Journal of Microbiology, Immunology and Infection (2017) 50, 321–326

Available online at www.sciencedirect.com

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ORIGINAL ARTICLE

Seroepidemiology of varicella among elementary school children in northern Taiwan

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Received 11 March 2015; received in revised form 7 July 2015; accepted 19 July 2015
Available online 31 July 2015

KEYWORDS
school children;
seroepidemiology;
Taiwan;
varicella

Abstract *Background/Purpose:* In Taiwan, varicella vaccine was included in the expanded program of immunization since 2004. A seroepidemiologic study in the postvaccine era is helpful to evaluate the efficiency of current varicella vaccination strategies.
Methods: We used a multistage stratified systematic sampling design to classify 29 administrative districts of New Taipei City into five strata. In 2013, a total of 936 students from 14 primary schools were recruited and had blood drawn for serology tests for varicella-zoster virus-immunoglobulin-G via indirect chemiluminescence immunoassays. A history of clinical varicella and information on varicella vaccination status were obtained.
Results: Overall, the seroprevalence was 64.1%. For the five strata, the seropositive rate ranged from 54.2% (Stratum 5) to 71.7% (Stratum 2) with no significant difference. For each participating school, the seropositive rate ranged from 44.4% to 72.9% with a statistically significant difference ($p < 0.005$). For school children in each grade, seropositive rate increased significantly from 53.2% for Grade 1 to 71.8% for Grade 3 ($p = 0.005$) and increased steadily from 61.2% for Grade 4 to 71.2% for Grade 6 ($p = 0.17$). A positive correlation was observed between the seropositive rate and geometric mean titers ($p = 0.035$). Geometric mean titers and the rate of a history of clinical varicella were positively correlated with increasing class grades.
Conclusion: Nine years after the introduction of the varicella vaccine into the expanded program of immunization in Taiwan, around two-thirds of elementary schoolchildren were

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http://dx.doi.org/10.1016/j.jmii.2015.07.007

seropositive for varicella-zoster virus. Further surveillance studies on clinical varicella cases are worthwhile to determine whether a second dose of varicella vaccine is needed in Taiwan.

Introduction

Varicella, also known as chicken pox, is a highly contagious but vaccine-preventable disease caused by primary infection with varicella-zoster virus (VZV), which belongs to the *Herpesviridae* family.[1–3] The incidence of varicella varies among regions with different climates, population densities and degree of economic-social development.[4,5] The clinical course after primary infection in children is relatively mild and self-limited with fever and a characteristic skin rash that forms small and itchy blisters.[6] However, varicella may occasionally cause severe complications including secondary skin and soft tissue infection, pneumonia, central nervous system disease, and even death in immunocompetent children.[7]

In Taiwan, the seroprevelance of varicella was ~83% in children aged 11–15 years in the prevaccine era.[8] A live attenuated vaccine, that was developed from the Oka strain of the varicella-zoster virus, was first licensed for administration to children in Taiwan in 1997. Initially, the varicella vaccine was used in the private sector and people received the vaccine at their own expense. Since 1998, public health authorities of some local governments in Taiwan, including Taipei City, Taichung City, Taichung County, etc., started to provide free varicella vaccine to children younger than 2 years. Since 2004, varicella vaccine was included in the expanded program of immunization in Taiwan and was freely provided as a routine childhood vaccination nationwide.[9] The incidence of varicella significantly declined from 66/1000 children aged 4–5 years between 2000 and 2003, to 23/1000 children aged 6 years in 2008.[10] However, there was still a 2.1% varicella breakthrough infection among those vaccinees.[11]

Seroepidemiologic data in the postvaccine era is helpful to understand the epidemiology of varicella and evaluate the efficiency of current varicella vaccination strategies. Therefore, we conducted this survey, which was supported by the Centers for Diseases Control of Taiwan (CDC-Taiwan), to evaluate immune status against VZV among school children aged 7–12 years in northern Taiwan.

Methods

We conducted a cross-sectional survey from September 2012 to June 2013 to investigate VZV-specific immunoglobulin (Ig)G antibody in the population of elementary school children aged 7–12 years in New Taipei City.

