

# Evaluation of immunogenicity, safety and breakthrough following administration of live attenuated varicella vaccine in two doses three months apart regimen in Indian children

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## Abstract

**Background:** In India, where varicella outbreaks are reported at a younger age, a two-dose vaccine schedule administered at an early age could be highly efficacious in preventing varicella infection. The aim of this study was to evaluate the immunogenicity and safety of live attenuated varicella vaccine (VR 795 Oka strain) in a two-dose, 3 months apart regimen.

**Methodology:** Healthy children ( $\geq 12$  months and  $\leq 12$  years; mean age: 4.4 years) of either sex were included. Geometric mean titers (GMT) were measured at baseline and 28 days post first- and second-dose, and seroprotection rates were measured 28 days post first and second dose. The incidence of breakthrough (BT) infections post vaccination was determined from 42 days post first and second dose of vaccine up to 12 months. Adverse events (AEs) were monitored and recorded throughout the study period.

**Results:** Of 305 subjects enrolled, 217 were seronegative. The seroconversion rate (a change from a seronegative to a seropositive condition) was 93.3% post first-dose and 100% post two-doses. High levels (9 times) of GMT were reported since post first-dose to post second-dose in children aged 12–18 months, 18–60 months (99.43%); and in and above 60 months (99.02%). The extent of rise of anti-VZV IgG antibody titer post 28 days of first-dose at two-fold, three-fold and four-fold rise was 93.39%, 90.56% and 80.66%, respectively and 100% 4-fold rise post second-dose. A single case, a day after the first-dose of vaccination of mild BT infection, was observed after close contact with a severe case. AEs were mild and none of the serious AEs were related to the study drug.

**Conclusion:** The two-dose schedule of varicella vaccine was safe and immunogenic when given 3 months apart. However, further comparative studies and follow up for both dosing schedules are needed to validate the advantage of early dosing.

**Keywords:** antibody titer, breakthrough, seroprotection, vaccine, varicella

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## Introduction

Varicella-zoster virus (VZV) is a highly contagious herpes virus that causes varicella (chickenpox), usually during childhood, and herpes zoster (shingles), usually much later in adult life. As per

the World Health Organization (WHO) position paper 2014, approximately 4.2 million severe complications leading to hospitalization and 4200 related deaths occur globally each year due to varicella.<sup>1</sup>

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Varicella is vaccine-preventable, and varicella vaccines have been highly effective at preventing chickenpox caused by VZV. The US Food and Drug Administration introduced varicella vaccine in their national immunization schedule in 1995 as a single dose for susceptible children aged 12 months to 13 years and two dose for susceptible older persons.<sup>2</sup> Before the introduction of VZV vaccines, approximately 30.9 per 100,000 cases with varicella were hospitalized in the United States that drastically decreased by 53% to 14.5/100,000 population post-licensure of varicella vaccine.<sup>3</sup> In Taiwan, the implementation of the universal varicella vaccine program resulted in the reduction of crude varicella incidence from 5.68 in 2003 (pre-vaccination era) to 2.23 per 1000 persons in 2007.<sup>4</sup>

Though, the universal vaccination program led to a reduction in morbidity and mortality resulting from varicella infection in the US, active surveillance data from sites and states with well-implemented vaccination programs indicated that the number of reported varicella cases remained constant or declined minimally.<sup>5</sup> Further, outbreaks in schools with high vaccine coverage were reported.<sup>6</sup> It was noted that the outbreaks mostly occurred in elementary schools and younger students. The highest incidence of VZV disease was recorded between the age of 12 months to 12 years as majority of children are seronegative at an early age of <12 months, hence susceptible to VZV infection.<sup>7</sup>

The data prompted the Advisory Committee on Immunization Practices (ACIP) to rethink that the single dose vaccination program could not prevent varicella outbreaks completely; thus, recommending two-dose schedules for children >12 months, adolescents, and adults not showing evidence of immunity was the need of the hour.

