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Hepatitis B virus surface antigen glycan isomer (HBsAgGi) as a new glyco-biomarker to detect infectious HBV virions

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ABSTRACT

In HBV patients' sera, non-infectious particles are much dominant than infectious particles containing HBV DNA (Dane particles). Dane particles contain all HBsAgs (L-, M- and S-HBsAg), but non-infectious particles mainly contain S-HBsAg. Thus, general HBsAg test using antibodies recognizing S-HBsAg does not always distinguish infectious and non-infectious HBV particles. It has been shown that a lectin recognizing *O*-glycans could enrich DNA-containing particles. This study aimed to develop a new marker to measure infectious particles which contain *O*-glycosylated M-HBsAg [HBsAg glycan isomer (HBsAgGi)].

First, we developed a new HBsAgGi antibody recognizing M-HBs but not L-HBs that is not modified with *O*-glycan on the PreS2. Mutation analysis also confirmed that HBsAgGi partly recognized *O*-glycans. HBsAgGi localized in ER to Golgi, suggesting generation of HBsAgGi depends on glycosylation pathway.

Next, we developed ELISA system and measured HBsAgGi in sera of chronic hepatitis B (CHB) patients before and after nucleos(t)ide analog (NA) treatment to investigate clinical utility of the HBsAgGi.

Immunoprecipitation with HBsAgGi antibody confirmed that both HBV DNA- and HBV RNA-containing particles were collected by the antibody. At baseline, serum HBsAgGi level was higher in HBe-positive patients than HBe-negative patients. HBsAgGi levels were significantly correlated with the HBV DNA level (p=0.002, n=32).

Taken together with above results, HBsAgGi would be a new glyco-biomarker to monitor viral kinetics in CHB patients during therapy.

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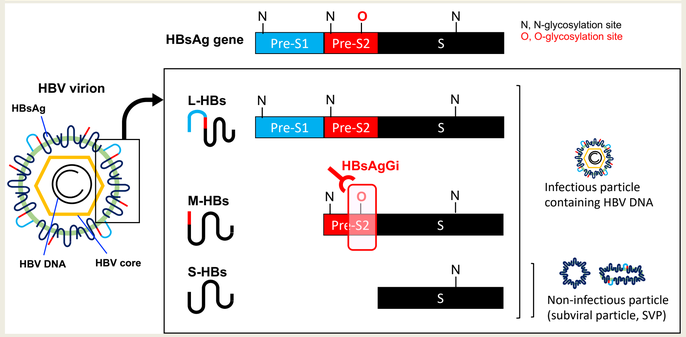
INTRODUCTION

Hepatitis B virus surface antigen (HBsAg) gene generates L-, M-, and S-HBsAg. In HBV patients' sera, non-infectious particles are much dominant than infectious particles containing HBV DNA (Dane particles). Dane particles contain all HBsAgs, but non-infectious particles mainly contain S-HBsAg. Thus, general HBsAg test using antibodies recognizing S-HBsAg does not always distinguish infectious and non-infectious subviral particles. *O*-glycosylated M-HBsAg was determined by mass spectrometry, because a lectin recognizing *O*-glycans could enrich DNA-containing particle. This study aimed to develop a new marker to measure infectious particles which contain *O*-glycosylated M-HBsAg [HBsAg glycan isomer (HBsAgGi)].

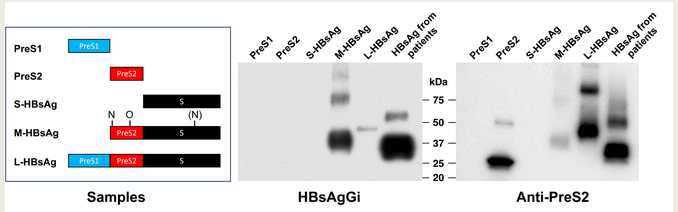
Glycosylation of HBsAg: *O*-glycosylated PreS2 (HBsAgGi) presents on M-HBsAg in HBV virion.



METHODS AND MATERIALS

To generate HBsAgGi antibodies, PreS2 glycopeptides modified with *O*-glycans were generated. To analyze the HBsAgGi antibody, Western blotting, immunostaining, and ELISA were performed. To characterize target particles of HBsAgGi, immunoprecipitated (IP) particles by HBsAgGi antibody was analyzed by qPCR and reverse-transcription PCR for quantifying HBV DNA and HBV RNA, respectively. Furthermore, a new HBsAgGi ELISA system measured sera of chronic hepatitis B (CHB) patients before and after nucleos(t)ide analog (NA) treatment to investigate clinical utility of the HBsAgGi.

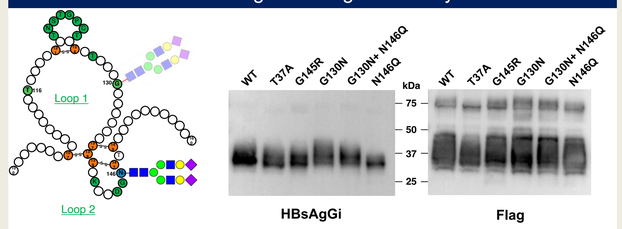
HBsAgGi antibody recognizes M-HBs but not L-HBs, which is not modified with *O*-glycan on the PreS2.



