論　文　内　容　要　旨

Epidemiological assessment of hepatitis E virus Infection among 1565 pregnant women in Siem Reap, Cambodia using an In-house double antigen sandwich ELISA.

（カンボジア王国シェムリアップ州 1,565 人の妊婦を対象にした In-house 二重抗原サンドイッチ ELISA 法を用いた E 型肝炎ウイルス感染の疫学的評価）

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【Background】

The Hepatitis E virus (HEV) affects roughly a third of the world's population, presenting varied epidemiological profiles across different areas, including hyperendemic regions, endemic zones, areas with distinct epidemiological features, and countries with autochthonous cases. Cambodia is known to be a hyperendemic region for HEV.

The virus is divided into eight genotypes, with 1 to 4 primarily affecting humans. Genotype 1 is common in many southern and southeast Asia countries, including Cambodia, while genotype 2 is mainly found in Africa. In contrast, genotype 3 is prevalent across Europe, the United States, and Japan, and genotype 4 is frequently seen in Japan and China. The transmission of genotypes 3 and 4 often occurs through the consumption of undercooked meat due to their intermediate hosts, whereas genotypes 1 and 2 are associated with poor sanitation practices.

The identification of HEV genotypes demands advanced laboratory infrastructure and specialized personnel, which incurs significant costs and presents a challenge in resource-limited settings. The link between genotype 1 and increased maternal mortality rates during the third trimester underscores the importance of developing an in-house ELISA method.

The new ELISA method, aimed at being affordable and easy to use, provides an effective strategy for large-scale screening in hyperendemic areas. Pregnant women the risk of development of severe HEV infection, yet data on the prevalence of HEV among pregnant women in Cambodia is lacking.

【Methods】

Study Design and Methods

The study capitalized on preserved serum samples from a previous research project on mother-to-child transmission of hepatitis B virus (HBV) in Cambodia. Blood samples were collected from participants across three hospitals in Siem Reap in 2020, and a structured questionnaire was used to collect five sociodemographic and two anamnestic information items.

Development of In-house ELISA

The in-house ELISA used specific recombinant HEV antigens. The primary coating antigen was a recombinant HEV capsid protein (ORF2) with a C-terminal mouse Fc-Tag, and the secondary antigen was a recombinant HEV antigen protein with a His Tag. The secondary antigen was biotin-labeled and used with polyclonal Streptavidin HRP to enhance the chemiluminescent signal. The ELISA plates were coated with the antigens, blocked, and then incubated with serum samples. The reaction was developed using TMB solution and read at 450 nm.

Comparison with Commercial Test Systems

For this phase, we randomly selected a subset of 262 serum samples from the total 1565 pregnant women for this comparison. The In-house ELISA was evaluated against the two commercial kits (anti-HEV IgG EIA, Institute of Immunology, Co. Ltd, Tokyo, Japan; anti-HEV IgG RecomLine LIA, Mikrogen GmbH, Germany) by analyzing sensitivity, specificity, and the level of concordance using percentage agreement and Cohen's kappa coefficient.

After accuracy assessment, we estimated the prevalence of total anti-HEV using the in-house ELISA across all 1565 participants. Next, we conducted the detecting IgM among those who tested

positive for total anti-HEV immunoglobulins, using an anti-HEV IgM RecomLine LIA, Mikrogen GmbH, Germany. For the final phase, the positive anti-HEV IgM cases were tested by RT-PCR to confirm HEV RNA presence.

Additionally, in the study, we estimated an epidemiological trend of HEV transmission and association between HEV infection prevalence and various socio-demographic factors based on previously developed questionnaire for the study on mother-to-child transmission of hepatitis B. This questionnaire gathered demographic and health-related information, including age, education, occupation, family size, and history of blood transfusions and surgeries, from pregnant women.

Statistical Analysis

The study employed Receiver Operating Characteristic (ROC) curves, agreement percentages, and Cohen's kappa coefficient to evaluate the diagnostic accuracy of the in-house ELISA against two commercial kits. A significant level of 0.05 was considered the threshold for statistical significance.

【Results】

The newly developed In-house ELISA showed a sensitivity of 76% and specificity of 94.1% against the Institute of Immunology kit, with a Cohen's kappa of 0.61. Against the RecomLine LIA by Mikrogen, it demonstrated a sensitivity of 71.4% and specificity of 98.6%, with a Cohen's kappa of 0.76. Both tests had an area under the curve (AUC) of 0.85, indicating good diagnostic accuracy.

The prevalence of total anti-HEV among 1565 pregnant women was found to be 11.6% (181/1565). The prevalence of anti-HEV IgM among 181 total anti-HEV was 22.7% (41 cases), indicating recent or ongoing infection.

The prevalence of total anti-HEV varied significantly across age groups, with higher rates observed in older women. Multivariate analysis revealed no significant association between total anti-HEV immunoglobulins positivity and socio-demographic factors such as education level, occupation, family size, or history of blood transfusion and surgical operations, except age and occupation of head of household as public officer.

The anti-HEV IgM presence was not associated with any of those factors.

HEV RNA was not detected in any of the 41 anti-HEV IgM positive samples, suggesting the absence of active viral replication among the participants.

【Conclusion】

In conclusion, our investigation into the seroprevalence of HEV among pregnant women in Siem Reap, Cambodia, reveals a prevalence rate of 11.6% for total anti-HEV. This prevalence underscores the significant public health concern posed by HEV infection, particularly given its severe complications during pregnancy, such as acute liver failure and increased maternal and fetal mortality. Our study's strength lies in its comprehensive approach, utilizing a newly developed in-house ELISA method for serological analysis, which demonstrated good diagnostic accuracy against commercial assays.

The absence of HEV RNA among anti-HEV IgM positive cases suggests a lack of active HEV infection within our cohort, highlighting the potential for false-positive results or the transient nature of HEV viremia. Additionally, our analysis identified older age and the occupation of the household head as public officer as significant factors associated with HEV seropositivity. Being exposed to HEV

infection throughout the life may contribute to a higher occurrence of total anti-HEV among older pregnant women.

Considering the high seroprevalence of HEV among pregnant women and the potential severe outcomes associated with infection during pregnancy, our findings emphasize the need for upgrading preventive measures as promoting hygienic practices, ensuring food safety, and water supply options. The recently developed double antigen sandwich ELISA might prove to be a valuable instrument for detecting HEV infection in developing countries, thereby enhancing the comprehension of HEV infection's epidemiological patterns.