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**Genetic diversity of Hepatitis C virus in Pakistan using next generation sequencing**

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In Pakistan, HCV disease is considered a major public health issue with about 10-17 million people suffering with this infection and rate is increasing every day without any hindrance. The currently available pyrosequencing approach is used to analyze complex viral genomes as it can determine minor variants. It is crucial to understand viral evolution and quasispecies diversity in complex viral strains. Intra-host viral diversity of HCV was determined using Next Generation Sequencing (NGS) from 13 chronically HCV infected individuals. NGS of three different regions (E2 (HVR1), NS3 and NS5B) of HCV-3a allowed for a comprehensive analysis of the viral population. Phylogenetic analysis of different HCV genes revealed great variability within the Pakistani population. The average nucleotide diversity for HVR1, NS3 and NS5B was 0.029, 0.011 and 0.010, respectively. Our findings clearly indicate that patient-2 had greater quasispecies heterogeneity than other patients of same genotype-3a using phylogenetic and one step network analyses. Initially phylogenetic analysis showed that genotype 3a samples have greater genetic diversity. However, no significant difference was determined when nucleotide variability of genotype 3a compared with other genotypes (1a, 1b, 2a and 4a).

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