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ORIGINAL ARTICLE

Comparison of Four Serological Tests for Detecting Antibodies to Japanese Encephalitis Virus after Vaccination in Children

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Abstract
Objectives: Several different methods are currently used to detect antibodies to Japanese encephalitis virus (JEV) in serum samples or cerebrospinal fluid. These methods include the plaque reduction neutralization test (PRNT), the hemagglutination inhibition (HI) test, indirect immunofluorescence assay (IFA), and enzyme-linked immunosorbent assay (ELISA). The purpose of this study was to compare the performance of each method in detecting vaccine-induced antibodies to JEV.
Methods: The study included 29 children who had completed a primary immunization schedule with an inactivated vaccine against JEV derived from mouse brain ($n = 15$) or a live attenuated SA14-14-2 vaccine ($n = 14$). Serum samples were collected between 3 months and 47 months after the last immunization. The serum samples were tested by performing the PRNT, HI test, in-house IFA, and commercial ELISA. The antibody detection rates were compared between tests.
Results: All 29 serum samples were positive with the PRNT, showing antibody titers from 1:20 to 1:2560. The HI test showed positive rates of 86.7% (13/15) and 71.4% (10/14) in the inactivated and live attenuated vaccine groups, respectively. The results of the IFA for immunoglobulin (Ig)G were positive in 53.3% (8/15) of children in the inactivated vaccine group and 35.7% (5/14) in the live attenuated vaccine group. Neither the IFA nor ELISA detected JEV IgM antibodies in any of the 29 children.

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Conclusion: These results show that detection rates of vaccine-induced antibodies to JEV have a wide range (0−100%) depending on the testing method as well as the time since immunization and individual differences between children. These findings are helpful in interpreting serological test results for the diagnosis of Japanese encephalitis in situations where vaccines are widely administered.

1. Introduction

Japanese encephalitis (JE), a mosquito-borne disease, is caused by the JE virus (JEV), which belongs to the *Flavivirus* genus within the *Flaviviridae* family [1]. An estimated 67,900 cases of JE with 20−30% fatality occurred throughout the Asian and the Pacific regions in the early 2010s [2]. Vaccination is the most effective measure for disease control and extensive immunization programs have been implemented in Korea, Japan, and Taiwan [3−5]. Two types of vaccines have been used in Korea. Inactivated vaccines derived from mouse brain have been used since the late 1960s. Currently, a three-dose primary vaccination course at 1−3 years of age (0 day, 7−30 days at 12−23 months of age, and 1 year after the 2nd dose) and two booster immunizations at the ages of 6 years and 12 years are recommended by the National Immunization Program (NIP) [3]. A live attenuated SA 14-14-2 vaccine has been administered in the private sector since the late 1990s and was included in the NIP from 2013. The latest regimen is a one-dose primary vaccination at 12−23 months of age and one booster immunization 12 months after the first dose.

There are several methods used to detect the JEV antibodies induced by vaccination and natural infections; these tools have been used to evaluate the efficacy of the vaccine and to diagnose patients with the disease [6−8]. These include the plaque reduction neutralization test (PRNT), the hemagglutination inhibition (HI) test, an indirect immunofluorescence assay (IFA), and an enzyme-linked immunosorbent assay (ELISA). The PRNT has been considered to be the most reliable method for the evaluation of the efficacy of the vaccine or patient diagnosis, although its turnaround time is 5−7 days for most flaviviruses and quality control remains difficult [7,9]. The PRNT is advantageous in regions where two or more flaviviruses circulate together. Because strong cross-reactions occur between antibodies induced by flavivirus infections, it is hard to identify the exact pathogens with tools other than the PRNT [10]. However, more rapid and easier methods such as ELISA and IFA may be preferable when only one or two distantly related flavivirus species are transmitted. Serological tests other than PRNT may therefore be useful in Korea, where only JE has been reported, although tick-borne encephalitis virus (TBEV) has been isolated in ticks and rodents [11,12]. Although many papers have addressed the detection performance of each testing method for patient diagnosis [6,7,13−16], no standard or guideline has been established for choosing a methodology to detect vaccine-induced antibodies, although some investigators have demonstrated a high correlation of HI, ELISA, and IFA results with that of the PRNT [17,18].

In this study, we compared the performance of each test in detecting vaccine-induced antibodies to JEV in vaccinated children.

2. Materials and methods

2.1. Serum samples

A total of 29 serum samples was collected from healthy children who completed the primary JE vaccine schedule between June 2001 and August 2006. Fifteen children were vaccinated with an inactivated vaccine derived from mouse brain (3 doses) and 14 children were vaccinated with live attenuated SA14-14-2 vaccine (2 doses). Serum samples were collected from study participants between 3 months and 47 months after the last vaccine dose (between May 2006 and March 2007). The medical history of all participants showed no apparent history of infection with JEV or other flaviviruses during the study. This study was approved by the Institutional Review Board and written informed consent was obtained from the parents of the children (IUH IRB 06-390, Inha University).

2.2. PRNT

BHK-21 cells (ATCC, CCL-10) were initially inoculated at 4.5 × 10^5cells/well in six-well tissue culture plates and propagated for 48 hours at 37°C in a CO_2 incubator. Serum samples were inactivated for 30 minutes in a 56°C water-bath and serially diluted two-fold from 1:5 to 1:1280 in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS) and penicillin/streptomycin antibiotics (Gibco, Grand Island, NY, USA). A 100-μL aliquot of JEV (Nakayama strain) with 100 plaque-forming units (pfu) was mixed with equal volumes of diluted serum samples and incubated for 1 hour at 37°C. Each virus/serum mixture (total volume 200 μL) was inoculated onto the BHK-21 cell monolayer after draining the culture medium and was allowed to settle for 1 hour at 37°C in a CO_2 incubator. The mixture was removed from the cell monolayer and each well washed once with phosphate-buffered saline (PBS). Then 4 mL of pre-warmed overlay medium consisting of 0.9% Noble agar, penicillin/streptomycin,