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Original article

Varicella zoster virus antibody detection: A comparison of four commonly used techniques

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ABSTRACT

Background: Antibody tests for the varicella zoster virus (VZV) include neutralization, fluorescent antibody to membrane antigen (FAMA), immune adherence hemagglutination (IAHA), enzyme immunoassay (EIA), glycoprotein-based enzyme-linked immunosorbent assay (gpELISA), and complement fixation (CF) tests. Of these, FAMA is considered the most sensitive. However, in Japan, the EIA method is most frequently employed.

Objective: The VZV antibody detection rate of the FAMA, EIA, gpELISA, and IAHA methods was compared.

Methods: Four types of antibody tests were conducted with sera collected from 83 college students. The relationships between two antibody tests were examined using Pearson's correlation coefficients.

Results: All 83 subjects were observed to be VZV antibody-positive using the FAMA method. The Pearson correlation coefficients of gpELISA, EIA, and IAHA relative to FAMA were 0.808, 0.782, and 0.356, respectively. The positive agreement rate of IAHA relative to FAMA was 88.0% (73/83), whereas those of gpELISA and EIA were both 97.6% (81/83). Furthermore, EIA showed 100% positive agreement with gpELISA and a high correlation coefficient of 0.911, whereas these values for IAHA compared to gpELISA were much lower (90.1% and 0.530). The calculated Pearson correlation coefficient for comparison of the EIA and IAHA methods was 0.498, with a positive agreement rate of 90.1% (73/81).

Conclusions: The EIA method should be employed in Japan based on the similarity of the positivity between EIA and gpELISA, as it is more available and practical than gpELISA.

1. Introduction

Primary infection with varicella zoster virus (VZV) manifests clinically as varicella. The virus maintains a lifelong latency in the neurons of the sensory ganglia. Reactivation of latent VZV causes herpes zoster as a consequence of declining VZV-specific cell-mediated immunity [1].

The fluorescent antibody to membrane antigen (FAMA) method, developed by Williams et al. [2], is considered the "gold standard" test for varicella antibodies [3]. This method determines the presence or absence of viral immunity in both healthy and infected (through natural causes) individuals, but has not previously been employed to examine immunity after vaccination [4]. In fact, the Centers for Disease Control and Prevention (CDC) [5] do not recommend antibody testing in individuals vaccinated twice because they are considered fully immunized to VZV. Thus, antibody testing is infrequently conducted in the United States, where universal immunization is employed. In contrast, in Japan, varicella vaccination rates are low [6], and antibody testing is frequently

Abbreviations: CDC, centers for disease control and prevention; CF, complement fixation test; CMI, cell mediated-immunity; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; FAMA, fluorescent antibody to membrane antigen; gpELISA, glycoprotein-based enzyme-linked immunosorbent assay; IAHA, immune adherence hemagglutination; VZV, varicella zoster virus.

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employed to immunologically examine VZV immunity. For this purpose, an antibody enzyme immunoassay (EIA) is often utilized. However, it is currently unclear which, among EIA and the other methods used for protective antibody detection, is the most efficient, reliable, and cost-effective technique for evaluating VZV immunity.

In the present study, we sought to compare the various antibody testing methods employed in Japan, namely FAMA, glycoprotein-based enzyme-linked immunosorbent assay (gpELISA), immune adherence hemagglutination (IAHA), and EIA, to determine which test is the most useful. To our knowledge, this is the first time that all of these methods have been compared.

2. Materials and methods

2.1. Study population

The present study was conducted using sera collected from 83 students (age: 18–25, 50 men and 33 women) enrolled at the Hyogo College of Medicine in 2011. Informed consent was obtained from all study participants. The vaccination and clinical histories of these subjects were unknown. The study design was approved by the Ethics Committee of the Hyogo College of Medicine and followed the guidelines of the Declaration of Helsinki.

2.2. Antibody tests

The following VZV immunity assays were performed as described previously: IAHA [7–9], FAMA [2,10], and gpELISA [11,12]. IAHA antigen was purchased from Denka Seiken Co., Tokyo (CF antigen) and prepared by the destruction of virus-infected cells. For the gpELISA test, VZV glycoprotein was purified by lentil lectin-sepharose 4B. A standard curve generated from the absorbance data for a standard serum sample was used to determine the antibody titers for each sample analyzed with gpELISA. Furthermore, EIA values were determined using a commercially available ELISA kit (Denka Seiken Co., Tokyo) in which purified virion was used as an antigen; the analysis of these data was outsourced to a major commercial laboratory (SRL, Inc., Tokyo).

