行政院國家科學委員會補助專題研究計畫成果報告

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※ B 型肝炎病毒基因型及表面抗原 T 細胞抗原決定部 ※

※位基因變異慢性 B 型肝炎病毒感染病程的影響(1/3) ※

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計畫類別：☐個別型計畫　　☐整合型計畫

計畫編號：NSC90－2314－B－002－154

執行期間：　90 年 8　月　1 日至　91 年 7　月　31　日

計畫主持人：張美惠

共同主持人：崔道貞

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主持人：張美惠教授　台大醫學院小兒科
共同主持人：崔道貞博士　台大醫學院小兒科
計畫參與人員：黃秀萍　台大醫學院小兒科

一、中文摘要

在 B 型肝炎病毒感染之後，不同的病人會有不同的病程及預後。本研究之目的要探討 B 型肝炎病毒之基因型對於兒童慢性 B 型肝炎病毒感染臨床病程及 e 抗原抗體轉變的影響。

我們長程追蹤 250 名慢性 B 型肝炎病毒感染之病童之肝功能及 B 型肝炎標記，以及 B 型肝炎之基因型。我們將這些兒童分成四組：第一組含 68 名兒童，在追蹤過程中一直保持 e 抗原陽性的狀況；第二組 156 名兒童，在追蹤過程中由 e 抗原陽性轉變成 e 抗體陽性；第三組 26 名兒童，在進入本研究時已轉變成 e 抗體陽性的狀況。另有 25 名兒童為肝細胞癌患者(第四組)，均為 B 型肝炎慢性感染者。

我們先以基因型特異性聚合 °鏈(PCR)反應作基因型分類，無法分類者再用限制 °切割 PCR 產物，判斷其基因型。結果顯示在第一、二、三、四組者屬基因型 B 型者分為 71%，73%，92%及 68%；屬基因型 C 型者，則分別為 25%，7%，0%及 11%。在長程追蹤中，基因型之轉變發生於 10.8%的孩童。

總之，在台灣慢性感染 B 型肝炎病毒的兒童中，基因型 B 型之 B 型肝炎病毒是常見的。基因型 C 型可能會延遲 e 抗原抗體轉變。肝癌兒童之 B 型肝炎病毒仍以基因型 B 型為主。

關鍵詞：B 型肝炎病毒、基因型、e 抗原抗體轉變、肝癌、兒童

Abstract

Background/Aim: During the natural history of hepatitis B virus (HBV) infection, clinical course and outcome differs in different individuals. The aim of this study is to investigate the effect of HBV genotypes on the clinical course and hepatitis B e antigen/antibody seroconversion during chronic HBV infection. *Subjects and Methods:* We longitudinally followed up 250 HBsAg carrier children and performed HBV genotype study in these children (Group I, 67 children with persistently positive HBeAg; Group II, 148 children who have been spontaneously seroconverted from HBeAg seropositive to anti-HBe positive during follow-up; Group III, 24 children who have been anti-HBe seropositive when they entered our long term follow-up study). Another 25 children (Group IV) with HBV-related hepatocellular carcinoma (HCC) were also recruited. The genotyping was performed by polymerase chain reaction (PCR) using type-specific primers. *Results:* Genotype B was 71%, 73%, and 92% in Group I, II, and III respectively. Genotype C was 25%, 7%, and 0% in Group I (p=0.01), II, and III respectively. During long-term follow-up Genotype changing occurred in 10.8% of these 239 study subjects. For the HCC group (group IV), Genotype B is still predominant (68%) than Genotype C (11%). *Conclusion:* HBV Genotype B is the commonest one in children Taiwan. Genotype C might delay HBeAg seroconversion. The role it played in the pathogenesis of chronic liver damage is worthy of further attention. Genotype changing seemed not an uncommon event in the natural history of childhood HBV chronic infection. For childhood HCC, Genotype B is the dominant one.

Key Words: hepatitis B virus, genotype, HBeAg seroconversion, hepatoma, children

.**INTRODUCTION**

Chronic hepaitits B virus (HBV) infection is a worldwide health problem. It may leads to chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC) (1). In hyperendemic areas, chronic HBV infection begins mainly during infancy and early childhood (2,3). HBV in early childhood tends to cause persistent infection (4). Although complications of chronic HBV infection manifest mostly during adulthood, liver histologic changes begin early since childhood (5). Study in children is therefore very important to enhance the understanding of the mechanism of persistent HBV infection and the clinical course shortly after primary infection.

We have already investigated the role of HBV in the long-term follow-up course of chronic HBV infection in children. According to our previous longitudinal follow-up studies, mutation of the precore gene (10), basal core promoter gene, and core gene deletion (11) precedes or are accompanied with HBeAg seroconversion, some with more severe liver diseases. Additional determinant factors have to be explored.

