary for the determination of their roles in the rate of unwinding and determination of key residues for helicase inhibitors.

Analysis of the final model and the underlying alignment among PK-NCVI/Pk3a NS3 and reported genotypes of NS3 indicates that the mutations occur in the nonfunctional regions of PK-NCVI/Pk3a NS3 and almost conserved interactions between the residues have been seen. Hence, the substituted residues in PK-NCVI/Pk3a NS3 are not part of the functional regions of protein and are involved in the structure stability [11]. However, experimental techniques should be used to confirm the implications of structural differences and to investigate the congruency of our in silico results.

The IFN treatment response against the HCV genotype 3 is higher (~80%) in comparison to other strains (40%) [43]. However the new class of protease inhibitors seems to be less efficacious against genotype 3a, requiring higher concentration to achieve threshold level of inhibition as measured by qPCR of viral load. This may be due to the differences in the amino acids composition between genotype 1 versus genotype 3, as we have reported thus generating variable architecture and variable response. The biological relevance of the genotypic differences in NS3 has not been addressed in this study, but several intriguing ideas can be gleaned from the resultant data. These regions have less frequent mutations and possible substitutions are either positive in nature or have similar IM contact architecture. Although such ideas are highly speculative but they could be feasibly tested by measuring mutation rates in HCV replicon systems and could be measured in the presence and absence of ribavirin or other antiviral compounds. Altogether, this exercise reflected the interaction patterns of substituted residues along with implications for PK-NCVI/Pk3a NS3 in its role of disease profile and further studies with other genotypes should be carried out to define a complete map of IM noncovalent interactions responsible for the variable disease transmission, severity, and resistance to antiviral therapies.

**Abbreviations**

HCV: Hepatitis C virus  
NS3: Nonstructural protein 3  
kb: Kilo base  
PK: Pakistan  
3a: Genotype 3a  
NS3: Nonstructural protein 3  
DNA: Deoxyribonucleic acid  
ssRNA: Single stranded ribonucleic acid  
QM/MM: Quantum mechanics/molecular mechanics  
PDB: Protein data bank  
IM: Intermolecular  
ATP: Adenosine triphosphate  
ADP: Adenine dinucleotide  
NTPase: RNA nucleoside triphosphatases  
NTP: Nucleoside triphosphate.

**Conflict of Interests**

The authors declare that they have no competing interests.

**Authors’ Contribution**

Ishtiaq Qadri conceived the idea, designed the project, and analysed results. Kaneez Fatima performed the experimental work. Kaneez Fatima wrote the paper and Ishtiaq Qadri and Esam Azhar helped in literature review for the paper. Shilu Mathew and Ghazi Damanhouri did the copy editing and proof reading of the paper.

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