Molecular Study of Hepatitis C Viral RNA Extracted From Local Isolates In Pahang, Malaysia: *Genotyping, Subtyping And Base Sequencing*

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BACKGROUND

Hepatitis C virus infection affects approximately 170 million individuals constituting about 3% of the world's population. Most of those infected face the risk of developing liver cirrhosis and/or liver cancer. In Malaysia, hepatitis C prevalence is 1.6% and is still the foremost infection among multiple blood transfusion groups. The current mainstay treatment of HCV is pegylated alpha-interferon in combination with ribavirin, incurring considerable expense on local health services. In fact, less than 50% of treated patients respond favorably to the given therapy. Understanding the characteristics of the RNA genome of the local HCV genotypes can serve as foundation for future development of rapid diagnostic techniques. In addition, it has the potential for helping in designing small interfering RNA (siRNA) to be utilized in studies related to specific silencing of vital viral genes. However, despite the plethora of global HCV studies, there is relative scarcity of HCV research in Malaysia. Currently, we present results of study of HCV isolates from infected haemodialysis (HD) patients focusing on the characterization of their genomes, by genotyping and base-sequencing.

METHODS

Serum samples collection

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Viral RNA Extraction

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RT-PCR assay of 5'UTR and NS5B region of HCV genome

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Sequencing

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MEGA 4 and MFOLD software analysis

RESULTS

Fig A

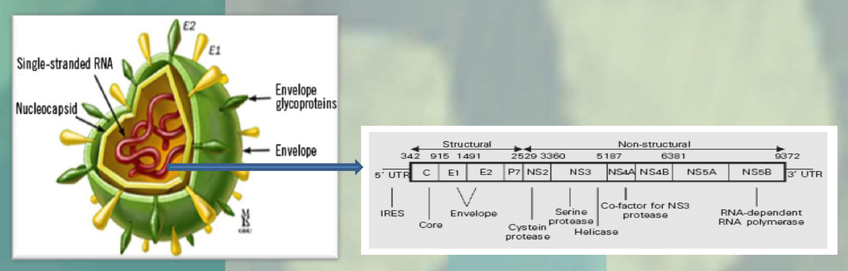


Fig B

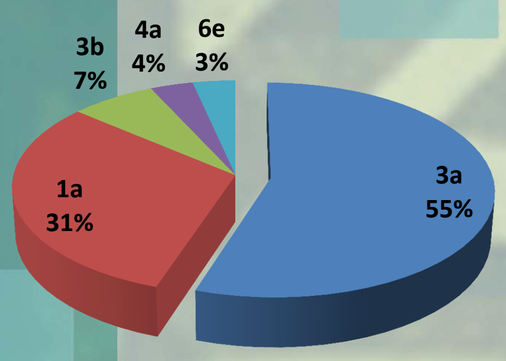
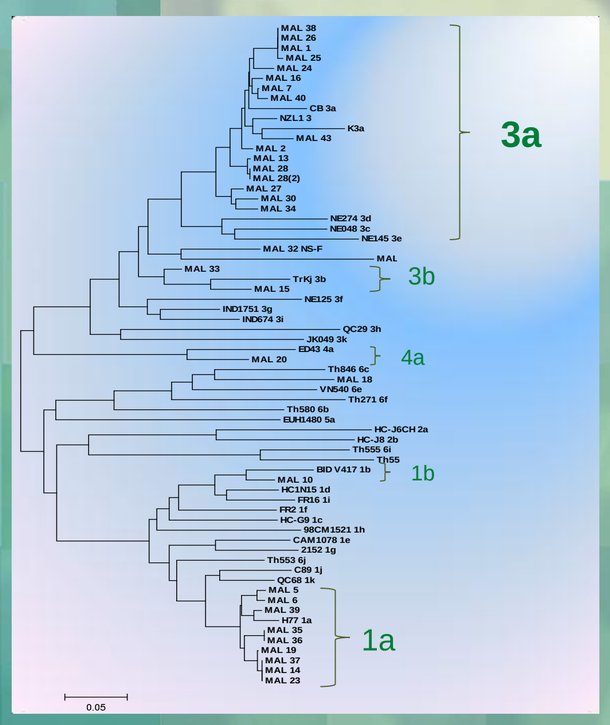