Genotypes of hepatitis C virus (HCV) were closely related to the virus load, disease course, and the response to antiviral therapy (12,13). The recommended protocol for antiviral therapy by National Institute of Health, U.S.A. was different for patients with different genotypes of HCV (14). However, studies concerning genotypes of HBV and clinical correlation are very limited. Okamoto et al. first described HBV genotypes and classified it into genotypes A to D on the basis of a nucleotide divergence of greater than 8% of the whole genome (15). HBV can be divided into at least seven genotypes, A to G (16,17). Genetic variability of HBV may influence the viral antigen expression, which will affect the host immune response to HBV.. In addition, different HBV genotypes are associated with different mutations in the HBV precore and core promoter gene regions during HBeAg seroconversion (16,18,19). Previous Japanese studies showed that those infected with HBV *adr* strain (genotype C) had a severer form of liver diseases than those with *adw* strain (genotype B) (20).

Studies for the clinical and pathogenetic correlation of genotypes of HBV are limited and are all in adults. Recently, cross sectional studies in adults revealed that different genotypes are very likely related to disease severity. In Asian people, comparing to genotype B, genotype C was associated with a higher frequency of core promoter mutation, higher levels of aminotransferases, and lower response rate to interferon therapy (21). Genotype C was prevalent in patients with cirrhosis and HCC >50 years, while genotype B prevalent in patients with HCC less than 50 years (22). Longitudinal studies would be more revealing than cross-sectional data, but were lacking.

Our aim of this study was: (1) To study the influence of HBV genotypes in the clinical course of chronic HBV infection in children. (2) To test the possibility of mixed infection of different genotypes of HBV or the transition from one genotype to the other during long-term follow-up.

SUBJECTS AND METHODS

250 HBsAg carrier children, under parental consents, were followed every 6 months. At each visit, physical examination, blood test for liver function profiles and
HBV markers. The serum samples at enrollment into this study and the latest one were checked. These 250 children were divided into three groups: Group I (n=68) consisted of the children with persistent seropositive HBeAg during follow-up. Both of the serum samples checked were in the pre-HBeAg seroconversion phase. Group II (n=156) consisted of the children who underwent HBeAg seroconversion during the follow-up period. Also, their serum samples at enrollment and the latest one were checked, but unlike Group I, the latest one was

after HBeAg seroconversion; Group III (n=26) consisted of children who were already seropositive for anti-HBe at enrollment. Both of the serum samples checked were in the post-HBeAg seroconversion phase. The HCC group (group IV) consisted of 25 patients (M:F=18:7) and the serum samples checked were withdrawn at the time of diagnosis.

HBV Markers (including HBsAg, anti-HBs, anti-HBc, HBeAg, anti-HBe) were examined by radioimmunoassay using the commercially available kits (Abbott Company, North Chicago, IL, USA). Alanine transferase (ALT) was checked by using an autoanalyzer (Hitachi 7450, Tokyo, Japan).

Determination of HBV Genotype by Nested PCR.

The genotype of HBV was determined by PCR using type-specific primers (23).

In brief, DNA was extracted from 30ul of serum by the SDS-proteinase K/phenol/chloroform method. 10 µl of the product was used for PCR in a reaction volume of 50ul containing 1.5 mM $MgCl_2$, 50 mM KC1, 10 pmol each of first pair of primers P1 and P2 covering nucleotide 2823 to 704 of the HBV genome. The condition of first run PCR was described previously (23). The product of the first PCR subjected for 2^{nd} PCR using mix A (for identifying Genotype A, B, C) and mix B (for identifying Genotype D, E, F) with a universal sense primer and type-specific antisense primers. The procedure condition was the same as the first PCR.

If the results were not interpretable after double check, the PCR plus restriction fragment length polymorphism (RFLP) method was then used for feuther confirmation (24).The PCR-RFLP method used PCR by primers spanning from HBV nucleotide position 256 to 796. The PCR product was digested with Tsp509I, and Hinf I separately. The samples were run on a composite gel (2% NuSieve agarose and 1% standard agarose). Different genotypes of HBV DNA fragments could be marked by different length when digested with different enzymes (24). All experiments were done at least in duplicate to confirm the results.

RESULTS

Basic and biochemical data.. The basic and peak aminotransferase (ALT) data of the three groups of chronic HBV-infected children and the HCC group were listed in Table 1. Their ages at enrollment and at diagnosis were not different statistically (chi square test, $p>0.05$). The age of HCC group was not different from the three chronic infection groups' age at enrollment (chi square test, $p>0.05$).

Genotype determinations. Genotype B is the predominant strain of HBV in Taiwan, but the distribution was not even in the three chronic infection groups. Genotype B was 71%, 73%, and 96% in Group I, II, and III respectively. Genotype III was 25%, 7%, and 0% in Group I, II, and III respectively (Fig. 1). For the HCC group, 8 cases had undetectable DNA while the remaining 17 belonged to : Genotype B 82% (14/17), Genotype C 12% (2/17), Genotype A 6% (1/17). There were 26 events of genotype changes for the 250 subjects (10.4%).

