Genotype and variations in core promoter and pre-core regions are related to progression of disease in HBV-infected patients from Northern Vietnam

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Abstract. Vietnam is one of the countries with a high rate of hepatitis B virus (HBV) infection, but there are only a few reports about relation of HBV genotypes and mutations to clinical course in Northern Vietnam. The characteristics of HBV and its relationship to clinical outcome in patients from Northern Vietnam were analyzed. Serum samples were collected from 183 HBV-infected Vietnamese patients. They were clinically categorized into 4 groups: hepatocellular carcinoma (HCC), liver cirrhosis (LC), chronic hepatitis (CH), and asymptomatic carriers (ASC). HBV serology, α-fetoprotein, HBV genotypes, HBV-DNA level and mutations in the core promoter and pre-core regions of HBV-DNA were examined. The majority of sera contained HBV genotype B (67.8%) and C (27.9%). The median age was matched between genotype B and C (38.2 vs. 42.9 years). The rates of HBeAg seroconversion and G1896A for genotype B were significantly higher than those for genotype C ($P<0.05$). Genotype C had a higher HBV-DNA level than genotype B. C1858 was frequent, especially in genotype C (62.7%). The most prevalent genotype in ASC and CH was genotype B. The presence of the mutation A1762T/G1764A correlated with disease progression. The triple mutation T1753C/A1762T/ G1764A was quite common and was more prevalent in LC and HCC than in CH and ASC. In Northern Vietnamese, HBV genotypes B and C were prevalent. Genotype C and mutations in the core promoter region were associated with progressive, severe liver diseases.

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Introduction

Hepatitis B virus (HBV) infection is one of the most common infectious diseases in the world, and more than 350-million people are chronic HBV carriers (1). HBV infection is associated with socioeconomic conditions. Southeast Asia, China and Africa are the regions with high endemic HBV infection (1,2). Vietnam is a developing country and is located in Southeast Asia, and the frequency of HBV surface antigen (HBsAg)-positivity in Vietnam may be as high as 16% in rural communities (3), where more than 70% of the population dwells. HBV is the major leading cause of chronic hepatitis (CH), liver cirrhosis (LC) and hepatocellular carcinoma (HCC) in Vietnam.

HBV is classified into eight genotypes, from A to H (4-7). In East Asia, HBV genotypes B and C prevail, and recently these genotypes have been divided into sub-genotypes (8-10). Genotype B is classified into Bj and Ba (8,9); while genotype C is divided into Cs and Ce (10), and these sub-genotypes have different geographical distribution. The HBV genotype has an effect on long-term clinical outcome (11-13). Genotype C is associated with more progressive liver disease (13) and higher risk of HCC (14). Genotype B has earlier hepatitis B e antigen (HBeAg) seroconversion compared with genotype C (12,15). Pre-core mutation (G1896A) and dual core promoter mutations (A1762T/G1764A) can affect the clinical outcome and response to therapy. These mutations are closely associated with negative HBeAg (16-18). G1896A is seen at a higher rate in genotype B, while A1762T/G1764A is predominantly observed in genotype C (13,15,19,20) and is associated with an increased risk of HCC (21-24).

There have not been many studies of HBV genotype in Vietnamese but the data from reported studies showed that genotypes B and C prevailed (19,25-28). Most patients in those studies were from Southern Vietnam (25-27). A study of

Table I. Characteristics of Vietnamese patients at baseline.

Characteristic	Total (n=183)	HCC (n=48)	LC (n=44)	CH (n=29)	ASC (n=62)
Sex (M/F)	154/29	46/2	40/4	22/7	46/16
Age (years)	39.5±16.5 (17-75)	49.5±11.3[a,b] (26-74)	50.9±12.2[a,b] (18-75)	42.4±13.8[a,b] (17-70)	22.3±6.2[b] (19-51)
HBeAg (+)	55 (30.1%)	10 (20.8%)	14 (31.8%)	1 (3.4%)	30 (48.4%)
Anti-HBe (+)	97 (53.0%)	22[a] (45.8%)	28[a] (63.6%)	20[a] (68.9%)	23[a] (37.1%)
Genotype					
B[d]	124/183[c] (67.8%)	27/48[a] (56.2%)	26/44[a] (59.1%)	24/29[a] (82.8%)	47/62[a] (75.8%)
C	51/183[c] (27.9%)	19/48[a] (39.6%)	17/44[a] (38.6%)	4/29[a] (13.8%)	11/62[a] (17.7%)
Mixed B+C	3/183 (1.6%)	1/48 (2.1%)	1/44 (2.3%)	0/29 (0%)	1/62 (1.6%)
Unclassified	5/183 (2.7%)	1/48 (2.1%)	0/44 (0%)	1/29 (3.4%)	3/62 (4.8%)

