

Brief Original Article

Serologic and molecular characteristics of hepatitis B virus infection in vaccinated schizophrenia patients in China

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Abstract

Introduction: Previous studies have indicated that the patients with psychiatric illness were at higher risk of hepatitis B virus (HBV) infection. However, the efficacy of hepatitis B vaccine in schizophrenia patients remains unclear.

Methodology: Between June 2014 and January 2015, 415 schizophrenia patients and 3,038 controls who had been routinely immunized as infants were recruited in the present study. Hepatitis B surface antigen (HBsAg), HBsAb, and HBV DNA were detected with commercial methods according to the manufacturer's protocol. A 600-bp region of the S gene (region nt236–nt835) was amplified by nested polymerase chain reaction (PCR). The genotypes of isolated HBV were identified using phylogenetic analysis by the neighbor-joining algorithm in the software MEGA version 4.1.

Results: The seroprevalence of HBsAg in schizophrenia patients was 6.75%, which was significantly higher than 3.32% measured in controls. HBsAg prevalence was 7.94% in male schizophrenia patients and 5.47% in female schizophrenia patients, while it was only 4.04% in males and 2.08% in females in the control group. The HBsAb seroprevalence rate was 58.31% in schizophrenia patients and 59.94% in non-schizophrenia controls. Moreover, one HBV strain in the schizophrenia group presented I126S vaccine escape mutation (5.88%), while three HBV isolates showed Q129H, M133L, and G145R vaccine escape mutations in the control group (6.81%).

Conclusions: Schizophrenia patients are at higher risk for HBV infection, even those who had received routine immunization. Therefore, a booster HB vaccination targeted at schizophrenia patients should be considered in the future.

Key words: hepatitis B virus; hepatitis B vaccine; schizophrenia; seroprevalence.

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Introduction

Hepatitis B, caused by hepatitis B virus (HBV), is one of the most prevalent viral infections in humans. HBV infection can be complicated by long-term carriage, chronic hepatic insufficiency, cirrhosis, and hepatocellular carcinoma (HCC) [1]. Worldwide, about 350 million people are chronically infected with HBV, and the number of HBV-infected people continues to increase, with 4.5 million new infections every year [2,3]. In regions highly endemic for HBV, perinatal, child-to-child and parenteral transmission, blood transfusion, surgical interventions, dental extraction, and wet cupping are considered to be the main routes of HBV transmission [4].

In order to control HBV infection in China, the national expanded vaccination program was launched

in 1992. It has had a dramatic impact on the reduction of HBV infection and is estimated to have prevented over four million deaths [5,6], especially in children and adolescents. However, epidemiological studies revealed that some specific groups might be at higher risk for HBV infection, including patients undergoing dialysis, parenteral drug users, immunosuppressed patients, male homosexuals, and those suffering serious mental illness [7-9].

Because of psychiatric impairment, inadequate hygienic habits, patterns of aggressiveness, and immunological deficiencies, studies from various countries have detected an increased prevalence of HBV, hepatitis C virus (HCV) and hepatitis E virus (HEV) infection in patients with psychiatric illness compared to those without [9-11]. Recently, we

reported that the seroprevalence of HBsAg was 11.0% in Chinese adult schizophrenia patients, compared with 7.18% in the general population [12]. Furthermore, although hepatitis B vaccination results in protection of a high proportion (95%) of infants, children, and young adults, the efficacy of hepatitis B vaccine in schizophrenia patients remains unclear. In the present study, we further investigated the serologic and molecular characteristics of HBV infection in hepatitis B-vaccinated schizophrenia patients.

Methodology

Study subjects

Between June 2014 and January 2015, 415 patients who met the criteria of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, text revision (DSM-IV-TR) for schizophrenia were recruited from the Huai’an Third Hospital, the largest mental hospital in Jiangsu, China. Meanwhile, 3,038 persons without mental disease were identified from the annual health examination population living in the same region. All participants were asked to provide a copy of their newborn vaccination records. The details of the study were fully explained to all the subjects, and written informed consent was obtained. This study was approved by the ethical committee of Huai’an Third Hospital in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Laboratory testing

The serum samples obtained from all patients were tested for HBV surface antigen (HBsAg) and HBV surface antibody (anti-HBs) by enzyme-linked immunosorbent assay (ELISA) with a commercial ELISA kit (3V Biotechnology, Weifang, China). When an ambiguous result was found, the sample was retested and confirmed as positive only if one of the repeats (2/3 of the total tests) was positive.

HBV DNA isolation and sequence analysis

HBV DNA was extracted by Viral RNA/DNA Extraction Kit Version 5.0 (TaKaRa Biotechnology, Dalian, China) from 200 µL of plasma. A 600-bp region of the S gene (region nt236–nt835, reference strain HBV-Chi89, AB073826) was amplified by nested PCR. The first round of PCR was performed using primers DZ-1: 5’-CTAGGACCCCTGCTCGTGTTAC and B8: 5’-ACATACTTTCCAATCAATAGG, and the nested PCR used primers DZ-2: 5’-GACAAGAATCCTCACAATAC and B7: 5’-CCAATTACATATCCCATGAA. The genotypes of isolated HBV were identified using phylogenetic analysis by the neighbor-joining algorithm in MEGA version 4.1 software. The reliability of the phylogenetic tree was tested by bootstrap analysis with 1,000 replicates. HBV reference wild-type sequences of genotype B (AB073826), genotype C (AF286594), and genotype D (AB090270) were deduced from the full-length genomes of different genotypes HBV in GenBank and used to identify nucleotide and amino acid variability [13].