Study populations and the selection of participants

New Taipei City is the second largest directly controlled municipalities in northern Taiwan, which comprises 29 administrative districts and 3,921,580 people resided in. In total, 225,234 students resided in this city, which accounted for 16.5% of all primary school children in Taiwan in 2012. A multistage stratified systematic sampling design was employed to obtain samples. The 29 administrative districts of New Taipei City were arbitrarily classified into five strata based on 14 variables, which included six medical facilities' variables (nursing staff/10,000 people, medical personnel/10,000 people, all staff in health centers/10,000 people, number of colleges or universities/10,000 people, proportion of agriculture population, and proportion of population with college degree or above), four socio-educational variables (number of physicians, nursing staff, low-income households, and near-poor households per 10,000 people), and four demographic variables [population density (persons/km^2), proportion of population older than 65 years, younger than 15 years, and younger than 6 years]. Elementary schools in each stratum were selected with selection probability proportional to their size. From each stratum, elementary schools were selected as the primary sampling unit, and then classes were selected as the second sampling unit. One class was drawn from each grade in a sampled school, that is, six classes in total were drawn from each sampled school. In each selected school, the numbers of students in each class were also selected with probability proportional to their size. Finally, students were randomly selected in each class. Accordingly, 558 students were selected from six schools in Stratum 1, 130 students from two schools in Stratum 2, 66 students from two schools in Stratum 3, 140 students from two schools in Stratum 4, and seven students from two schools in Stratum 5. In total, at least 901 students were obtained. Subsequently, we recruited a total of 936 students from the selected schools.

All participants were healthy without acute illness. Past medical history including clinical varicella was obtained using a questionnaire from each participant. The information on varicella vaccination status of students from Grade 1 to Grade 4 was obtained from the CDC-Taiwan since the recoding of administration of vaccines included in expanded program of immunization should be mandatorily transmitted to the CDC-Taiwan.

Determination of varicella-specific antibodies

Each selected students had 5–10 mL blood drawn and the sera was stored at −20°C before being measured. We used indirect chemiluminescence immunoassays (Liaison, DiaSorin, Italy), which is a quantitative test to determine the VZV-specific IgG antibody. Those with an antibody titer of > 160 mIU/mL were classified as positive, a titer between 140 mIU/mL and 160 mIU/mL was grouped as equivocal,

and a titer of < 140 mIU/mL was considered negative. All procedures were conducted by following the original company's recommendation. The test was repeated for those samples with the indeterminate results.

Ethics statement

The study proposal was reviewed and approved by the Institutional Review Board of Chang Gung Memorial Hospital, Taoyuan, Taiwan in 2012. A written informed consent was obtained from all participants, as well as their guardians.

Statistical analysis

During the study period, the students in Grade 1 were usually born between September 2005 and August 2006, and the students in Grade 6 were usually born between September 2000 and August 2001. Accordingly, all the students from Grade 1 to Grade 3 and some students in Grade 4 were supposed to have a provision of free varicella vaccine. Seroprevalence data for genders and age groups were analyzed as percentages with 95% confidence intervals. We used the Chi-square test to compare the seropositive rate, as well as age-specific between different groups. VZV-specific antibody titers were presented as geometric mean titers (GMTs). The statistic deviances between GMT levels in different grades were compared using the independent *t*-test. Data analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). A p value < 0.05 was considered statistically significant.

Results

A total of 936 students from 14 elementary schools were recruited for the serology test of VZV-IgG, of whom 452 (48.2%) were boys. Overall, the result of the VZV-IgG test was positive for 600 children and equivocal for 13 children. The seroprevalance was 64.1%. There was no significant difference in the seroprevalence by gender (63.7% in males vs. 64.5% in females; $p = 0.06$). Among the five strata, the seropositive rate ranged from 54.2% (Stratum 5) to 71.7% (Stratum 2) and the difference was not statistically significant ($p = 0.804$). For each participating school, the seropositive rate ranged from 44.4% for Gan-Yuan school to 79.2% for Ju-Guang school and a statistically significant difference was observed among the 14 schools ($p < 0.005$; Table 1).

The varicella seroprevalence and the level of GMTs of the children stratified by school grades are shown in Figure 1. The seropositive rate increased significantly from 53.2% for Grade 1, 62.2% for Grade 2, to 71.8% for Grade 3 ($p = 0.005$), and increased steadily from 61.2% for Grade 4, 64.1% for Grade 5, to 71.2% for Grade 6 ($p = 0.17$). The seropositivity rate for students from Grade 1 to Grade 4 (389/624, 62.34%) was lower than that for students in Grades 5 and 6 (211/312, 67.63%), but the difference of rates did not reach statistically significant ($p = 0.45$). However, the mean level of GMTs also increased significantly from 345.8 mIU/mL for Grade 1 to 758.4 mIU/mL for Grade 4 ($p < 0.005$) and steadily from 737.3 mIU/mL for Grade 5 to 848.9 mIU/mL for Grade 6 ($p = 0.89$) respectively. A positive correlation was observed between the seropositive rate and the mean level of GMTs ($p = 0.035$).