HCC, hepatocellular carcinoma; LC, liver cirrhosis; CH, chronic hepatitis; ASC, asymptomatic carrier; M, male; F, female; +, positive; -, negative. [a]P<0.05, [b]P<0.0001, [c]P<0.0001, [d]123/124 (99.2%) were genotype Ba, 1/124 (0.8%) was genotype Bj.

patients in Hanoi in Northern Vietnam showed that genotype B was more prevalent than genotype C; however, the number of patients was quite small (28). Among the studies of the HBV genotype in Vietnamese, that of Lindh *et al* suggested that genotype A was present (17), but Hannoun *et al* later analyzed those HBV strains in more detail and concluded that it was not certain that genotype A was present (29). To the best of our knowledge, the HBV genotype A in Vietnamese was previously reported in only four patients: three of them were originally from Southern Vietnam (30) and the other was a Vietnamese immigrant in the United States (31); and most recently one study reported that HBV genotypes from A to G were detected in Vietnamese but the prevalence of genotype A was only approximately 18% (32). In contrast to the findings of the above studies in Vietnamese, a study by Song *et al* reported that genotype A was very commonly observed in HBV-infected Vietnamese patients (98.7%), that the pre-core mutation (G1896A) was not found, and dual core promoter mutations (A1762T/G1764A) were very rare in HCC patients (8%) and were not found in LC patients, CH patients or asymptomatic carriers (ASC) (33). The results reported by Song *et al* were very surprising, since the results of many other studies in East Asia and some in Vietnam, with only the exception of studies in the Philippines, showed that genotypes B and C were the major HBV genotypes in this region and mutations in the core promoter and pre-core regions were frequently found (8-10,12-15,17-31,34-38).

In order to clarify the frequencies of the various HBV genotypes in Vietnam in general, and particularly in the Northern area, and the frequency of mutations in the core promoter and pre-core regions of HBV-DNA, we carried out a study examining patients from Northern Vietnam. We also investigated the relationship between HBV genotype and mutations in the core promoter and pre-core regions in HBV-infected patients at different stages of the clinical course.

Materials and methods

Patients. HBV-infected Vietnamese patients (n=183) from 16 different provinces in Northern Vietnam (154 males and 29 females, median age 39.5±16.5 years) were enrolled in this study. Patients' sera were collected in Bach Mai Hospital, Hanoi, Vietnam from March 2004 to May 2005. Based on clinical symptoms, blood examinations and imaging features, patients were clinically categorized into 4 groups: hepatocellular carcinoma (HCC; n=48), liver cirrhosis (LC; n=44), chronic hepatitis (CH; n=29) and asymptomatic carriers (ASC; n=62). Patients with a positive test for antibody to hepatitis C virus or human immunodeficiency virus were excluded. None of the patients had received previous interferon therapy, nucleoside analogue treatment or immuno-suppression treatment.

Assay for HBeAg, anti-HBe test, AFP, HBV genotyping and quantitation of HBV-DNA level. Patients' sera were stored at -40˚C until use. HBeAg and anti-HBe antibody were assayed using an enzyme linked immunosorbent assay (ELISA) kit (Immunis EIA, Tokushumeneki Kenkyusho, Tokyo, Japan), according to the manufacturer's instructions. α-fetoprotein (AFP) level was determined by an ELISA kit following the recommendation of the company (Microwell ELISA AFP test, Hope laboratories, CA, USA). HBV-DNA was extracted by using a QIAamp DNA blood mini kit (Qiagen GmbH, Germany). HBV genotypes were determined by using the restriction fragment length polymorphism (RFLP) method for the S gene sequence amplified by PCR with nested primers, as previously described (39). The ELISA method with monoclonal antibodies was carried out in cases in which the genotype was not determined by RFLP (40). In addition, genotype B was sub-classified into Ba or Bj according to the method of Sugauchi *et al* (9). The quantitation of HBV-DNA level was determined by real-time PCR with a set of primers and Taq Man probe located in the S gene as previously reported (41).