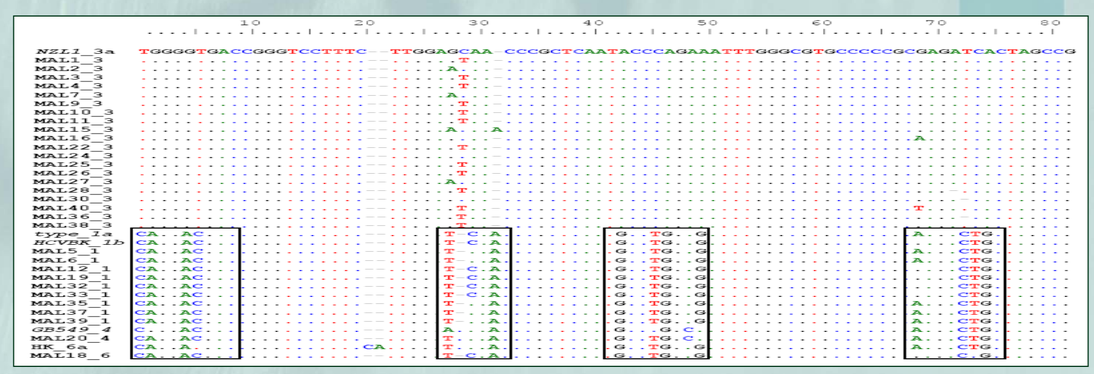
Fig C



The study focused on 5'UTR and NS5B region of HCV genome (Fig A). HCV Genotype 3a was predominant of as compared to other genotypes (Fig B). The construction of the phylogenetic tree of the HCV isolates was based on the nucleotide sequence of their NS5B region using MEGA 4.1 software (Fig C). Genotype 1a comes second in prevalence followed by the other genotypes.

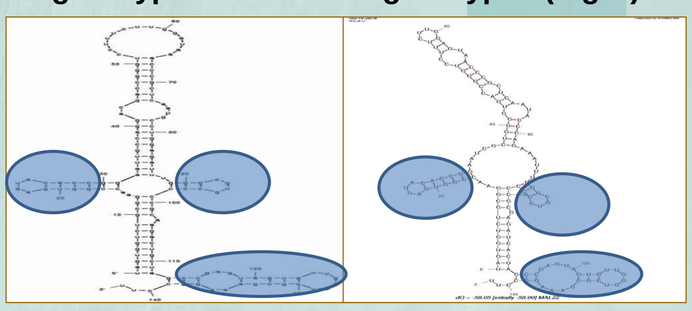
RESULTS

Fig D



The nucleotide sequence of the conserved 5'UTR region of HCV genome revealed several sequence patterns across the 4 main HCV genotypes available in the study panel. The revealed sequence patterns have the potential for designing probes that could differentiate the predominant HCV genotype 3 from other genotypes (Fig D)

Fig E



Analysis of the secondary structure of genotype 3a, as determined by MFOLD software, showed conserved loop (circled blue) structures that could be targeted by small interfering RNA molecules (Fig E).

DISCUSSION

The high prevalence of HCV genotype 3a among the study isolates from infected haemodialysis patients is probably a reflection of its known high frequency in Malaysia, thus it does not constitute a special risk factor for the above patients. Consequently, the HD group can be a model for studying the infection in general. Molecular characterization of 5'UTR revealed a potential target regions that can be utilize for further studies such as subtyping for epidemiology investigation and siRNA designation.

CONCLUSION

In conclusion, molecular studies of local HCV strains provide a new dimension for the improvement of current HCV detection and genotyping methods, aid in better understanding of the molecular epidemiology of the virus infection and may form the basis for future in-vitro studies on viral molecular pathogenetic mechanisms and discovering pathways for inhibiting viral replication.

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