



## Commentary on: A study of genotype, mutants, and nucleotide sequence of HBV in Pakistan

Seyed Mohammad Jazayeri <sup>1\*</sup>

<sup>1</sup> Hepatitis B Molecular Laboratory, Department of Virology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran

### ARTICLE INFO

**Article Type:**  
Letter to Editor

**Article history:**  
Received: 9 Mar 2011  
Revised: 15 Mar 2011  
Accepted: 25 Mar 2011

**Keywords:**  
Genotype  
Hepatitis B virus  
Epidemiology  
Mutants

► **Implication for health policy/practice/research/medical education:**

Knowing the HBV genotypic distribution in a certain population is of importance because it has been shown that they influence the clinical prognosis, response to therapy and, the transmission routes of the disease.

► **Please cite this paper as:**

Jazayeri SM. Commentary on: A study of genotype, mutants and nucleotide sequence of HBV in Pakistan. *Hepat Mon.* 2011;11(4):289-91.

© 2011 Kowsar M.P.Co. All rights reserved.

### Dear Editor,

I read with interest the article "A study of genotype, mutants and nucleotide sequence of HBV in Pakistan" by Mumtaz *et al.* (1) in your journal. The study revealed a prevalence of 96.2% of genotype D in different areas of Pakistan. Hepatitis B virus (HBV) has been classified into 10 genotypes (based on the intergroup divergence of > 8% to < 17%), with a characteristic geographic distribution that largely coincides with human history and migration. Among the different HBV genotypes, genotype D has been found worldwide, with its highest prevalence in the Mediterranean, the Middle East, and Southern Asia (2). Mumtaz *et al.* stated that they wanted to determine the country-wide distribution of HBV genotypes in Pakistan. However, comparing the previous data from this country using larger sample sizes showed conflicting results. We collected all the available data regarding the

prevalence of HBV genotypes in Pakistan population and we found somewhat contradictory results on the type and prevalence of different HBV genotypes circulating in this region (Table 1). The majority of studies carried out on the determination of HBV genotypes utilized a nested-multiplex PCR using genotype-specific primers. The rates of genotype D in these studies ranged between 13% and 70% (Table 1). However, authors who used an INNO-Lipa methodology found prevalence rates between 96.2% and 100% (Table 1). The genotype prevalence was found to be different in the Sind and Punjab provinces in three reports (Table 1). We consider that these discrepancies may be explained in part by the selection of patients as well as the differences in the sensitivity of the assays used to detect signature sequences within the isolates.

Moreover, Mumtaz *et al.* randomly selected 3 samples, followed sequencing in a phylogenetic analysis, and stated that they compared the nucleotide sequences of isolates from Pakistan to isolates from the rest of the world. We think that this type of selection imposes a bias on the phylogeny results. First, despite being a gold standard for methodology, direct sequencing does not detect the viral mixture pool (quasispecies). Second, how do these 3 sequences reflect the dominant sequences from Paki-

\* Corresponding author at: Seyed Mohammad Jazayeri, Hepatitis B Molecular Laboratory, Department of Virology, School of Public Health, Tehran University of Medical Sciences, PO Box: 14155-6446, Tehran, IR Iran. Tel/Fax: +98-2188992660.

E-mail: [jazayerism@tums.ac.ir](mailto:jazayerism@tums.ac.ir)

© 2011 Kowsar M.P.Co. All rights reserved.

**Table 1.** Prevalence of HBV genotypes in Pakistan's population

Author	Samples (No.)	Location (Predomiant Genotype)	Genotype D	Genotype A	Method
Baig S <i>et al.</i> (2008)(5)	201	-	128 (64%)	47 (23%)	Type-specific primer
Baig S <i>et al.</i> (2009)(6)	129	-	98 (76%)	24 (18.6%)	Type-specific primer
Baig S <i>et al.</i> (2009)(7)	315	Sind	219 (70%)	65(20%)	Type-specific primer
Baig S <i>et al.</i> (2007)(8)	295	-	208 (70%)	59 (20%)	Type-specific primer
Alam MM <i>et al.</i> (2007)(9)	110	-	66 (65.3%)	5 (4.9%)	Type-specific primer
Alam MM <i>et al.</i> (2007)(10)	56	NWFP	35 (62.5%)	15 (8.9%)	Type-specific primer
Awan Z <i>et al.</i> (2010)(4)	269	Sind (A)/Punjab (C)/Baloochistan (B)/Kheybar (C)	39 (13%)	43 (14.3%)	Type-specific primer
Noorali S <i>et al.</i> (2008)(12)	180	Sind	150 (83.3%)	0	Type-specific primer Se- quencing
Noorali S <i>et al.</i> (2008)(13)	180	Sind	151 (83.8%)	0	PCR
Abbas Z <i>et al.</i> (2006)(14)	109	Sind	109 (100%)	0	INNO-Lipa
Mumtaz K <i>et al.</i> (2011)(1)	257	Sind/Punjab/Baloochestan/NWFP	247 (96%)	1	INNO-Lipa
Ahmed CS <i>et al.</i> (2009)(15)	236	Sind (D)/Punjab (D)	220 (93.2%)	2 (0.8%)	RFLP-Sequencing

stan with such a large number of diversifications and recombinations (Table 1)? We think that a higher number of sequencing data with a higher number of sequencing data with fewer mutations, should have been employed for more precise comparisons and to eliminate the noise of the software on the processing data. Also, the authors used genotype G for outgrouping; however, they should have used genotype E for this purpose because genotype E is more precise for outgrouping of suspected genotype D isolates (Norder, personal communication, April 25, 2010).

