

## Appropriate Genotyping of Hepatitis B Virus in Iran

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### Dear Editor

We read, with great interest, the article by Karimi et al. (1) published in your journal recently, regarding the characterization of hepatitis B virus (HBV) genotypes in a group of patients with chronic hepatitis B infection. They found a prevalence of 73.9% and 26.1% for genotypes D and C, respectively, in Iranian patients. Bearing this context in mind, some points deserve to be considered.

The patients were HBsAg-positive and a conventional polymerase chain reaction (PCR) methodology was used to detect DNA-positive samples. Subsequently, the authors applied genotype-specific real-time PCR to identify the genotypes. Only a fraction of the HBsAg-positive patients (19.82%) tested positive by conventional and real-time PCR. The use of standard PCR as a protocol for detecting DNA-positive samples was the major concern. Basically, all HBsAg-positive individuals should be tested positive by PCR, regardless of the type of PCR applied. The first weakness of the study was the sensitivity of PCR. In the field of clinical virology, nucleic acid amplification technology (NAT) is considered superior to conventional serology; therefore, samples testing positive by serological tests but negative by NAT is indicative of an error in the type of NAT used. Based on the article, it seems that the authors used multiplex real-time PCR to identify HBV genotypes; however, there is no data regarding the optimization of the method. Moreover, there is no information on the sequence of primers and probes used or any references that support the reliability of the kit. In such situations, real-time PCR can provide erroneous results, especially in the identification of minor variants. Therefore, the second weakness of the study was that the real-time PCR results were not confirmed by a specific approach such as sequencing.

Previous and recent investigations carried out by the Iranian Hepatitis Network on the distribution of HBV among patients across different clinics have indicated that the main circulating genotype in Iran is D (2,3). Some

reports on the presence of genotypes apart from D in Iran have been published; however, they suffer from the same weaknesses as those mentioned above with respect to the methodology used. Previous surveys used fragment-based conventional PCR (4) and restriction fragment length polymorphism (RFLP) (5, 6). Although the sensitivity of RFLP is highly appreciated, RFLP and primer-specific methods are not very helpful in identifying minor variants in a quasispecies. Essentially, when a dominant variant exists in a population, the minor species might be ignored owing to their low proportion compared to that of the master sequence. Thus, sequencing is the gold standard method for the identification of genotypes. Owing to its adequate sensitivity, it is highly suitable for such purposes. Moreover, even the sensitivity of sequencing is not optimal for identifying all the possible variants. Therefore, the best option for accurate genotyping might be subjecting the amplicons to cloning plus sequencing.

The Iranian hepatitis network suggests that all national labs and research centers use a standard (not necessarily unique) methodology for HBV genotypic characterization. A sensitive real-time PCR method could be used as an appropriate screening technique. Thus, regardless of the NAT type, we propose subjecting the amplified cloned extracted material to direct sequencing.

### Authors' Contributions

All authors contributed equally to the preparation of the letter.

### References

1. Karimi A, Salimzadeh L, Bagheri N. Hepatitis B virus genotyping among chronic hepatitis B individuals with resistance to Lamivudine in shahrekord, iran. *Jundishapur J Microbiol.* 2014;7(4):e10196.
2. Khedive A, Norouzi M, Ramezani F, Karimzadeh H, Alavian SM, Malekzadeh R, et al. Hepatitis B virus surface protein muta-

- tions clustered mainly in CTL immune epitopes in chronic carriers: results of an Iranian nationwide study. *J Viral Hepat.* 2013;**20**(7):494-501.
3. Norouzi M, Ramezani F, Khedive A, Karimzadeh H, Alavian SM, Malekzadeh R. Hepatitis B virus genotype D is the only genotype circulating in Iranian chronic carriers, the unique pattern of genotypic homogeneity. *J Gastroenterol Hepatol Res.* 2014;**3**(9):1238-43.
  4. Haghshenas M, Mosavi T, Rafiee A, Hosseini V, Hosseinikha Z. Prevalence of Hepatitis B virus genotypes with HBsAg positive patients in the Northern of Iran (Mazandaran) during 2010-2011. *Healthmed.* 2012;**6**(5):1568-73.
  5. Neisi N, Makvandi M, Samarbaf-Zadeh AR. A study on genotypes of hepatitis B virus among hemodialysis patients in Khuzestan province. *Jundishapur J Microbiol.* 2011;**4**(2):65-70.
  6. Dokanehiifard S, Bidmeshkipour A. Study of hepatitis b virus (hbv) genotypes in kermanshah province, west of Iran. *J Biol Sci.* 2009;**1**:113-20.

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