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Title: Studies on hepatitis C virus non-structural gene 3 mutations and HCV host pathogenesis.

Abstract:

The Hepatitis C virus (HCV) is a major public health problem worldwide. It causes HCV-mediated chronic liver disease and end-stage liver diseases like hepatocellular carcinoma. Approximately, 58 million people are suffering from HCV infection throughout the world. In India, it is estimated that 6-12 million people are HCV-infected which accounts for a large portion of global HCV prevalence. Transmission of HCV is mainly due to blood to blood contact like, injecting drugs, poor blood transfusion methods, and unsafe clinical practices. No vaccine for HCV is available till now. The high error rate of RNA-dependent RNA polymerase (RdRp) of HCV gives rise to 8 genotypes and more than 86 subtypes. The high genetic variation of HCV is the main challenge for vaccine development against HCV. In India, HCV prevalence and genotype distribution is still under reported. HCV non-structural protein 3 (NS3) is a bi-functional enzyme (protease and helicase) which plays a major role in viral replication. It has long been targeted for therapeutic intervention of HCV. NS3 protein-specific T-cell response was found to be associated with viral clearance. Mutations of NS3 protein are also said to have a connection with HCV pathogenesis and cancer. HCV genotype 3 is very common in Southeast Asian nations and challenging to treat with DAAs. Epitope-based vaccinations have recently demonstrated promising outcomes. Beside, vaccine for HCV is an early need of hour.

The major focus of this thesis is to spotlight the epidemiology and genotype distribution of HCV, virus-mediated pathogenesis, mutational analysis of NS3 protein and evaluate MHC-I and MHC-II epitopes on the NS3 gene of HCV genotype 3 using in-silico and *In-vitro* approaches.

It was found that, out of 661 anti-HCV-positive samples examined, 535 samples (80.39%, 535/661) belonged to the high-risk groups and the remaining 126 were from the general population with chronic liver diseases (CLD). Out of 661 samples, 403 samples (60.96%) were RNA-positive. The highest viremia was observed in the People Who Inject Drugs (PWIDs) population (70.70%), followed by haemophilia (62.50%) and thalassemia (65.21%). Genotype distribution of HCV in different population groups revealed an interesting finding. HCV subtype 3a (76.69%) is mostly prevalent in the thalassemia population, whereas subtype 1c (67.95%) was predominant in the CKD population and the case of general population subtypes 3a (36.71%) and 3b (34.18%) were found to be almost equally prevalent. Overall, Genotype 3 was found to be the most prevalent genotype in this region.

The phylogeographic study reveals that subtype 3a strains were related to Sri Lanka, Russia, Pakistan, Myanmar, and Thailand. Whereas, subtype 1c showed a resemblance with isolates from Indonesia, China, Cameroon, and Myanmar. HCV subtype 3b isolates found in this region shared common ancestors with China, Myanmar, Vietnam, Thailand, and other Southeast Asian isolates. Subtype 1b seemed to share similar ancestors with Japan, Myanmar, and Thailand isolates. Subtype 1a had links with other nations' isolates, including China, Venezuela, Germany, and Iran. Subtype 4a very possibly, drifted from Saudi Arab.

HCV also showed disease complexities in high-risk groups like thalassemia and Chronic Kidney Disease (CKD), an NS3 mutation N224T might relate to decompensated liver disease progression. HCV also showed to augment ESRD in CKD patients and further liver inflammation in thalassemia patients.

In this study, The MHC-I and MHC-II epitopes were found utilizing the IEDB server. Antigenicity, non-allergy, non-toxicity, non-human homology, and other important factors were utilized to choose anticipated epitopes. Conservancy analysis was also performed on filtered epitopes. Docking was carried out on highly conserved epitopes utilizing several MHC-I and MHC-II allele structures obtained from PDB. The top-scoring epitopes from docking data were synthesized and tested in *In-vitro* utilizing CFDA-SE cell proliferation and IFN-γ gamma assays.

The top-scoring MHC-I and II epitopes showed encouraging outcomes in T cell proliferation and IFN-γ response *in vitro*.

This thesis comprised HCV epidemiology, pathogenesis, genotype distribution and mutational changes within the NS3 protein across the different genotypes. Epitopes predicted and validated in this thesis may be further utilized to produce vaccination against HCV in the future.

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