For children aged 12 months to 12 years, a dosing schedule with a minimum interval of 3 months between the two doses is recommended.<sup>8</sup>

Further, a global meta-analysis of 42 studies estimated that the single dose of varicella vaccine was moderately effective in preventing all varicella (81%) and highly effective at preventing combined moderate and severe varicella (98%), while two doses of varicella vaccine were highly effective at preventing all varicella.<sup>9</sup> A two-dose

schedule is also widely applied and included in national routine immunization programs for children  $\geq$  12 months of age in countries like the USA, Japan, China, and Canada due to various documented cases of varicella “breakthrough” (BT) infection that may occur in some persons vaccinated with varicella vaccine after exposure to wild-type (WT)-VZV.<sup>10–13</sup>

In India, varicella vaccine for single-dose schedule was studied by a few researchers and proven to be safe and effective in preventing disease, but a second dose is yet not introduced as mandatory in the national immunization schedule.<sup>14–16</sup> As per WHO, countries deciding to introduce routine childhood varicella immunization should administer vaccination at an early age of 12–18 months, and two doses are recommended for decreasing mortality and severe morbidity, and to further reduce the number of cases and outbreaks.<sup>17</sup>

As per the Indian Academy of Paediatrics (IAP) immunization schedule (2018), the minimum age of administering varicella vaccine is recommended as 12 months with the minimum dosing interval of 3 months for children aged 12 months through 12 years.<sup>18</sup> It is imperative to note that varicella vaccination in early childhood could lower the incidence of institutional outbreaks as children of this age are more prone to varicella infection, and transmission of infection is facilitated through close contact with infected children.<sup>19</sup>

Further, administering a second dose of vaccine at an early age could also ensure compliance as most of the vaccines are administered in early childhood.

Kuter *et al.* studied the rate of varicella and persistence of varicella antibody after a one-dose *versus* a two-dose regimen of varicella virus vaccine and followed-up approximately 2000 recipient children for 9–10 years. The two dose regimen given 3 months apart was found to be significantly more effective than a single injection.<sup>20</sup> The current study is designed on similar lines to evaluate the immunogenicity and safety of a two-dose, 3 months apart regimen in the Indian population using live attenuated varicella vaccine (VR795Oka strain). A single dose regimen of live attenuated varicella vaccine (VR 795 Oka strain) has been already studied in the Indian population and was

found to be comparable with the control vaccine (Oka-RIT strain; Varilix) in immunogenicity and safety<sup>15</sup>

## Methodology

### *Inclusion/Exclusion criteria*

Healthy Indian children of age group  $\geq 12$  months to  $\leq 12$  years of either gender whose parent(s)/guardian(s) were willing to give written informed consent (audio and video) or complying with all the study related procedures were included in the study. Subjects with a history of chicken pox disease and herpes zoster infection in the previous 4 weeks prior to vaccination, those who were pre-vaccinated with varicella vaccine, or those in the close vicinity of any person who is at high risk of developing varicella (like an immune compromised sibling) were not included in the study. Subjects who showed an axillary temperature  $\leq 37.5^\circ\text{C}$  at the time of vaccination were also excluded.

In addition, those with any established or clinically suspected immunosuppressive disorder for which they were receiving any parenteral immunoglobulin or any immunosuppressive drugs in the last 3 months, those with any major congenital abnormality, those with any allergy, and those who had a bacterial/viral/ fungal infection were excluded from the study.

### *Study design and procedure*

This was an open label, non-comparative, single arm, single center, investigator-initiated study conducted at the Institute of Child Health, Kolkata from 2 January 2017 to 27 April 2018. The study was carried out after approval from Drug Controller General India (DCGI) and was registered with the clinical trial registry, India [CTRI/2016/11/007452 dated 08/11/2016].

Being an investigator-initiated study, the study documents were submitted to the DCGI by the site institutional ethics committee.

The study was conducted following the principles of the Schedule Y of the Drugs and Cosmetics Act, good laboratory practices, the ethical guidelines for biomedical research on human participants (Indian Council of Medical Research, 2006), and the Declaration of Helsinki. The

informed consent form was designed as per Schedule Y with all the essential elements and an audio-visual consent was taken.

### *Study visits, dosing schedule ad blood sampling*

The study comprised 4 scheduled visits; Visit 1 (Day 0); Visit 2 (Day 28+7), Visit 3 (Day 84+7), and Visit 4 (Day 112+7) and follow up visits at 6, 9, and 12 months post first-dose of vaccination. At every visit, clinical examination and vitals were assessed. All subjects provided three blood samples on Visit 1 (Day 0), Visit 2 (Day 28  $\pm$  7) and Visit 4 (Day 112  $\pm$  7).