2.3. Statistics

To calculate the correlation between two sets of immunologic test results, a Pearson product-moment correlation analysis was used. All statistical analyses were performed using SPSS software (version 19; IBM Co., Armonk, NY, USA).

3. Results

In the present study, the following criteria were used to determine whether the subject was positive for VZV antibodies using the test specified: FAMA (1:4) [4], gpELISA (≥50) [12], EIA (≥2) [Denka Seiken: positive ≥4, intermediate 2–4], and IAHA (>2) [13]. Using these criteria, all 83 subjects were positive for VZV antibodies using the FAMA method. The other three methodologies investigated all indicated less than 100% immunity in the population studied, with only 81 of the subjects (97.6%) found to be positive for VZV antibodies using both the gpELISA and EIA methods, whereas only 73 (88.0%) were positive using the IAHA method (Fig. 1). Furthermore, the Pearson correlation coefficients calculated relative to the FAMA method for the gpELISA, EIA, and IAHA methods were 0.808, 0.782, and 0.356, respectively (Fig. 1).

Relative to the gpELISA methods, the Pearson correlation coefficients calculated for the EIA and IAHA methods were 0.911 (positive agreement rate of 100, 81/81) and 0.530 (positive agreement rate of 90.1%, 73/81), respectively. Lastly, the calculated Pearson correlation coefficient comparing the EIA and IAHA methods was 0.498, with a positive agreement rate of 90.1% (73/81) (Fig. 2). These data indicate that the gpELISA and EIA methods provided the most similar results, having the highest correlation coefficient and positive agreement rate.

4. Discussion

The varicella vaccine was developed in 1974 [14]. In Japan, it was licensed in 1986, and a single vaccination was approved for healthy children aged 1 year or older in 1987, whereas routine two-dose immunization was not started until 2014. In the United States, the vaccine was approved in 1995, and routine vaccination with a single dose started in infants aged 12–18 months in 1996. This was followed by the approval of two-dose vaccination in 2007, and vaccination in people aged 60 years or older in 2008 [15]. The U.S. Food and Drug Administration licensed the zoster vaccination for use, and it can be administered to persons aged 50 years or older, but ACIP recommends that vaccination begin at the age of 60 years [16]. Varicella vaccination has been epidemiologically demonstrated to reduce the number of varicella patients [17] worldwide, particularly in the United States, where the number of varicella patients greatly decreased as the vaccination rate increased [4]. However, as this decrease resulted in a decrease in the possibility of contracting varicella, fewer patients were documented to return for their booster immunization against VZV [18]. This decrease in booster immunization subsequently resulted in an increase in the number of zoster patients. It is likely that cell-mediated immunity is involved in the reactivation of VZV [1], making the booster immunization for VZV essential.

The CDC does not recommend antibody testing at all after vaccination, using the following criteria as evidence of immunity in health care personnel: 1) written documentation of vaccination with 2 doses of varicella vaccine, 2) laboratory evidence of immunity or laboratory confirmation of disease, and/or 3) diagnosis or verification of history of varicella or herpes zoster by a health-care provider. The CDC has also indicated that although commercial assays can be used to assess disease-induced immunity, they often lack sensitivity to detect vaccine-induced immunity [4]. Moreover, Behrman et al. [19] also observed a difference in antibody avidity between subjects that were vaccinated and those that had been naturally infected, but these differences were not detected by commercially available tests. Therefore, we believe that it is essential to determine what VZV protection antibody detection methods are optimal for clinical use. Thus, in the present study, we compared the positive detection rate of four VZV antibody detection methods: FAMA, gpELISA, EIA, and IAHA.

The antibody titer threshold used in this study was based on that of Gershon et al. [2], who demonstrated the presence of protection antibodies in healthy adults with a history of varicella at an antibody titer ≥1:4 using the FAMA method. However, varicella infection was not always prevented after vaccination in this previous study, even when an antibody titer of ≥1:4 was used, indicating that although it is the most sensitive method, it is not the optimal tool to measure protective antibodies after vaccination. Furthermore, the FAMA method is generally considered more difficult than the other methods for both economic and technical reasons.

In the present study, all 83 samples tested positive by the FAMA method, possibly because the subjects were young adults and therefore were likely to have been exposed to VZV in the past. However, there may have been false-positive cases because no negative case was included as a control. Therefore, we demonstrated that two ≥ 1-year-old children tested negative by the FAMA