論　文　内　容　要　旨

Existence of hepatitis B virus surface protein mutations and other variants: demand for hepatitis B infection control in Cambodia

（B 型肝炎ウイルス感染制御を目的としたカンボジア王国の母子集団における B 型肝炎ウイルス表面タンパク遺伝子変異・塩基多型に関する疫学的研究）

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【Background】

Global prevalence of hepatitis B virus (HBV) infection was 3.5% causing 1.34 million deaths in 2015. Among six world health organization (WHO) regions, western pacific region ranks the most hepatitis B surface antigen (HBsAg) prevalent area having 6.2%. Cambodia, one of the developing countries in WHO western pacific region (WHO-WPRO), has reported HBsAg prevalence from 3.2 to 8.5% before implementation of hepatitis B vaccine (HepB) to national immunization programme (NIP). After phasing-in of HepB vaccine in 2005, HBsAg prevalence among ≤ 5 years old children markedly reduced to 0.33-3.45%. In Cambodia, there is no specific surveillance system on infection control including viral hepatitis and no more reports on HBV infection were found after 2011. Therefore, in joint collaboration with WHO-WPRO, WHO Cambodia, United State Centers for Disease Control and Prevention (US-CDC) and Ministry of Health of Cambodia (MOH), Hiroshima University conducted the nationwide study on HBsAg prevalence among 5-7 years old children and their mothers in 2017. By the nationwide study, the reported HBsAg prevalence among 5-7 years old children was 0.56% and that among their mother was 4.39% using HBsAg rapid test. The nationwide study revealed the occurrence of HBV infection among children who received HepB birth-dose (HB-BD) within 24 hours after delivery and demanded further in-detail study on HBV genotype distribution and their mutant variants. Therefore, this study aimed to detect HBV genome sequences and their variants as of nationwide scale using dried blood spot (DBS) samples and to provide up-to-date reference data for infection control and surveillance in Cambodia.

【Methods】

Using multi-stage random sampling strategy, the nationally representative cross-sectional study was conducted in 2017 and a total of 2,520 children and their 2,028 mothers were taken by questionnaires, rapid test and their blood samples using dried blood spot (DBS). Among them, 2 children samples and 5 mother samples were excluded for their insufficient amount of blood for measurement. All samples were tested for HBsAg using chemiluminescent enzyme immunoassay (CLEIA). Nucleic acid was directly extracted from one fin of HBsAg positive samples using SMITEST. The amplification was carried out by nested polymerase chain reaction (nested-PCR) using WA primer set targeting nt1-nt1779 and nt-1909-nt3215. For the missing portion of HBV circular DNA, second round of nested PCR were done using primer set targeting nt1548-nt2097. For those samples that could not be amplified at WA primer, second attempt of nested PCR was done targeting s-region. Then, direct sequencing was done using 3730xl DNA sequencer with BigDye Terminator v3.1 cycle sequencing kit. After analyzing the raw sequences data by GENTYX, the phylogenetic tree was employed by neighbor joining method using the standard strains retrieved from GeneBank. The recombination of circular DNA was detected using the SimPlot program and

boost scanning analysis with jumping profile Hidden Markov Model (jpHMM) and visualized in a circular form using Circos. The statistical analysis was performed using JMP. The X2 and Fisher's exact test were used to compare between group setting significance level at $p<0.05$.

【Results】

Using DBS samples, HBsAg positive rate was 4.7% in mothers and 0.52% in their children. The mean age of mothers was 32.36±6.01 years. 69.2% of children were 5 years old and the rest were 6 years old. Among 95 HBsAg positive mothers, nine of their children were positive to HBsAg so that mother-to-child transmission (MTCT) rate was 9.5%. Using DBS samples, 75.9% (82/108) of HBsAg positive samples can determine HBV genotype amongst which 51.2% (42/82: 32 mothers and 10 children) were full length genomes and the remaining were partial genomes. HBV genotype C (80.49%) was abundantly found throughout Cambodia whilst genotype B (19.51%) was exclusively found in regions bordering Vietnam. Almost all genotype C belong to subtype C1 except one for C8 and almost all genotype B are recombinant B4/C2 except one for B2/C2. Full length genome analysis revealed the homology of 99.62-100% in each mother-child pair. The overall mutation rate of "a determinant region" of S gene among HBV DNA positive sera was 24.3% in mothers and 16.7% in children. One mother-child pair has P127S mutation in both mothers and children but the other two pairs have mutation only in mother (G145R) or child (P120S). The mutation rate of "a determinant region" was higher in children if their mother was infected with mutant variants (5.9% Vs 1.9%, $p=0.39$) or if they received HepB vaccine (28.57% Vs 0.00%, $p=0.21$). The double mutation (A1762T/G1764A) in C1 strains was 48.39% and that of combination mutation C1653T or T1753C and A1762T/G1764A.

【Discussion】

This is the first report on genotype distribution of HBV and the existence of mutant strains in "a determinant region of S gene as of its nationwide scale in Cambodia. Both partial and full-length HBV genomes can be extracted from DBS which confer up to large scale molecular epidemiological study of HBV especially in resource limited settings. The evolutionary analysis of HBV genomes revealed its historical relation among neighboring countries. Understanding of predominant genotypes aware its geographic variation and calls for implementation of area-specific effective infection control against HBV. Existence of mutant strains in "a determinant region" of S gene alert the potential breakthrough infection among immunized children. 99.62-100% homology of HBV strains from mother-child pairs strongly demands to promote mother-to-child prevention strategies on HBV in Cambodia. Moreover, the occurrence of double and combination mutation in C1 strains isolated in this study which are significantly related to hepatocellular carcinogenesis superimposes the needs of effective infection control and its surveillance system including

PMTCT so that Cambodia can straightforwardly move to WHO's elimination goal of viral hepatitis by 2030.