Statistical analysis

Statistical analysis was performed using SAS version 9.2 software. All statistical tests were two-sided, conducted at a significance level of 0.05.

Results

Seroprevalence of HBsAg in study population

In total, 415 schizophrenia patients (214 males and 201 females), between 16 and 20 years of age (mean age: 18.47 ± 1.62 years), and 3,038 general controls (1,932 males and 1,106 females), between 16 and 20 years of age (mean age: 18.58 ± 1.71 years), with complete HB vaccination records were recruited in the present study. As Table 1 shows, the overall prevalence of HBsAg was 6.75% in schizophrenia patients, which was significantly higher than that in controls after adjustment for age and gender (3.32%, *p* < 0.001).

Table 1. Comparison of HBsAg and HBsAb positive rates between schizophrenia patients and non-schizophrenia controls.

Variable	HBsAg		Adjusted*		HBsAb		Adjusted*	
	Positive n (%)	Negative n (%)	OR (95% CI)	P	Positive n (%)	Negative n (%)	OR (95% CI)	P
Schizophrenia	28 (6.75%)	387 (93.25%)	1 (Ref)	0.000	242 (58.31%)	173 (41.67%)	1 (Ref)	0.270
Non-schizophrenia	101 (3.32%)	2,937 (96.68%)	2.24 (1.45,3.47)		1,821 (59.94%)	1,217 (40.06%)	0.89 (0.72,1.10)	
Gender								
Male								
Schizophrenia	17 (7.94 %)	197 (92.06%)	1 (Ref)	0.009	122 (57.01 %)	92 (42.99%)	1 (Ref)	0.270
Non-schizophrenia	78 (4.04 %)	1,854 (95.96%)	2.06 (1.20,3.56)		1,168 (60.46 %)	764 (39.54%)	0.85 (0.64,1.13)	
Female								
Schizophrenia	11 (5.47 %)	190 (94.53%)	1 (Ref)	0.018	120 (59.70 %)	81 (40.30%)	1 (Ref)	0.650
Non-schizophrenia	23 (2.08%)	1,084 (97.92%)	2.47 (1.17,5.21)		653 (59.04%)	453 (40.96%)	0.93 (0.68,1.27)	

* OR was adjusted for age and gender. When compared in different gender groups, the OR was adjusted for age.

Moreover, HBsAg prevalence was 7.94% in male schizophrenia patients and 5.47% in female schizophrenia patients, while it was only 4.04% in males (78/1,932) and 2.08% in females (23/1,106) in the control group ($p = 0.009$ for males and $p = 0.018$ for females).

Seroprevalence of HBsAb in study population

The seroprevalence rate HBsAb was 58.31% in the schizophrenia group, including 122 males (57.01%) and 120 females (59.70%). In the non-schizophrenia control group, 1,168 (60.46%) males and 653 (59.04%) females were positive for HBsAb. No significant difference in HBsAb positivity was observed between the schizophrenia patients and controls (Table 1). In addition, 12 and 41 persons showed co-existing HBsAg and HBsAb in the schizophrenia and control groups, respectively ($p = 0.830$).

Phylogenetic analysis

A total of 129 HBsAg-positive samples were then analyzed to determine the existence of HBV DNA by nested PCR. A 600-bp fragment of the HBV S gene was obtained from 61 persons, 17 schizophrenia patients and 44 controls. Of the 17 HBV strains isolated from schizophrenia patients, 16 sequences belonged to genotype C and the other strain belonged to genotype B. Among 43 HBV sequences amplified from controls, phylogenetic analysis indicated that 35 strains belonged

to genotype C, 8 sequences belonged to genotype B, and 1 strain belonged to genotype D. No other genotypes were detected (Figure 1). Compared with the reference HBV sequence of genotype B (AB073826) and C (AF286594), the results showed that nucleotide mutations happened more frequently in the control group than in schizophrenia patients (1.00% versus 0.63%, $p < 0.001$). However, there was no significant difference of genotype of HBV and HBC distribution between the two groups ($p = 0.728$).

Vaccine escape associated amino acid substitutions

The presence of vaccine escape associated amino acid substitutions in the major hydrophilic region (MHR) of the HBV S gene based on previous studies was investigated [14-16]. In total, 24 and 103 amino acid substitutions in amplified S gene fragments were observed in the viruses isolated from the schizophrenia and control groups, respectively. Results showed that one genotype C HBV strain in the schizophrenia group presented I126S vaccine escape mutation, and three genotype B HBV isolates showed Q129H, M133L, and G145R vaccine escape mutations in the control group. The amino acid mutation analysis is shown in Figure 2.