Amplification and sequencing of the core promoter and the pre-core regions of HBV-DNA. To assess mutations in the core promoter and pre-core regions, direct sequencing of amplified DNA by nested PCR was carried out as previously described (42). PCR products were directly sequenced by the dideoxy

Table II. Genotypes B and C in relation to variations in core promoter and pre-core regions, HBeAg seroconversion, AFP level and HBV-DNA level.

Characteristic	Genotype B (n=124)	Genotype C (n=51)	P-value
Age (years)	38.2±16.4 (17-74)	42.9±16.1 (18-75)	NS
C1858	2 (1.6%)	32 (62.7%)	<0.0001
A1762T/G1764A	56 (45.2%)	34[a] (66.7%)	<0.01
G1896A	39 (31.5%)	7[a] (13.7%)	<0.05
A1762T/G1764A/G1896A	17 (13.7%)	3 (5.9%)	NS
T1753C/A1762T/G1764A	11 (8.8%)	13 (25.5%)	<0.05
A1762T/G1764A/C1766T	3 (2.4%)	1 (1.9%)	NS
T1753C/A1762T/G1764A/C1766T	1 (0.08%)	1 (1.9%)	NS
HBeAg seroconversion	70 (56.5%)	20 (39.2%)	<0.05
AFP (ng/ml)	145.3±280.6 (0-901)	151.0±270.2 (0-853)	NS
HBV-DNA >5.0 (log copies/ml)	70 (56.5%)	42 (82.4%)	<0.01

[a]P<0.0001. AFP, α-fetoprotein; NS, non-significant difference.

chain termination method using Big Dye Terminator (Applied Biosystems) in an ABI PRISM 3100-avant analyzer. The sequencing was repeated at least once for confirmation in cases in which mutations were found.

Statistical analysis. Mann-Whitney U-test for ordinal scale, Fisher's exact test and Chi-square test for nominal scale were used to compute the data with STATA software, version 8.0 (Stata Corp). A P-value <0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of Vietnamese patients at baseline. The data characteristics of the patients are shown in Table I. The mean age of ASC (22.3±6.2 years) was significantly lower than that of other groups (P<0.0001). Additionally, the mean age of CH (42.4±13.8 years) was significantly lower than that of HCC (49.5±11.3 years) and LC (50.9±12.2 years) groups (P<0.05). Anti-HBe antibody was detected in 97 cases (53%), and the prevalence of anti-HBe antibody differed depending on the equal clinical category. Concerning genotyping, genotype B was the largest group (124 patients, 67.8%), followed by genotype C (51 patients, 27.9%). Among the cases of genotype B, 123 patients (99.2%) had genotype Ba, and only one patient (0.8%) had genotype Bj. Three patients (1.6%) contained mixed genotype B and C, and the HBV from five patients (2.7%) could not be classified into any of these genotypes. The most prevalent genotype in ASC and CH was genotype B, and the prevalence of genotype C in LC and HCC was higher than that in CH and ASC (P<0.05).

Comparison of genotypes B and C in relation to variations of HBV-DNA, HBeAg seroconversion, AFP level and HBV-DNA level. The data are shown in Table II. No significant difference was shown in mean age between genotypes B and C (38.2±16.4 vs. 42.9±16.1 years). Besides the most common mutations A1762T/G1764A and G1896A, C1858 was a relatively common variation and it was highly specific for genotype C (62.7%) compared with genotype B (1.6%, P<0.0001). Dual core promoter mutations (A1762T/G1764A) were significantly less frequent in genotype B than in genotype C (45.2% vs. 66.7%, P<0.01). However, the frequency of pre-core mutation (G1896A) in genotype B was significantly higher than that in genotype C (31.5% vs. 13.7%, P<0.05). In agreement with this result, the HBe seroconversion rate in genotype B was significantly higher than that in genotype C (56.5% vs. 39.2%, P<0.05). The frequency of triple mutation A1762T/G1764A/G1896A in genotype B was not significantly higher than that in genotype C (13.7% vs. 5.9%), while the frequency of T1753C/A1762T/G1764A in genotype B was significantly lower than that in genotype C (8.8% vs. 25.5%, P<0.05). Triple mutation A1762T/G1764A/C1766T and quadruple mutation T1753C/A1762T/G1764A/C1766T were found in only a few patients and there was not a significant difference between genotype B and C. The AFP (α-fetoprotein) level in genotype B was lower than that in genotype C but not significantly different (145.3±280.6 vs. 151.0±270.2 ng/ml). The prevalence of cases with HBV-DNA level >5.0 log copies/ml in genotype C was significantly higher than that in genotype B (82.4% vs. 56.5%, P<0.01).

