Authors' response

Sir,

We appreciate the various queries raised on our article by Bokharaei-Salim & Alavarian[1]. The various issues raised by them are clarified below:

Comment: Why the authors had not mentioned the genotypes/subtypes of HCV that could have been detected by the method of Chinchai *et al*[2]?

Response: Yes, we agree to the fact that the method of Chinchai *et al*[2] can detect genotypes/subtypes (1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 4a, 5a, 6a). However, we were only able to find genotypes 1a, 1b 1c, 2a, 3a, 3b in our population. We suggest the correspondents to refer the earlier publications of HCV genotyping from this geographical area[3,4], in which the same genotypes commonly occurring have been mentioned.

Comment: Why have the authors used second reference method (Ohno *et al*)?

Response: The second method was used for a few samples in which we were not very sure about the digestion pattern of RFLP. Hence, to rule out any discrepancies and to confirm our findings, we performed one more genotyping method.

Comment: "It seems that all samples have been sequenced. Nucleic acid sequencing of an appropriate subgenomic region is considered 'gold standard' for HCV genotyping".

Response: We did not perform direct sequencing for all the HCV RNA positive isolates. We have picked up a few strains randomly amongst the already genotyped samples because we wanted to assess the reliability and validity of our results with the gold standard method (*i.e*. direct sequencing). We have mentioned the accession no. of some (5/12) of the strains in the article also.

Comment: About detection of 3i and 3f HCV subtypes.

Response: We have detected only one isolate from each of subtype 3i and 3f by sequencing method, as the other two methods, the RFLP and the type specific PCR did not have the provision to detect the said subtypes. The present article deals with the association between the different HCV genotypes and corresponding viral load. The issue of association with individual subtypes is beyond the purview of this article.

Comment: Problems in explanation of the Fig. 2.

Response: Fig. 2 of our article appeared to have been misinterpreted by the correspondents. It is clearly mentioned in the legend below the Fig. that the entire pattern inclusive of all the five lanes is a part of a single RFLP run for the genotype HCV 1c. The bands are respective digested products of different molecular weights obtained by different restriction enzymes (lanes 2, 3, 4). Lane 1 was the negative control and lane 5 was the molecular weight marker.

Comment: Comparison of the results with the published data in Asia was not done.

Response: Actually, the authors have concentrated in comparing the regional data from within India, in which we have compared between various regional studies from India (see Ref. nos. 23-28 in our article). We have discussed in brief the comparison with the global data as a detailed review was not in the purview of the stated article. However, we appreciate the query and admit that next time intra-continental studies from Asia may also be reviewed in this context.

Comment: "…. using one of the methods for primary genotyping was enough. It is important to save limited resources we have in developing countries".

Response: The authors reserve the rights to compare the different methods along with the gold standards which provide the foundation for validity and reliability of the present study, which was a part of a clinical virology research work approved by the Institutional ethics committee. As at present, to the best of our knowledge there are no guidelines which deal with standard method of HCV genotyping as a routine

diagnostic test in mass scale, the authors believe that saving resources in developing countries is actually a matter of policy determination and can not be a reason to restrict our intellectual rights to validate a scientific research tool.

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References

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