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Commentary on: A study of genotypes, mutants, and nucleotide sequence of hepatitis B virus in Pakistan

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Dear Editor,

We read with interest the recent article by Mumtaz and colleagues published in Hepatitis Monthly (1). The authors attempted to describe an epidemiological pattern of hepatitis B virus (HBV) in Pakistan and compare this pattern to other parts of the world. To determine the genotype of 257 isolated HBV, a line probe assay was used. The HBV genotype D was determined among 247 cases, and 9 patients were infectedwith genotypes B and D. One strain with genotype A was isolated as well. To confirm this genotyping method, the authors constructed a phylogeny tree based on three full-length genome sequences. The phylogeny tree showed that two strains clustered with subgenotype D1 from India, China, Tunisia, and Belgium, and one strain could be grouped into subgenotype D3 from India, Indonesia, Spain, and France. The authors illustrated that genotype D is predominant in Pakistan, and they commented that ″phylogenetic analysis did not show any significant differences between isolated HBV genotype D from Pakistan and those isolated from other parts of the world.″ Additionally, they mentioned that ″HBV genotype D isolates from Pakistan are identical to the sequences from other regions.″Surprisingly, in their discussion, the authors hypothesized that there was the probable common source of infection for the study population. In our view, the study's title and broad conclusion do not coincide, although the study focuses on an important area of research. This motivated us to write this commentary. The development of new techniques to sequence genomes and to design different evolutionary models has led to a massive number of epidemiological studies across several geographical regions. Consequently, several new genotypes and subgenotypes were introduced for HBV (2, 3), and then several researchers reclassified HBV strains based on geographical origin (4, 5). In concordance with the references addressed by Mumtaz *et al.* recent evolutionary studies on HBV have revealed that genotype D could be the ancestor of other

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genotypes because of its worldwide distribution (6, 7). The geographical distribution of different subgenotypes of genotype D has identified three major groups: Asian, African, and European strains. Because of the immigration patterns over the last three decades, the circulating strains of genotype D in Europe originated primarily from Asia and Africa (8). Still, although line probe assay is a great technique for genotyping HBV, it is a genotype-restricted method. In other words, this method cannot identify the subgenotypes and recombination between two different genotypes. Indeed, recombinant strains could be detected in some geographical regions with more than one circulating genotype. In this case, using SimPlot and BootScan analyses of full-length genomes is necessary (9). We strongly stress that because Mumtaz *et al.* studied HBV genotype epidemiology, using a restricted number of full-length genome sequences (3 out of 257) is not sufficient to demonstrate homology between HBV strains circulating in Pakistan and genotype D strains from other geographical regions. For instance, our demographic follow-up demonstrated that the Belgian strain, which was utilized in Mumtaz *et al.'s* phylogeny tree, has been isolated from one immigrant of the Middle East (10). Moreover, the term "identical" is not relevant for cobranched strains in a phylogeny tree. In one of our own previous studies, we showed that demonstrating a common source of infection requires strong evidence consisting of evolutionary model tests of full-length genomes, genetic distance estimation, and powerful statistical models such as Poisson distributions (11). The detection of only two different subgenotypes (D1 and D3) is not enough to support Mumtaz *et al.'s* hypothesis. Finally, we would like to emphesize on fit epidemiological model and more accurate interpretation for restricted data.

Author's reply: A study of genotypes, mutants, and nucleotide sequence of hepatitis B virus in Pakistan

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Dear Editor,

I read with interest the comments of Dr. Pourkarim. It seems that he has concerns about the number of patients on whom the RFLP was performed for the study of whole genomic sequence of hepatitis B virus (HBV). The main reason for studying only four full-length genomic sequence was the limited budget. I agree with him that we cannot draw any solid conclusions based on only four genomic analyses, but it gives us at least the pattern. We wish we could check genomic sequence of HBV on more samples. He also asked to propose the common source of infection in our patients. There are multiple studies from Pakistan regarding the common source of transmissions of hepatitis B and C virus infections (12-14). However, since providing the source of transmission of HBV was beyond the scope of our study, we did not use genetic distance estimation, statistical models, etc.

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