Clinical outcome in relation to variations of HBV-DNA, HBeAg seroconversion, AFP level and HBV-DNA level. To examine the clinical importance of variations and mutations in the core promoter and pre-core regions, we compared the frequencies of these variations and mutations in each clinical category. The data are summarized in Table III. The highest prevalence of C1858 was observed in HCC and it was significantly higher than that in ASC (31.2% vs. 9.7%, P<0.01). The prevalence of A1762T/G1764A in ASC was significantly lower than that in other groups (P<0.01). The prevalence of G1896A was significantly lower in ASC than in LC and CH. The same tendency was shown for the HBeAg seroconversion

Table III. Clinical outcome in relation to variations in core promoter and pre-core regions, HBeAg seroconversion, AFP level and HBV-DNA level.

Characteristic	HCC (n=48)	LC (n=44)	CH (n=29)	ASC (n=62)
C1858	15[a] (31.2%)	10 (22.7%)	4 (13.8%)	6[a] (9.7%)
A1762T/G1764A	34[c] (70.8%)	30[a,c] (68.2%)	16[a] (55.2%)	13[a,c] (20.9%)
G1896A	11[c,d] (22.9%)	16[a,b] (36.4%)	13[b,d] (44.8%)	6[b] (9.7%)
A1762T/G1764A/G1896A	4 (8.3%)	9[a] (20.5%)	5[d] (17.2%)	2[a,d] (3.2%)
T1753C/A1762T/G1764A	13[a] (27.1%)	10[b] (22.7%)	1[a,b] (3.4%)	2[a,b] (3.2%)
A1762T/G1764A/C1766T	2 (4.2%)	1 (2.3%)	2 (6.9%)	0 (0%)
HBeAg seroconversion	26 (54.2%)	28[a] (63.6%)	19[d] (65.5%)	23[a,d] (37.1%)
AFP (ng/ml)	330.3±355.8[e] (0-894)	148.9±282.2[e] (0-863)	133.7±253.5[e] (0.1-901)	5.4±8.7[e] (0-46)
HBV-DNA >5.0 (log copies/mol)	32 (66.7%)	33[d] (75%)	13[d] (44.8%)	35[d] (56.5%)

HCC, hepatocellular carcinoma; LC, liver cirrhosis; CH, chronic hepatitis; ASC, asymptomatic carrier; ALT, almandine aminotransferase; AFP, α-fetoprotein. [a]P<0.01, [b]P<0.001, [c]P<0.0001, [d]P<0.05, [e]P<0.01.

Table IV. HBeAg status in ASC in relation to variations in core promoter and pre-core regions.

Characteristic	HBeAg (-) (n=32)	HBeAg (+) (n=30)
Age (years)	22.8±6.9 (19-51)	21.7±5.5 (19-50)
C1858	1 (3.1%)	5 (16.7%)
A1762T/G1764A	11[a] (34.4%)	2[a] (6.7%)
G1896A	6 (18.7%)	0 (0%)
A1762T/G1764A/G1896A	2 (6.2%)	0 (0%)

ASC, asymptomatic carrier; -, negative; +, positive. [a]P<0.05.

rate. Comparison between the rates of A1762T/G1764A and G1896A in HCC and LC revealed that the prevalence of A1762T/G1764A was significantly higher than that of G1896A (P<0.0001 and P<0.05), but no such tendency was seen in CH and ASC. The rates of triple mutation A1762T/G1764A/G1896A in LC and CH were significantly higher than those in ASC (P<0.01 and P<0.05). The triple mutation T1753C/A1762T/G1764A was quite common in this study, and this mutation was more prevalent in HCC and LC than in CH and ASC (P<0.001 and P<0.01). The AFP level was highest in HCC (330.3±355.8 ng/ml) and this level was significantly higher than that in LC, CH and ASC (P<0.01), most ASC patients had a normal range of AFP level (5.4±8.7 ng/ml). HCC and LC were more related with a higher HBV-DNA level than CH and ASC.

Generally, HBeAg-positive ASC and HBeAg-negative ASC are clinically different. Next, we compared these two groups (Table IV). The prevalence of A1762T/G1764A in HBeAg-negative patients was significantly higher than that in HBeAg-positive patients (34.4% vs. 6.7%, P<0.05). The mutation of G1896A and the triple mutation A1762T/G1764A/G1896A were only observed in HBeAg-negative patients.

