Sir,

We read with great interest the article by Chakravarti et al. The authors evaluated the distribution of different HCV genotypes and its association with HCV viral load in HCV RNA positive patients' plasma with three different methods. HCV type and subtype analysis was carried out by restriction fragment length polymorphism (RFLP) using the method of Chinchai et al., and in specimens with HCV-RNA positive results, HCV subtypes were detected by performing reverse transcriptase-nested polymerase chain reaction (RT-nPCR) using type-specific primers for the core fragment of the HCV genome, using two distinct reaction tubes containing different primer mixes and then HCV genotypes were confirmed by sequencing of core region. The RT-nPCR product and sense primer were used for sequencing.

According to the first reference method (RFLP) for HCV genotyping, the authors could detect HCV genotypes and subtypes 1a, 1b, 1c, 2a, 2b, c, 3a, 3b, 4a, 5a, and 6a, that were not mentioned in this article. The authors have mentioned that with RFLP method they could only identify HCV genotypes 1, 2, and 3. Using the second reference method (using two distinct reaction tubes containing different primer mixes) for HCV genotyping Ohno et al. could detect HCV genotypes and subtypes 1b, 2a, 2b, 3b in mix 1 tube, and 1a, 3a, 4, 5a, 6a in mix 2 tube, that were not detected by the present authors. The authors have not discussed about the results and have no reference to the progeny of the second method. It should be noted that two HCV genotyping methods were used by them, but in their study, there was no comparison between two HCV genotyping methods.

In this study, the analysis revealed the presence of genotypes 1, 2 and 3 using RFLP and type specific PCR followed by direct sequencing. It seems that all samples have been sequenced. Nucleic acid sequencing of an appropriate subgenomic region is considered the “gold standard” for HCV genotyping, so using one of the methods for primary genotyping was enough. It is important to save limited resources we have in developing countries.

The authors could not detect 3i and 3f HCV subtypes with these two methods, therefore, these subtypes were recognized by sequencing method. The authors have not discussed this important item in any part of the article.

There are some problems in explanation of Fig. 2. It would be better to mention that the bands are related to each HCV genotype; otherwise, it is not informative. In their study, there was a relationship between HCV viral load and HCV genotypes that looked interesting and significant. Additionally, the authors have mentioned some valuable points in discussion about the distribution pattern of HCV genotypes, the route and source of HCV infection in India. They have also compared their data with that of United States and Europe. It would be better and valuable if they compare their results with the published data from Asia too.

Farah Bokharai-Salim* & Seyed Moayed Alavian**,+
*Department of Virology
Tehran University of Medical Sciences &
**Baqiyatallah Research Center for Gastroenterology & Liver Disease
Tehran, IR Iran
*For correspondence:
editor@hepatmon.com, alavian@thc.ir

References
1. Chakravarti A, Dogra G, Verma V, Srivastava AP. Distribution pattern of HCV genotypes & its association with viral load. Indian J Med Res 2011; 133 : 326-31.
2. Chinchai T, Labout J, Noppornpanth S, Theamboonlers A, Haagemans BL, Osterhaus AD, et al. Comparative study of different methods to genotype hepatitis C virus type 6 variants. J Virol Methods 2003; 109 : 195-201.
3. Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, et al. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. J Clin Microbiol 1997; 35 : 201-7.

4. Amini S, Farahani Majd Abadi M, Alavian SM, Jouliaie M, Ahmadipour MH. Distribution of hepatitis C virus genotypes in Iran: A population-based study. Hepat Mon 2009; 9 : 95-102.

5. Mellor J, Holmes EC, Jarvis LM, Yap PL, Simmonds P. Investigation of the pattern of hepatitis C virus sequence diversity in different geographical regions: implications for virus classification. The International HCV Collaborative Study Group. J Gen Virol 1995; 76 : 2493-507.

6. Furione M, Simoncini L, Gatti M, Baldanti F, Grazia Revello M, Gerna G. HCV genotyping by three methods: analysis of discordant results based on sequencing. J Clin Virol 1999; 13 : 121-30.

7. Cavalheiro Nde P, Filgueiras TC, Melo CE, Morimitsu SR, de Araujo ES, Tengan FM, et al. Detection of HCV by PCR in serum and PBMC of patients with hepatitis C after treatment. Braz J Infect Dis 2007; 11 : 471-4.

8. Verbeeck J, Maes P, Wollants E, Van der Merwe S, Song E, Nevens F, et al. Use of a commercially available line probe assay for genotyping of hepatitis C virus 5a strains. J Clin Microbiol 2005; 43 : 6117-9.