GENOTYPES OF HEPATITIS B VIRUS AMONG CHILDREN IN CHIANG MAI, THAILAND

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Abstract. In sub-Saharan Africa, the Pacific, and particularly Asia, hepatitis B virus (HBV) infection is highly endemic, the most common route of transmission is perinatal. To minimize the number of horizontal transmissions, we determined the prevalence of HBV genotypes among children in northern Thailand. From a survey of 1,231 schoolchildren in Chiang Mai during 1998 to 2000, 55 (4.5%) were found positive for HBsAg. Fifty-three HBsAg-positive samples were available for this study. These came from 28 girls (52.8%) and 25 boys (47.2%), age 5-16 years, with a mean age of 12.8 (±2.6) years. The laboratory method was based on a multiplex-PCR for the detection of 6 HBV genotypes (A-F). Among 53 HBsAg positive cases, 48 (90.6%) were genotype C, followed by 4 cases of genotype B (7.5%), and 1 case (1.9%) with mixed infection with genotypes B and C. The high prevalence of HBV genotype C follow by genotype B is similar to that found among blood donors in northern Thailand and the nationwide epidemiological survey conducted in 2004. Perinatal transmission may play an important role in the spread of the virus in this area, as in other Asian countries, where genotypes C and B are highly prevalent.

INTRODUCTION

More than 2 billion people have been infected with the hepatitis B virus (HBV) worldwide, and of these, more than 350 million suffer from chronic HBV infection (WHO, 2000). Highly endemic areas (>8% of the population being HBV carriers) include Southeast Asia and the Pacific Basin (excluding Japan, Australia, and New Zealand), sub-Saharan Africa, the Amazon Basin, parts of the Middle East, the central Asian Republics, and some countries in eastern Europe (Chen *et al* 2000; Custer *et al*, 2004). In the highly endemic areas, the most common route of transmission is perinatal, or the infection is acquired during the preschool years. The majority of individuals become chronic HBV carriers. Children with chronic HBV infection are usually symptom free but nonetheless have a long-term risk of liver cirrhosis and hepatocellular carcinoma (HCC) (Merican *et al*, 2000).

HBV has been classified into genotypes A to H by sequence divergence greater than 8% for the entire genome (Schaefer, 2005). The prevalence of specific genotypes varies geographically. HBV genotype A is mainly found in Europe and North America. Genotypes B and C are prevalent in Asia. Genotype D has its highest prevalence in a belt stretching from southern Europe to India. Genotype E is restricted to Africa, and genotype F to South and Central America (Kidd-

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Ljunggren *et al*, 2002). Few cases of HBV infection with genotypes G and H have been identified (Schaefer, 2005). There is some evidence that the long-term prognosis, the initial clinical manifestations, and the response to treatment may differ depending on which genotype has infected the person (Kidd-Ljunggren *et al*, 2002). Most of HBV genotypic prevalences were reported from specimens taken from adult patients or blood donors, those who might have become infected by horizontal routes of transmission. Northern Thailand is a highly endemic area for HBV infection, and the seroprevalence of HBsAg among voluntary blood donors was 8.7% (Jutavijittum *et al*, 1999). Perinatal infection is the major route of transmission. To minimize the number of horizontal transmissions; we determined the prevalence of HBV genotypes among schoolchildren residing in Chiang Mai Province, northern Thailand.

MATERIALS AND METHODS

From July 1998 to August 2000, 1,231 schoolchildren age 4-16 years from 7 rural schools and 3 urban schools in Chiang Mai Province were interviewed to state the status of hepatitis B (HB) vaccination and blood samples were taken for serological studies. There were 55 (4.5%) children positive for hepatitis B surface antigen (HBsAg) using the ELISA kit, Monolisa Ag HBs (Sanofi Diagnostic Pasteur, Marnes la Coquette, France). All of the HBsAg-positive sera from the survey were kept at -20ºC. There were 53 serum samples available for DNA extraction and HBV genotype determination in this study.

DNA was extracted from 100 µl of serum samples using the commercial nucleic acid extraction kit: High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Mannheim, Germany). The resulting pellet was resuspended in RNase-free water and then subjected to nested PCR. The laboratory method was based on a multiplex-PCR for the detection of 6 major genotypes (A-F), as described by Naito *et al* (2001). The HBV genomes were amplified using the universal primers (P1 and S1-2) as outer primers, followed by two different mixtures (Mix A for genotypes A, B, and C; Mix B for genotypes D, E, and F) containing type-specific inner primers. The genotypes of HBV were determined by the migration patterns of the PCR products (Naito *et al*, 2001).