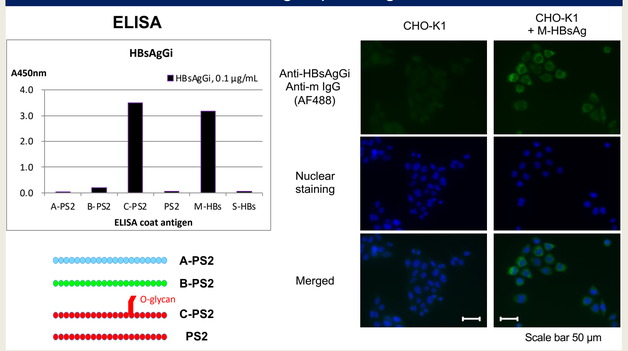
RESULTS

PreS2 peptides with or without glycans generated genotype specific antibodies. For genotype C, HBsAgGi antibody recognized M-HBs but not L-HBs that is not modified with *O*-glycan on the PreS2. Mutations in *O*-glycosylation site or removal of *O*-glycans resulted in decrease of the recognition by HBsAgGi antibody. HBsAgGi localized in ER to Golgi in MHBs-expressing cells, suggesting generation of HBsAgGi through glycosylation pathway. Immunoprecipitation experiments confirmed that both HBV DNA- and HBV RNA-containing particles were collected by HBsAgGi antibody. In treatment naïve CHB patients, serum HBsAgGi level was higher in HBe-positive patients (EPH) than HBe-negative patients (ENH) at baseline. HBsAgGi levels were significantly correlated with the HBV DNA level (p=0.002, n=32).

Mutations in antigenic loop in S-HBs did not affect the binding of HBsAgGi antibody.



HBsAgGi specific to genotype C localized in ER to Golgi in M-HBsAg-expressing cells



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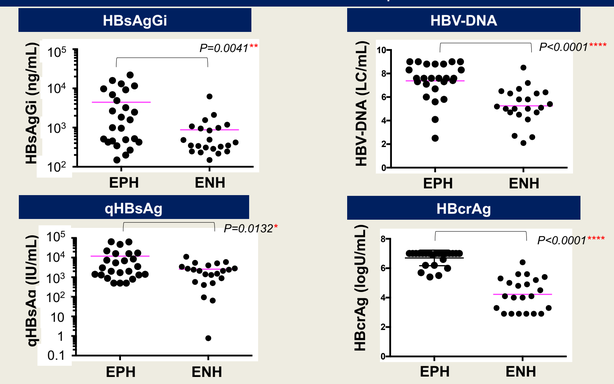
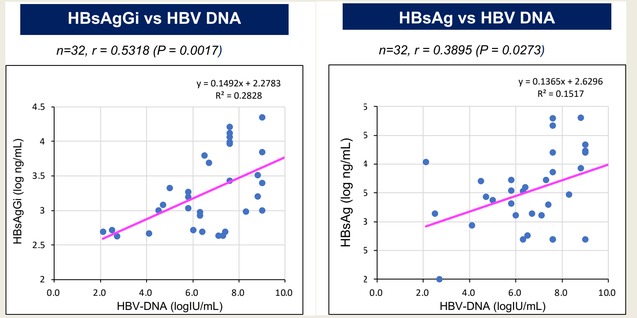
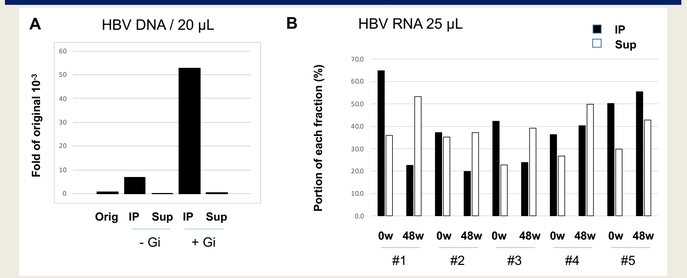
Distribution of HBsAgGi, HBV-DNA, qHBsAg and HBcrAg at baseline in NA naïve EPH and ENH patients



The correlation of HBsAgGi with HBV DNA at baseline in NA naïve CHB patients



HBsAgGi antibody can immunoprecipitated both HBV DNA- and HBV RNA-containing particles.



CONCLUSIONS

New HBsAgGi antibodies recognize HBV particles in genotype specific manner dependent on the presence of *O*-glycans in PreS2 domain. HBsAgGi specifically presents in minor infectious fraction of HBV virions containing HBV DNA or HBV RNA. Taken together with ELISA analysis of patients' sera, HBsAgGi would be a new glyco-biomarker to monitor viral kinetics in CHB patients during therapy.

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