Naturally occurring genotypic drug-resistant mutations of HBV in Huzhou, China: a single-center study

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China is an area with highly endemic hepatitis B virus (HBV) infection, with an estimated 93 million HBV carriers, resulting in approximately 330,000 deaths annually.¹ The predominant HBV genotypes in China are genotype B and C. Currently, nucleos(t)ide analogs are used for anti-HBV treatment. However, prolonged antiviral therapy may lead to drug resistance, which is associated with mutations in the reverse transcriptase region of the HBV genome. Several studies have shown that drug-resistant mutations existed in treatment-naïve patients with chronic hepatitis B (CHB). However, the prevalence rates of natural drug-resistant mutations varied in different reports.²,³ Furthermore, the prevalence and clinical profile of natural drug-resistant mutations in CHB patients are not quite clear. Thus, the purpose of this study was to investigate the prevalence and clinical feature of natural drug-resistant mutations among treatment-naïve CHB patients in a tertiary hospital in Huzhou, eastern China.

In this study, we recruited 218 CHB patients who had not received anti-HBV treatment in Huzhou Central Hospital. The diagnosis of CHB was done according to the Chinese consensus criteria. This study was approved by the ethics committee of Huzhou Central Hospital in accordance with the ethical guidelines of the Declaration of Helsinki. All patients provided written informed consent. Routine serological examination was performed by the technicians in Department of Laboratory Medicine. Serum HBV DNA levels were quantified using real-time polymerase chain reaction. The reverse transcriptase region amplification and sequencing was performed as described previously.⁴

Among 213 successfully sequenced sample from patients, natural drug-resistant mutations were detected in 6.1% (13/213) patients, and these included rtM204I/V (n=8), rtL180M (n=4), rtA181T/V (n=4), rtL80I/V (n=4), rtV173L (n=2), and rtN236T (n=2). The clinical information of these patients is shown in Table 1. The prevalence rates of natural drug-resistant mutations in CHB patients were found to be varied in different areas of China (from 2.01% to 8.9%).²,³ A meta-analysis revealed that the pooled incidence of natural resistance mutations in China is higher than those in other countries (8.00% vs 1.88%).⁵ The incidence rate of natural resistance mutations in our study was not in agreement with the results of other studies. The discordance between previous studies and our results might be due to discrepancy of sample size and differences in study method, genotypic distribution, and study population. The actual prevalence of natural drug-resistant mutations in treatment-naïve patients may vary.
be higher than our results show considering the low sensitivity of direct sequencing (mutation frequency >20%). Nonetheless, the current study provides a rationale for the further large-scale investigation on prevalence of natural resistance mutations by the next-generation sequencing technologies.

At present, we compared the clinical and virological characteristics between the patients with and without natural drug-resistant mutations (Table 2). No significant correlation was found between natural drug-resistant mutations and gender, age, HBsAg status, HBV DNA levels, proportion of genotype C, and liver function biochemical markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyl transpeptidase (GGT) (all P>0.05). To date, the clinical significance of natural drug-resistant mutations is unclear. Another study reported that natural resistance mutations may be correlated with HBV DNA levels and genotype.7 Hence, large-scale investigations on natural drug-resistant mutations are needed to further clarify the clinical significance of natural resistance mutations in CHB patients.

In summary, the present study shows that the primary drug-resistance mutations (rtM204V/I, rtA181T/V, and rtN236T) and secondary drug-resistance mutations (rtL80V/I, rtV173L, and rtL180M) existed in treatment-naive CHB patients in Huzhou, eastern China. Considering that pre-existing drug-resistant mutations may affect the efficiency of antiviral therapy, it is necessary to monitor the nucleos(t)ide analog resistance mutations before antiviral therapy.

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Disclosure
The authors report no conflicts of interest in this work.

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