

28019- SCIENTIFIC RESEARCH PROCESS

Title: *Role of RNA Secondary Structure in Emergence of Compartment Specific Hepatitis B Virus Immune Escape Variants*

Authors: *Sibnarayan Datta, Runu Chakravarty*

Name of Journal: *World Journal of Virology*

ESPS Manuscript No: 28019

1. What did this study explore?

In this study, we explore the compartment specific presence of HBV subgenotype A2 sequences in the PBL and the exclusive presence of the potent G145R mutation, from the perspective of RNA secondary structure analysis.

2. How did the authors perform all experiments?

HBV sequences isolated from serum/plasma and PBL compartments of HBV carriers during our previous study were retrieved from the GenBank and were subjected to RNA secondary structure analysis using the Vienna RNA secondary structure server, followed by annotation using the RNASHAPES program.

3. How did the authors process all experimental data?

All the experimental data generated in this study were visually examined and based on the comparative analysis of the base-pairing patterns of pgRNA of the two different HBV subgenotypes present in the two different compartments; the role of base-pairing in HBV subgenotype specific emergence of G145R mutation was explained.

4. How did the authors deal with the pre-study hypothesis?

Based on the comparative examinations of the sequences obtained from the serum/plasma and the PBL compartments, it was found that most of the nucleotide variations were mostly synonymous in nature. Hence their role in change in antigenic epitopes was ruled out. Furthermore, the invariable co-occurrence of one non-synonymous mutations (at nt 587^A) along with a synonymous mutation (at nt 358^C) led us to hypothesize that the changes in the subgenotype specific variations might lead to changes in the pgRNA base-pairing, resulting in emergence and selection of immune escape mutants advantageous for the virus for persistence in specific anatomical compartments.

5. What are the novel findings of this study?

The results of this study suggest a novel mechanism of the emergence and selection of HBV genotype/subgenotype associated immune escape mutations in a compartment specific manner. The results of the present study have important implications in HBV persistence, immune escape and transmission.