Table 1 Seroprevalence among 936 primary school children in five strata[a]

School (administration)**	*N*	Sex (M/F)	GMT	Std.	Positive, *n* (%)
Stratum 1*	576	0.98	616.57	742.37	374 (64.93)
Zhong-Shan (Ban-Qiao)	96	0.81	344.55	480.61	49 (51.04)
San-Zhong (San-Zhong)	96	0.92	593.23	789.25	59 (61.46)
Zhong-Gang (Xin-Zhuang)	96	0.85	639.40	705.92	64 (66.67)
Lu-Zhou (Lu-Zhou)	96	1.09	664.50	793.87	66 (68.75)
Ji-Mei (San-Zhong)	96	1.23	667.47	725.30	60 (62.50)
Ju-Guang (Ban-Qiao)	96	0.96	790.28	848.91	76 (79.17)
Stratum 2*	120	0.85	821.17	1064.64	86 (71.67)
Cheng-Fu (San-Xia)	60	0.62	636.32	827.62	41 (68.33)
Xin-Xing (Dan-Shui)	60	1.07	1006.03	1237.62	45 (75.00)
Stratum 3*	72	0.64	531.76	711.26	43 (59.72)
Jin-Mei (Jin-Shan)	36	0.71	436.74	563.85	21 (58.33)
Jin-Long (Xi-Zhi)	36	0.57	626.79	830.50	22 (61.11)
Stratum 4*	144	0.95	603.88	817.68	84 (58.33)
Gan-Yuan (Shu-Lin)	72	1.00	478.6	774.34	32 (44.44)
Wu-Gu (Wu-Gu)	72	0.89	729.16	845.67	52 (72.22)
Stratum 5*	24	4.00	539.31	844.36	13 (54.17)
Shi-Ding (Shi-Ding)	12	5.00	393.33	850.48	6 (50.00)
Shuang-Xi (Shuang-Xi)	12	1.00	685.29	849.04	7 (58.33)
Total	936	0.93	632.35	776.89	600 (64.1)

[a] The five strata were classified according to 14 variables (see text).
* $p = 0.807$ for seropositivity among the five strata.
** $p < 0.005$ for seropositivity among the 14 schools.
GMT = geometric mean titers; Std. = standard deviation.

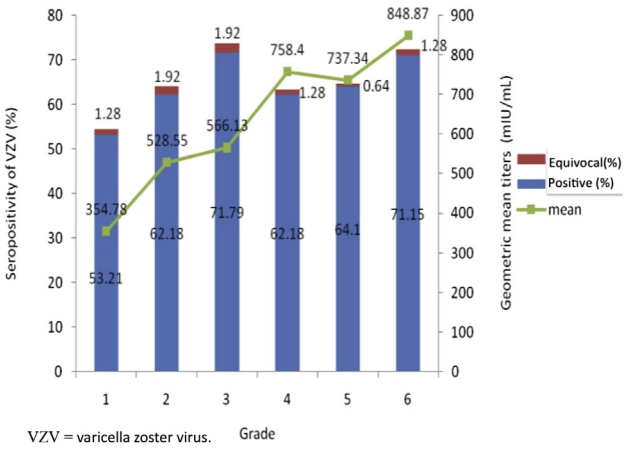

Figure 1. Grade-specific seropositivity and geometric mean titers of varicella zoster virus among 936 participants. The seropositive rate was positively correlated to mean level of geometric mean titers ($p = 0.035$). VZV = varicella zoster virus.

Of the 624 participants from Grade 1 to Grade 4555 (88.9%) had received the varicella vaccine, among whom 353 (63.6%) were seropositive and 26 (4.68%) had a history of clinical varicella. On the contrary, among 312 participants in Grades 5—6, 87 (94%) of 93 students who had a history of clinical varicella were seropositive—a rate significantly higher than those without a history of clinical varicella (124 among 218 students, 56.6%, $p < 0.001$; Table 2).