RESULTS

In our study, there were 1,231 schoolchildren age 4-16 years (mean age of 6.5±1.6 years), 327 boys and 343 girls. Of these, 371 schoolchildren (182 boys and 189 girls) were from 7 rural schools and 299 schoolchildren (145 boys and 154 girls) were from 3 urban schools in Chiang Mai Province. We found that the overall seroprevalence of HBsAg positivity among the schoolchildren age 4-16 years was 4.5%. Fifty-three HBsAg positive cases were available for genotype determination. These were comprised of 28 girls (52.8%) and 25 boys (47.2%) age 5-16 years, with a mean age of 12.8 (±2.6) years. Fourty-five children were born before 1989, the year in which HB vaccination was started as a pilot project in Chiang Mai, thus 43 children did not get the HB vaccine but one got 3 doses of the HB vaccine. Eight children were born in 1989 or after; 4 of them received 3 doses of the HB vaccine beginning within 7 days of birth, then at 2 months, and 6 months of age, while 4 of them did not receive HB vaccination. HBV genotypes were identified in all samples and their distribution is shown in Table 1. Forty-eight cases (90.6%) were genotype C, 4 cases were genotype B (7.5%) and 1 case (1.9%) was a mixed infection with genotypes B and C (in a 15 year old girl).

DISCUSSION

We examined the serum samples from schoolchildren in Chiang Mai, a province in northern Thailand, and found that the preva-

Table 1
Genotypes of hepatitis B virus among Chiang Mai children.

Genotypes	Numbers (%)
C	48 (90.6)
B	4 (7.5)
B and C	1 (1.9)

lence of HBsAg among them was 4.5%, which was lower than in the general population. Hepatitis B vaccinations in Chiang Mai children were given at birth, and 2 and 6 months of age starting with a pilot project begun in 1988 then to the expanded program of immunization (EPI), and later added to the nationwide EPI in 1992 (Poovorawan *et al*, 2001). The number of participants in our study was partly influenced by the EPI, with 45 of 53 children studied being born before the vaccination program started and failing to obtain hepatitis B vaccination. The declining prevalence of HBV infection in recent years is a result of universal hepatitis B vaccination in Thailand (Chongsrisawat *et al*, 2006).

Certain genotypes predominate within different geographic, regional, and racial groups. Genotypes B and C are prevalent in high endemic areas, such as Asian countries, where vertical transmission plays an important role in spreading the virus (Huy and Abe, 2004). In our study, we found a very high prevalence of genotype C (90.6%), followed by genotype B (7.5%), and 1.9% with mixed infection due to genotypes B and C. The distribution of HBV genotypes in Chiang Mai children was almost identical to that of adults in our published data of voluntary blood donors in northern Thailand age 16-52 years (mean age of 25.4 years), 89.3% had genotype C and 7.4% genotype B, 1.9% mixed infection of genotypes B and C, and 0.5% with genotype A (Jutavijittum *et al*, 2006). These values are similar to a nationwide epidemiological survey conducted in 2004, genotypes C, B and A accounted for 87.1%, 11.6 and 1.3% of total infections, respectively (Suwannakarn *et al*, 2008). Recent data suggests that genotype C induces a more severe disease course, causes higher scores on the necroinflammatory activity scoring system and fibrosis stage, is more prevalent in patients with cirrhosis, and progresses more often to hepatocellular carcinoma (HCC). HBV disease progression with genotype B appears to be slower than with genotype C, however the life-long risk of progression to advanced fibrosis and HCC may be similar. Previous reports describe that although HCC patients with genotype B responded well to embolization therapy, and had no recurrence of HCC for a prolonged period of time, HCC patients with genotype C showed poor responses and died of hepatic failure because of rapid HCC progression (Kao *et al*, 2000; Schaefer, 2005; Tangkijvanish *et al*, 2005).

One case (1.9%) in our study had a mixed infection with genotypes B and C. Genotype-specific PCR, as used in multiplex-PCR approaches, was reported to be superior in detecting double infections (Schaefer, 2005).

In conclusion, our data indicate that HBV due to genotype C was more common than genotype B infection among schoolchildren in northern Thailand. Genotypes B and C are prevalent in highly endemic areas of HBV infection, such as Asian countries, where vertical transmission plays an important role in spreading the virus (Kidd-Ljunggren *et al*, 2002).

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