Comparison of mutation in relation to HBeAg status, AFP level and HBV-DNA between genotypes B and C. The data are shown in Table V. The median age was not significantly different among compared groups between genotype B and C. In the triple mutation T1753C/A1762T/G1764A or dual core promoter mutation A1762T/G1764A group, in comparison to genotype C, genotype B had a significantly higher frequency of negative HBeAg (81.1% vs. 38.5%, P<0.05; and 91.1% vs. 52.9%, P<0.0001, respectively). Genotype B also had a higher frequency of positive test to anti-HBe than genotype C. In all compared groups, even though it was not a significant difference, the AFP level in genotype B had a lower trend than that in genotype C. In dual core promoter mutation A1762T/G1764A, the percentage of patients with HBV-DNA level >5.0 log copies/ml in genotype B was significantly lower than that in genotype C (50% vs. 88.2%, P<0.0001). In triple mutation T1753C/A1762T/G1764A and pre-core mutation G1896A, in comparison to genotype B, genotype C was also more associated with a higher level of HBV-DNA.

Discussion

The various HBV genotypes have different geographical distributions worldwide (6,7,43-45). In East Asia, genotype B has been classified into genotypes Ba and Bj: genotype Bj was mainly found in Japan and genotype Ba was observed in other Asian ethnic groups (8,9,46). Genotype C has been sub-grouped into genotype Ce and Cs: genotype Ce was mainly found in Japan, Korea and Northern China, and genotype Cs was present in Southern China (10). Recently, HBV genotypes found throughout the world have been classified into sub-genotypes (47). This classification has provided abundant information about the geographical distribution of

Table V. Mutations in core promoter and pre-core regions in relation to HBeAg status, AFP level and HBV-DNA level in genotypes B and C.

Characteristics	T1753C/A1762T/G1764A		A1762T/G1764A		G1896A	
	Geno-B (n=11)	Geno-C (n=13)	Geno-B (n=56)	Geno-C (n=34)	Geno-B (n=39)	Geno-C (n=7)
Age (years)	49.5±8.1[a] (33-60)	55.2±8.5[a] (45-75)	44.8±15.4[a] (19-71)	48.4±13.2[a] (20-75)	44.2±15.3[a] (17-74)	46.7±13.6[a] (22-66)
HBeAg (-)	9[b] (81.1%)	5[b] (38.5%)	51[c] (91.1%)	18[c] (52.9%)	39 (100%)	6 (85.7%)
Anti-HBe (+)	6 (54.5%)	4 (30.8%)	38[b] (67.9%)	14[b] (41.2%)	30 (76.9%)	5 (71.4%)
AFP (ng/ml)	229.2±341.5 (0.8-835)	267.3±333.8 (1.8-853)	216.7±335.3 (0-901)	220.9±312.8 (0-853)	196.2±317.1 (0-863)	122.8±291.6 (0.6-784)
HBV-DNA >5.0 (log copies/ml)	8 (72.7%)	13 (100%)	28[c] (50%)	30[c] (88.2%)	25 (64.1%)	7 (100%)

-, negative; +, positive; AFP, α-fetoprotein; Geno-B, genotype B; Geno-C, genotype C. [a]P>0.05, [b]P<0.05, [c]P<0.0001.

HBV genotypes and the role of HBV genotypes and sub-genotypes in relation to the pathogenesis of liver diseases.

So far, our study is one of the largest-scale population studies examining the HBV genotype and having the largest population of determining variations in the core promoter and pre-core regions of HBV-DNA in Vietnamese. In agreement with previous studies (25-28), our results showed that genotypes B and C were the major genotypes in Vietnamese, and that genotype B was more prevalent than genotype C. In the study no cases with genotype A, D, E, F or G were detected. This is the first study to sub-classify genotype B into sub-genotype Ba or Bj in Vietnamese and showed that nearly all cases (99.2%) of genotype B were sub-classified into genotype Ba. Our results corresponded to those of previous studies of native Vietnamese and Vietnamese immigrants from other countries (17,19,25-31), but were inconsistent with the report that genotype A was very common in Vietnamese (33). It was reported that genotype C in East Asia was more strongly associated with severe liver disease and risk of HCC than genotype B (12-15,20,24,35,37,48,49). Most recently studies showed that HBV-DNA level was a strong risk predictor of liver cirrhosis and hepatocellular carcinoma and was independent of HBeAg status and serum alanine transaminase (ALT) level (50,51). In the present study, the frequency of HCC and LC in genotype C was significantly higher than that in genotype B. In contrast, ASC and CH patients had a significantly higher frequency of genotype B. In addition genotype C was already more related to a significantly higher level of HBV-DNA than genotype B in Vietnamese patients. Our data provide more information than previously available for comparing genotypes B and C regarding the role of genotype in pathogenesis and the progression of liver diseases, and regarding the influence on clinical outcome in HBV-infected Vietnamese.