DISCUSSION

Genotype B is regarded as the commonest type of HBV in adults in Taiwan. This childhood study reconfirmed this point. It is expected since either perinatal transmission or horizontal parenteral transmission is strongly related to the adult population. Genotype C is the second common one.

The most interesting finding of this study was the genotype changes during the follow-up period of the childhood study. The genotype was assumed very uncommon to change during the infection course since the

chance of superinfection of other type of HBV in carriers is not high (10%) (25). Such an incidence of genotype changes is compatible with the data in this prospective study (10.4%). This may imply there is still quite a chance of repeated horizontal infection in childhood. We demonstrated that horizontal transmission of HBV mostly occurred in childhood previously (26,27). We believe such an infection may not be a single event in a susceptible subject. If HBV infection can repeat in one single subject, childhood would be an appropriate time to observe the changing of genotype. The other possibility is because this study is the longest prospective study (more than 10 years) up to now. All these patients lived in the HBV hyperendemic area, Taiwan. Therefore, they were prone to have superimposed HBV infection and subsequent genotype changes. Our long-term follow-up study design is right for recording these changes. Such an observation was hard to be done by cross-sectional studies.

We classified the study subjects according to their HBeAg seroconversion status. HBeAg seroconversion can mark the active and quiescent HBV replication stages (28). Group I consisted of children who did not undergo HBeAg seroconversion in the whole follow-up period. That means their age of HBeAg seroconversion would be after 20 years in average (Table 1). Both Group II and III had already undergone HBeAg seroconversion but Group II was witnessed to undergo this event during the follow-up, which was at the age of 10-20 years approximately while Group III had already undergone this event before the age of nine (Table 1). Genotype C occupied a relatively larger proportion in Group I in comparison to that in Groups II and III. Thus, we speculate that Genotype C is more resistant to HBeAg seroconversion. The delayed seroconversion may prolong the inflammatory process and consequently a more severe liver damage. From this point of view, we may expect Genotype C to be a more virulent form of HBV.

HCC is regarded to be the gravest complication of chronic HBV infection. Genotype B is the most common one found in this patient group. Kao et al. reported that Genotype B is the predominant type in young adult with HCC (age<35 years). This childhood HCC study also support such a finding while we still had no clue in the carcinogenesis of Genotype B HBV.

In conclusion, HBV Genotype B is the commonest one in Taiwan undoubtedly. The role Genotype C played in the pathogenesis of chronic liver damage is worth further attention while the current data showed it might delay HBeAg seroconversion. For childhood HCC, Genotype B still the dominant one while some unusual genotypes might play some roles.

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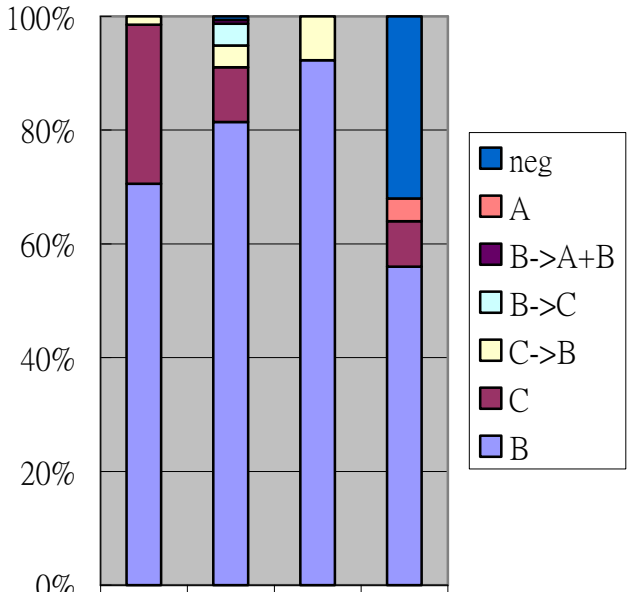
FIGURE LEGENDS

Figure 1. Genotype analysis in the three groups of chronic HBV-infected children (Group I: persistent HBeAg seropositive, Group II: underwent HBeAg seroconversion during follow-up, Group III: already seroconverted at enrollment) and HCC children. No HBV DNA could be detected by PCR in six HCC children.



	Group I	Group II	Group III	Group IV
neg		1		8
A		0		1
B->A+B		1		0
B->C		6		0
C->B	1	6	2	0
C	19	15		2
B	48	127	24	14

ronic HBV-infected and HCC children

At enrollment		Latest check	
Age (mean±SD)	HBeAg/anti-HBe	Age (mean±SD)	HBeAg/anti-HBe
10.6±3.8	+/-	20.1±3.8	+/-
9.6±4.7	+/-	20.2±5.1	-/+
9.2±4.9	-/+	22.1±4.7	-/+
9.7±2.9	-/+*		