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# Seroprevalence rate after one dose of varicella vaccine in infants

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**Summary** *Background:* Live, attenuated varicella vaccine has been used since 1988 in Korea. However, varicella is still prevalent among both vaccinated and unvaccinated individuals, despite a relatively high level of immunization rate up to 80%. A recent report has demonstrated ~ 20% of primary vaccine failure rate after one dose of varicella vaccine using the fluorescent antibody to membrane antigen (FAMA) assay.
*Methods:* The seroprevalence rate was determined using the FAMA and ELISA assays in 67 Korean infants following one dose of varicella vaccine. Positive fluorescence at a serum dilution of 1:4 or greater was considered as seropositive.
*Results:* The median age at vaccination was 12 months and the post-immunization sera were obtained on average 6.3 months (range 6 weeks–12 months) after vaccination. Among the 67 vaccinated infants, 56 (83.6%) were seropositive by FAMA assay while 30 (44.8%) were seropositive by ELISA. The geometric mean titers (GMTs) of the seropositive vaccinated infants ($n = 56$) were significantly lower than the GMTs of 9 individuals with a history of varicella (1:17.0 *vs.* 1:74.7, $P = 0.001$). Although there were no significant differences in seropositive rates according to intervals, there was a decreasing trend in the GMTs over time among the 56 seropositive recipients ($r^2 = 0.154$, $P < 0.001$).
*Conclusions:* These data can be useful for optimizing the immunization strategy against varicella and should be confirmed by a prospective study including a large number of immunized infants.


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## Introduction

Varicella is a highly contagious disease caused by the varicella-zoster virus (VZV), and annual epidemics among susceptible children occurred during winter and spring, in the prevaccine era.[1] The varicella vaccine has become widely used for the prevention of varicella since Oka strain of live, attenuated varicella vaccine was developed by Dr. Takahasi in 1974.[2] However, immunization policies against varicella are quite variable worldwide. The World Health Organization recommends that routine childhood immunization against varicella may be considered in countries where varicella is a relatively important public health and socioeconomic problem, where the vaccine is affordable, and where high immunization rate (85–90%) can be achieved for the long-term.[3] Currently, 10 countries have included varicella vaccine as part of their national immunization program (NIP), while many of European countries and Japan recommend varicella vaccination only to certain high-risk individuals.[4,5] In contrast, 2 dose of routine immunization program has been employed since 2006 in the United States by changing initial recommendation to use one dose of vaccine.[1,6]

In Korea, the immunization strategy against varicella has evolved since 1988. The varicella vaccine was initially introduced for optional use among healthy children aged $\geq$12 months and high-risk groups. The immunization rate of varicella vaccine was relatively high around 70% among 12–15 month old infants even before the introduction into NIP, at year of 2005.[4] The current immunization rate is estimated to be greater than 80%, as of 2007.[7] The reported number of varicella cases ranged from 180,000 to 250,000 per year which accounts approximately 40–50% of annual birth cohort between 2003 and 2008 in the absence of catch-up vaccination.[8] Among the several methods used to evaluate VZV immunity after immunization, the fluorescent antibody to membrane antigen (FAMA) assay and the glycoprotein enzyme-linked immunosorbent assay (gpELISA) are used to measure seroprevalence, which is thought to be a marker of immunity.[9]

This study was performed to determine seroprevalence rate using the FAMA assay in previously healthy Korean infants following one dose of varicella vaccine.

## Materials and methods

### Subjects

A total of 86 subjects (83 children and 3 adults) who visited the Seoul National University Bundang Hospital located in Gyeonggi province, Korea from November 2007 to December 2008 were included in the study. Study subjects were categorized into three groups, as follows: 6 children and 3 adults with a documented history of varicella (positive control group), 10 infants 11–13 months of age with no history of natural VZV infection and no history of immunization against varicella (negative control group), and 67 infants 13–24 months of age who received one dose of varicella vaccine and had no history of varicella (study group). Children with underlying immunodeficiency disorders or a history of systemic steroid treatment were excluded from the study.

The study was conducted under protocols approved by the Institutional Review Board of the Seoul National University Bundang Hospital and samples were collected with written informed consent in the Department of Pediatrics. Serum samples were obtained from all infants in the study group at a minimum of 6 weeks since immunization. Immunization status was confirmed by immunization records (90%) or interviews with parents (10%). History of varicella disease was obtained from the interview with parents. Vaccine recipients were immunized with one of four varicella vaccines; the Oka strain of Varilix™ (2000 pfu/dose, Glaxo Smith Klein, Brussels, Belgium), Varivax™ (1350 pfu/dose, Merck, White station, USA), Vari-L™ (1400 pfu/dose, Changchun Institute of Biological Products, Chengdu, China), and the MAV strain of Suduvax™ (1400 pfu/dose, Green Cross Corporation, Yongin, Korea). Overall seroprevalence rate was evaluated regardless of the vaccine components because limited information on vaccine types was available in the immunization records.

### Assays to detect anti-VZV antibodies

#### The FAMA assay

The FAMA assay was performed to determine seroprevalence rate following varicella vaccination using previously described methods with minor modification.[10,11] Briefly, confluent monolayers of MRC-5 cells (American Type Culture Collection, Manassas, USA) were infected with Oka VZV strains (American Type Culture Collection). The virus infected cells were harvested when a cytopathic effect was >75% of the monolayers. Heat-inactivated serum samples were serially diluted in 2-fold aliquots (from 1:2 to 1:128 of each sample) in a 96-well U bottom plates (Nunc, inc., Roskilde, Denmark), to which VZV-infected MRC-5 cells were added and incubated at room temperature for 30 min. Following incubation, a fluorescein-5-isothiocyanate (FITC)-conjugated goat IgG fraction to human IgG (Cappel, Inc., Ohio, USA) was added to the washed cell pellets and incubated for 1 h. Membrane fluorescence was read independently by two different people under the fluorescent microscope (Microscope Research + Digital Camera, Axioskop 40 + Axiocam Mrc5, Carl Zeiss, Inc., Goettingen, Germany).

A bright fluorescent ring around the surface of cells was graded on a scale of 1 + to 4 + on the basis of width and intensity. Cells with specific but vague fluorescent staining were defined as grade 1; grade 2, specific and weak fluorescence; grade 3, bright and specific fluorescence; grade 4, thick brilliant fluorescent membrane. Damaged cells with cytoplasmic fluorescence and no distinct membraneous fluorescence were regarded negative. Grade 2 + or greater fluorescence is considered seropositive. Diluted anti-VZV antibody ($3 \times 10^{-3}$ IU/mL, National Institute for Biological Standards and Control, Hertfordshire, UK) was used as a grade 2 positive standard and phosphate buffered solution was used as a negative standard for the FAMA assay. In addition, the FAMA assay was regarded as reliable when the FAMA titer of a seropositive adult serum, for which the FAMA antibody titer is known, had a variation in the $\leq$2-fold range. Seropositivity of the FAMA assay was defined by a positive fluorescence at a serum dilution of