We further analyzed the seroprevalence of the students from Grade 1 to Grade 4 stratified by the history of clinical varicella and varicella vaccination. The vaccination rate of students in Grade 4 was significantly lower than the others (64.7% vs. 97%, $p < 0.001$). The percentage of clinical varicella among the study students was significantly decreased from Grade 4 (15.4%) to Grade 1 (3.2%, $p = 0.002$). Students with a history of clinical varicella had a higher seropositive rate than those without a history of clinical varicella (84.2% vs. 59.2%, $p < 0.001$). For further analysis, this difference was not significant among students from Grade 1 to Grade 4 (67.9% vs. 60.7%, $p = 0.27$) but among students from Grade 5 to Grade 6 (93.5% vs. 56.6%, $p < 0.001$). Among students without vaccination, the seropositive rate was higher for students with a history of clinical varicella than those without clinical varicella ($p < 0.001$). By contrast, among students without a history of clinical varicella, the vaccinees had a higher seropositive rate than those without vaccination ($p < 0.001$; Table 3).

Table 2 Comparison of seroprevalence among children who report a history of clinical varicella and varicella vaccination[a]

Category	*N*	GMT	Std.	Seropositive *n* (%)
Grades 1—4	624	551.96	763.44	389 (62.34)
With vaccination	555	536.63	748.79	353 (63.6)
With a history of clinical varicella	53	969.32	981.48	36 (67.9)
Without a history of clinical varicella	571	516.54	732.55	344 (60.2)
Grades 5—6[b]	312	793.11	859.91	211 (67.63)
With a history of clinical varicella	93	1199.29	783.56	87 (93.55)
Without a history of clinical varicella	219	620.62	833.98	124 (56.6)

[a] The seropositive rate was significantly higher in students with a history of clinical varicella than those without a history of clinical varicella ($p < 0.001$).

[b] For students in Grades 5 and 6, the seropositive rate was significantly higher in those with a history of clinical varicella than those without a history of clinical varicella ($p < 0.001$).

GMT = geometric mean titers; Std. = standard deviation.

Discussion

We conducted the first varicella seroepidemiology study in the postvaccine era in northern Taiwan. The overall seroprevalence of VZV IgG among elementary school students was 64.1%, a rate lower than expected in the postvaccine era. There was a significant difference in seropositive rates among 14 participating schools, with a discrepant rate up to 30%. This scenario might be related to the different vaccination rates of the different administrative districts. Tsen et al[12] conducted a study in 2003, when only some local government provided free varicella vaccine to children younger than 2 years, to evaluate the age-specific VZV seropositive rate in children aged 0—12 years in Taiwan. The results showed that seroprevalence was significantly different between the areas with and without provision of free vaccine in children aged 0 years (64.4% in free vaccine area and 38.0% in private vaccine area, $p < 0.005$) and in children aged 0—5 years ($p < 0.05$). The present study also indicated that among students without a history of clinical varicella, the seropositive rate was significantly higher for students with vaccinations than those without vaccination.

In Taiwan, varicella vaccine was included in the expanded program of immunization since 2004. Thus, all the students from Grade 1 to Grade 3 and some students in Grade 4 in this study were supposed to have a provision of free varicella vaccine, then had a higher vaccination rate (555/624, 89%), and were expected to have a higher seropositive rate than those in Grades 5 and 6. However, the results were beyond expectation. The seropositive rate was not significantly different between the students from Grade 1 to Grade 4 and those in Grades 5 and 6. Even the seropositive rate of the students significantly increased from Grade 1 to Grade 3, then slightly declined from Grade 3 to Grade 4, not statistically insignificant ($p = 0.07$), and increased again throughout Grade 6. Also, the levels of GMTs and the rate of a history of clinical varicella were positively correlated with increasing class grades. Apparently, in addition to natural infection, a certain portion of students in Grades 5 and 6 might have received self-paid varicella vaccine since the seropositivity rate (67.6%) for these students was much higher than the rate (30%) of a history of clinical varicella. Altogether, these findings suggest that with mass vaccination of varicella vaccine, the varicella cases of natural VZV infection decreased year by year; then the students of younger age had not only fewer

Table 3 Seroprevalence among elementary school students from Grade 1 to Grade 4 stratified by history of clinical varicella and varicella vaccination[a]