Discussion

Herein, we reported that the prevalence of HBsAg was 6.75% in vaccinated schizophrenia patients, which was significantly higher than that detected in controls (3.32%) and vaccinated children (2.08%) in China. It could have implications because schizophrenia patients

Figure 1. Phylogenetic analysis based on nucleotide sequencing of the 600 bp (nt237–nt836) of S region. One strain of genotype B HBV and 16 genotype C HBV strains from schizophrenia patients are presented as ●. Eight strains of genotype B HBV and 43 strains of genotype C HBV from non-schizophrenia controls presented as ○. The classification of genotype of virus according to 89 reference sequences genotype A1–A3 (4), genotype B1–B8 (43), genotype C1–C7 (30), genotype D1–D2 (4), genotype E (2), genotype F (2), genotype G (2), genotype H (2), and genotype I (2).

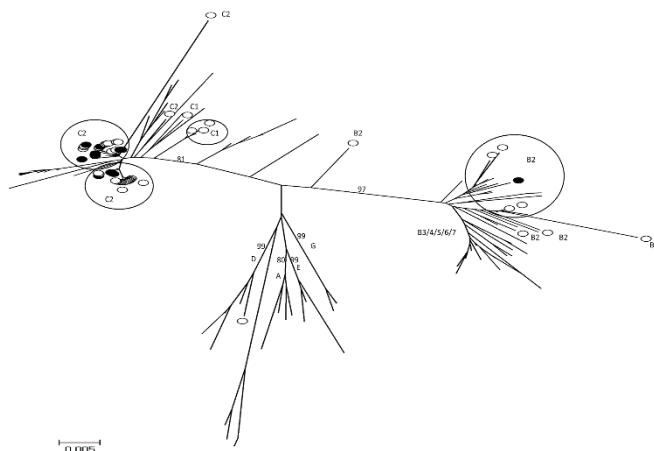


Figure 2. (A) Amino acid substitutions within the major hydrophilic region (99-169 aa) in genotype B HBV infected subjects. The consensus amino acid sequence of genotype B HBV was deduced from AB073826. One strain was isolated from schizophrenia patients and eight sequences were amplified from non-schizophrenia controls were presented. (B) Amino acid substitutions within MHR (99–169 aa) in genotype C infected subjects. The consensus amino acid sequence of genotype C HBV was deduced from AF286594. A total of 16 strains from schizophrenia patients and 35 sequences amplified from non-schizophrenia controls were presented.

Genotype B	DYQGMLVPCPLIQGSSSTSTGPKCTCTTPAQGTSMFPSCCCTKPTDGNCTCIPFPSWAFARFLWEWASVR
Schizophrenia	
WTC (None)	
KR920625P.....M.....
Control	
WTC (8 strains)	
KR920626M.....
KR920627A.....
KR920628F.....
KR920630H.....
KR920651L.....R.....K.....A.....
KR920632L.....R.....K.....A.....
(A)	
Genotype C	DYQGMLVPCPLLPGTSTSTGPKCTCTTIPAQGTSMFPSCCCTKPSDGNCTCIPFPSWAFARFLWEWASVR
Schizophrenia	
WTC (15 strains)	
KR920644S.....
Control	
WTC (29 strains)	
KR920657V.....
KR920677T.....
KR920678T.....
KR920679V.....
KR920680T.....
KR920681T.....
(B)	

are at increased risk of HBV infection, even with the implementation of routine vaccination. Previously, many factors such as cognitive impairment, financial instability, and poverty were reported to be associated with higher detection rates of HBV infection in schizophrenia patients [9-11]. As noted in earlier studies by Rosenberg *et al.* [10,11], the risk factors for HBV infection in schizophrenia patients included geographic location, gender, and other behavioral risk factors. In the present study, we further observed that nucleotide mutations happened more frequently in the HBV S gene in control groups than in schizophrenia patients. It is well known that schizophrenia patients usually have disorders of immune function such as abnormal CD4⁺/CD8⁺ T cells ratio and an imbalance of Th1/Th2 [17,18]. Therefore, our results indicate that the significant difference of immune pressure might exist between the two groups, which could partially explain the different nucleotide substitution rates of the S gene in schizophrenia patients.

The MHR, situated in a central region of the S gene, has been recognized as an important genetic element associated with virus infection issues, such as vaccine escape, failure of anti-viral treatment, and progress of chronic infection [19-21]. Chang *et al.* reported that the rate of HBV S gene mutants in HBV DNA positive children increased gradually from 7.8% before the vaccination program to 19.6%, 28.1%, and 23.1% at 5, 10, and 15 years, respectively, after the start of the vaccination program in Taiwan [22]. In this study, we found that one schizophrenia patient carried vaccine escape I126S mutation and three controls carried Q129H, M133L, and G145R mutations, suggesting that the circulation of HBV strains carrying vaccine-selected mutants can cause some level of concern in China. They have the potential of spreading and affecting the effectiveness of the national vaccination program.

Conclusions

Overall, we concluded that schizophrenia patients could be at higher risk for HBV infection, even those patients who have been routinely immunized. Considering that China is a hyperendemic region for HBV infection, our findings highlight the fact that booster hepatitis B vaccination targeted at schizophrenia patients should be considered in the future.

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