

## **Molecular characteristics of serum hepatitis B virus (HBV) RNA: a novel biomarker for chronic HBV infection**

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**Background and Hypothesis:** HBV is a major etiological agent for viral hepatitis and hepatocellular carcinoma. HBV is a DNA virus per se but viral pregenomic RNA (pgRNA) has been recently found in patient blood. The serum pgRNA is hypothesized to function as a novel biomarker for the activity of HBV covalently-closed-circular DNA (cccDNA), which is the intracellular persistent form of HBV DNA and the template for producing viral proteins responsible for the chronic virulence of HBV. However, the molecular characteristics of serum HBV RNA remains elusive.

**Experimental Design and Project Methods:** HBV RNA were extracted from patient's sera. RT-PCR and 3' RACE were utilized to amplify the internal sequence and 3' terminus of HBV pgRNA, respectively. The PCR products were gel purified and cloned into T vector for sequencing.

**Results:** After comparing the sequencing data to the reference HBV sequence, we found that the serum HBV RNA are spliced fragments of the original intracellular full-length pgRNA. The prevalence of the fragmented serum pgRNA was found in greater concentrations in untreated patients, and the ratios of different spliced forms of serum HBV RNA vary during the course of antiviral treatment.

**Conclusion and Potential Impact:** Our study demonstrated that the serum HBV RNA are spliced/truncated forms of pgRNA, indicating that the RNA-containing virion is noninfectious. The characterization of serum HBV RNA sequence provides important insights into the assay design for serum HBV RNA detection. Future study will focus on the mechanism underlying the selective egress of spliced pgRNA-containing virus particles.