Varicella history	Grade	Participant No.	Seropositive	Vaccinated	Seropositive	Nonvaccinated	Seropositive
Yes	1	5	2 (40.0)	3 (60.0)	1 (33.3)	2 (40.0)	1 (50.0)
	2	6	6 (100.0)	4 (66.7)	4 (100)	2 (33.3)	2 (100)
	3	13	10 (76.9)	10 (76.9)	7 (70.0)	3 (30.0)	3 (100)
	4	24	18 (75.0)	9 (37.5)[b]	5 (55.6)	15 (62.5)	13 (86.7)
	Total	53	36 (67.9)[a]	26 (47.1)	18 (72.0)	28 (52.8)	25 (89.3)[c]
No	1	149	81 (54.4)	149 (100)	81 (54.4)	0 (0)	0
	2	144	87 (60.4)	139 (96.5)	85 (61.2)	5 (3.4)	2 (40.0)
	3	141	100 (70.9)	140 (99.3)	100 (71.4)	1 (0.7)	0
	4	131	79 (60.3)	91 (69.5)[c]	64 (70.3)	40 (30.5)	15 (37.5%)
	Total	560	340 (60.7)	519 (92.9)	329 (63.3)	46 (8.2)	11 (23.9%)
Unclear	1	2	1 (50.0)	2 (100)	1 (50.0)	0 (0)	0
	2	6	3 (50.0)	5 (83.3)	3 (60.0)	1 (16.7)	0
	3	2	0	2 (100)	2 (100)	0	0
	4	1	0	1 (100)	0 (100)	0	0
	Total	11	4 (36.4)	10 (90.0)	6 (60.0)	1 (0.9)	0
No & unclear	Total	571	344 (60.2)	529 (92.8)	335 (63.2)	47 (0.8)	15 (31.9)[d]
Overall	Total	624	380 (60.9)	555 (88.9)	353 (63.6)	69 (11.1)	36 (52.2)

[a] The incidence of clinical varicella significantly decreased from Grade 4 to Grade 1 ($p = 0.002$).
[b] Vaccination rate of students in Grade 4 were significantly lower than the others ($p < 0.001$).
[c] Among students without vaccination, students with a history of clinical varicella had a higher seropositive rate than those without a history of clinical varicella ($p < 0.001$).
[d] Among students without a history of clinical varicella, the vaccinees had a higher seropositive rate than those without vaccination ($p < 0.001$).
Data are presented as *n* (%).

chances of exposure to VZV but also fewer chances for natural immunity boosting.

In the United States where universal varicella vaccination was implemented since 1995, Chaves et al[13] performed a 10-year active surveillance from 1995 to 2004 and included 350,000 participants. The results showed that the annual incidence of breakthrough varicella infection increased significantly with time since vaccination. The incidence increased from 1.6 cases per 1000 person-years within 1 year after vaccination to 9.0 per 1000 person-years at 5 years. Compared with participants who had been vaccinated $<$ 5 years, those who had been vaccinated $\geq$ 5 years previously, were 2.6 times as likely to have moderate-to-severe disease ($p = 0.01$). Thereafter, a second dose of varicella vaccine is recommended for children aged 4–6 years since 2007. In this study, 26 of 555 (4.68%) vaccinated students from Grade 1 to Grade 4 had a history of clinical varicella, which might imply waning of vaccine immunity. Further surveillance studies on clinical varicella cases should be conducted in Taiwan and a second dose of varicella vaccine should be taken into consideration if clinical cases due to breakthrough infection increased significantly.

There were some limitations in this study. Firstly, the study participants may not accurately represent all school children nationwide even though we used a multilevels design by using 14 variables to minimalize selecting bias. Secondly, the serologic result may be influenced by the laboratory method used for analysis and confounded by the recalling bias of the history of VZV infection, including subclinical infection. Thirdly, the vaccination status cannot be completely clarified in some participants. Initially, we also planned to collect the vaccination status using the questionnaires; however, when comparing the data collected from the questionnaires with those from CDC-Taiwan, the discordant rate was 20% in average, up to 30% for those from students in Grades 5 and 6. Thus, we just chose the data from CDC-Taiwan for students from Grade 1 to Grade 4 for final analysis. Fourthly, the social-educational status of participants might influence the vaccination rate especially among those in Grades 5 and 6. However, we did not obtain this data from our initial questionnaire.

In conclusion, the current study provided a seroepidemiologic profile for varicella within elementary school students in northern Taiwan. The relatively low seroprevalence, around 64.1%, and the incidence of 5% of possible VZV breakthrough infection might suggest the waning immunity. Further studies for the continuity of vaccine antibodies and surveillance of breakthrough infections should be conducted to provide guidance for national public health authorities in determining a vaccination strategy on whether a second dose of varicella vaccine is indicated in Taiwan.

Conflicts of interest

All authors declare that they have no conflicts of interest associated with the materials discussed in the article.

Acknowledgments

This study was supported in part by a grant from Centers for Disease Control, Taiwan (DOH101-DC-1016). We would like

to thank Ms. Kun-Ju Tsai, a member of Public Health Department of New Taipei City Government, for her helpful support.

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