Dual core promoter mutations (A1762T/G1764A) and pre-core mutation (G1896A) were reported to be closely associated with negative HBeAg and HBeAg seroconversion (16,41). Our data also confirmed that G1896A was closely associated with HBeAg seroconversion. C1858 is observed in genotypes A and F, while by contrast, T1858 is mainly found in genotypes B, C and D, and it was reported that the variation of nt1858 could predict the frequency of G1896A (17). Our data showed that the frequency of G1896A in genotype B was significantly higher than that in genotype C, which could be explained by the fact that the frequency of C1858 was higher in genotype C. Recent studies showed that C1858 was quite common in genotype C, especially genotype Cs (10,27); however, it was only analyzed in a small number of patients. As for the prevalence of C1858, our results were very similar to those in the previous study of patients mainly from Southern Vietnam (27), and it would be interesting to carry out a study in a large population to compare the characteristics of genotype C among countries in East Asia.

In both genotypes B and C, the frequency of A1762T/-G1764A was higher than that of G1896A. When comparing A1762T/G1764A to G1896A, the frequency was converse in genotypes B and C (Table II). The findings on the frequency of A1762T/G1764A and G1896A in relation to genotypes B and C in Vietnamese were similar to those in studies of people from Hong Kong, Taiwan and Japan (13,20,22,23,41,52), and confirmed that A1762T/G1764A was relatively common and G1896A was prevalent in genotype B in Northern Vietnam. In addition, the HBeAg seroconversion rate in genotype B was significantly higher than that in genotype C, accompanied by different HBV-DNA levels between genotypes B and C. These findings might imply that HBeAg seroconversion happens more easily and liver disease progresses more slowly in genotype B than genotype C. The different frequencies of mutations in the core promoter and pre-core regions between genotypes B and C in Vietnamese might contribute to the different roles of these genotypes in the pathogenesis of liver diseases.

It was reported that the mutations in the core promoter region were related to the risk of severe liver disease and the generation of HCC (23,24,27,35). Our results were also consistent with those previous data, and the prevalence of

A1762T/G1764A was highest in HCC, followed by LC. Among patients with A1762T/G1764A, the HBeAg seroconversion rate in genotype B was significantly higher than that in genotype C, by contrast genotype C was more associated with higher HBV-DNA level than genotype B, so it is possible that patients with A1762T/G1764A and genotype C without seroconversion have a high risk for HCC. Since HBe status and HBV-DNA level are the factors for assessing the stage of liver diseases, the finding that HBeAg seroconversion in genotype B was significantly higher and HBV-DNA level in genotype B was significantly lower could indicate a higher risk of genotype C causing progressive liver diseases than genotype B in Vietnamese. The frequency of mutations in the core promoter and pre-core regions and of HBeAg seroconversion were the lowest in ASC among Vietnamese, and this could be explained by the fact that the mean age of ASC was significantly lower than that of other groups.

Recently, some studies showed that triple mutations T1753C/A1762T/G1764A or A1762T/G1764A/C1766T and quadruple mutations T1753C/A1762T/G1764A/C1766T were closely associated with increasing the capacity of HBV genome replication and reducing HBeAg expression (53,54). In our study, the triple mutation T1753C/A1762T/G1764A was quite common, especially in genotype C, and the frequency in HCC and LC was significantly higher than that in CH and ASC. In addition, in the group of patients with T1753C/A1762T/G1764A, in comparison to genotype B, genotype C had a lower rate of HBeAg seroconversion and was closely related to higher HBV-DNA level. This result contributed more evidence that the mutation of T1753C/A1762T/G1764A was related to genotype C and severe progression of liver diseases in HBV-infected Vietnamese.

In conclusion, genotypes B and C were prevalent in Northern Vietnam. While genotype C and mutations in the core promoter region were more closely associated with progressive, severe liver diseases, genotype B had a higher prevalence of pre-core mutation and was correlated with HBeAg seroconversion, lower HBV-DNA level and lower risk of severe liver disease than HBV genotype C.

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