The evolution of HBV genotypes in the world (especially the widespread genotype D in Southern Asia) is confusing. Summarizing the reports from Pakistan, we found an average prevalence of genotypes D (62%), A (14%), C (6%), other genotypes, including B (4%), and recombinants (10%) (3). Furthermore, the E and F genotypes (4) are unusual in the Pakistan population. These unusual genotypes deserve further investigation. This diversity of genotypes and the high rate of recombination (10%) between different genotypes in Pakistan indicate a high rate of intermixing among infected populations, perhaps driven by the T-cell selection in this distribution, reflected by the divergence of amino-acid substitutions in the signature sequences of the HBV genome. We propose that the HBV-infected ancestors of Caucasians acquired genotype D, then migrated in 3 directions: one group moved west towards Europe, another group moved south to Persia,

and the last group migrated to India. People infected with type D of the virus before migrating then transmitted the virus generation by generation after migration. This is why the dominant genotype in southern Asia (including India, Iran, and Pakistan) and most parts of Europe is D (3). Based on molecular epidemiology studies, knowing the HBV genotypic distribution in Pakistan's population is of importance because it has been shown that they influence the clinical prognosis, response to therapy and, the transmission status of the disease.

## References

- Mumtaz K, Hamid S, Ahmed S, Moatter T, Mushtaq S, Khan A, et al. A study of genotypes, mutants and nucleotide sequence of hepatitis B virus in Pakistan. *Hepat Mon.* 2011;**11**(1):14-6.
- Norder H, Courouche AM, Magnius LO. Molecular basis of hepatitis B virus serotype variations within the four major subtypes. *J Gen Virol.* 1992;**73**(Pt 12):3141-5.
- Jazayeri SM, Carman WF. Evolution of Hepatitis B Genotype D in the Middle East and South Asia. *Hepat Mon.* 2009;**9**(1):9-11.
- Awan Z, Idrees M, Amin I, Butt S, Afzal S, Akbar H, et al. Pattern and molecular epidemiology of Hepatitis B virus genotypes circulating in Pakistan. *Infect Genet Evol.* 2010;**10**(8):1242-6.
- Baig S, Siddiqui AA, Chakravarty R, Moatter T, Unnissa T, Nazr ul H. Phylogenetic analysis of hepatitis B virus in Pakistan. *J Coll Physicians Surg Pak.* 2008;**18**(11):688-94.
- Baig S, Siddiqui AA, Ahmed WU, Qureshi H, Arif A. Frequency of hepatitis C and D super infection in patients with hepatitis B related complex liver disorders. *J Coll Physicians Surg Pak.* 2009;**19**(11):699-703.
- Baig S, Siddiqui A, Chakravarty R, Moatter T. Hepatitis B virus subgenotypes D1 and D3 are prevalent in Pakistan. *BMC Res Notes.*

- 2009;**2**:1.
8. Baig S, Siddiqui AA, Ahmed W, Qureshi H, Arif A. The association of complex liver disorders with HBV genotypes prevalent in Pakistan. *Virology*. 2007;**4**:128.
  9. Alam MM, Zaidi SZ, Malik SA, Shaikat S, Naeem A, Sharif S, et al. Molecular epidemiology of Hepatitis B virus genotypes in Pakistan. *BMC Infect Dis*. 2007;**7**:115.
  10. Alam MM, Zaidi SZ, Shaikat S, Sharif S, Angez M, Naeem A, et al. Common genotypes of Hepatitis B virus prevalent in injecting drug abusers (addicts) of North West Frontier Province of Pakistan. *Virology*. 2007;**4**:63.
  11. Idrees M, Khan S, Riazuddin S. Common genotypes of hepatitis B virus. *J Coll Physicians Surg Pak*. 2004;**14**(6):344-7.
  12. Noorali S, Hakim ST, McLean D, Kazmi SU, Bagasra O. Prevalence of Hepatitis B virus genotype D in females in Karachi, Pakistan. *J Infect Dev Ctries*. 2008;**2**(5):373-8.
  13. Noorali S, Hakim ST, McLean D, Kazmi SU, Bagasra O. Prevalence of Hepatitis B virus genotype D in females in Karachi, Pakistan. *J Infect Dev Ctries*. 2008;**2**(5):373-8.
  14. Abbas Z, Muzaffar R, Siddiqui A, Naqvi SA, Rizvi SA. Genetic variability in the precore and core promoter regions of hepatitis B virus strains in Karachi. *BMC Gastroenterol*. 2006;**6**:20.
  15. Ahmed CS, Wang ZH, Bin Z, Chen JJ, Kamal M, Hou JL. Hepatitis B virus genotypes, subgenotypes, precore, and basal core promoter mutations in the two largest provinces of Pakistan. *J Gastroenterol Hepatol*. 2009;**24**(4):569-73.