The first dose of the vaccine was given at 12–15 months of age as per the vaccine schedule of ACIP and Advisory Committee on Vaccines and Immunization Practices. The second dose of the two-dose vaccine schedule was administered 3 months post first-dose. BIOVAC-V<sup>TM</sup> varicella vaccine (live) I.P. freeze dried 0.5ml/vial marketed by Wockhardt Limited, Mumbai, containing Oka strain (VR 795) was used in this study. After reconstitution, each 0.5ml/dose contained varicella not less than 3.4 Lg Plaque Forming unit (PFU) of the Oka strain of the varicella virus. Immediately after reconstitution with sterile water for injection as per the manufacturer's instructions, 0.5 ml dose of the vaccine was injected by subcutaneous route at deltoid insertion area of the lateral upper arm, the best site for subcutaneous injection in children 1–3 years of age. All subjects provided three blood samples including a pre-vaccination blood sample at Day 0 for baseline antibody titer estimation and complete blood count. Subjects provided a post-vaccination blood sample at Day 28 for antibody titer estimation and at Day 112, that is, 28 days post second-dose of vaccination.

*Study objectives.* The primary objective of the study was to assess the immunogenicity of two doses of the vaccine by estimation of seroprotection rates, considering a cut-off of  $\geq 5$  gp ELISA or  $\geq 10$  mIU/ml of anti-varicella (VZV) IgG antibody and the rise of geometric mean titer (GMT) from baseline values to 4 weeks after the first and second dose of vaccine, respectively. The secondary objective included determination of incidence of “Break Through” infections of varicella occurring post vaccination (from 42 days after first and second dose of vaccine to study end, i.e. up to 12 months) and assessment of safety by monitoring for solicited and

unsolicited vaccine related serious and non-serious adverse effects from first-dose vaccination to 6 weeks of second-dose post-vaccination.

#### *Evaluation of immunogenicity*

*Endpoint.* Immunogenicity was the primary endpoint of the study. The immunogenicity of the vaccine was computed by estimating the rise in GMT of anti-VZV IgG antibody at baseline and 4 weeks following first and second dose vaccination. However, there was a subpopulation of the study population that was exposed to natural infection because of the occurrence of varicella outbreak during the conduct of study.

Seroprotection rate was defined as the percentage of subjects that achieved an antibody level at or above the  $\geq 10$  mIU/ml (equivalent to  $\geq 5$  gp ELISA) of the anti-VZV IgG antibody. The cut off levels of 10 IU/ml of 5 GP ELISA were considered as per the literature and the previous phase III study conducted on same vaccine in India.<sup>15</sup> It was computed at 4 weeks after first and second dose of vaccination. The estimation of a two- to four-fold rise in antibody titer was analyzed to assess the seroconversion. Quantitative estimation of VZV IgG antibody titer was done by an enzyme immunoassay. The titer was estimated using VaccZyme, VZV glycoprotein, IgG Low Level Enzyme Immunoassay Kit MK092 (manufactured by The Binding Site Ltd, Birmingham, and UK). The secondary endpoint was estimated by determining the incidence of “BT” infections of varicella occurring post vaccination (from 42 days after first and second dose of vaccine to end of the study, i.e. up to 12 months).

#### *Evaluation of safety*

The guardians of the enrolled subjects used a subject diary for recording solicited reactions from first day of vaccination up to 7 days post-vaccination and unsolicited vaccine related serious and non-serious adverse effects from first-dose vaccination to 6 weeks of second-dose post-vaccination. Information on adverse events (AEs) was collected throughout the study. All enrolled subjects were followed up to 12 months post first-dose of vaccination.

#### *Statistical analysis*

*Determination of sample size.* Children in the age group  $\geq 12$  months but  $\leq 12$  years of either sex

were screened. It was computed that with a sample size of 260 evaluable subjects, the 95% confidence interval (CI) of the anticipated seroconversion rate of 98% would be 96.41–99.8%. Considering a drop-out rate of 15%, a sample size of 305 subjects was recruited. The precision-based sample size calculation was performed by statistical software. Since this was an uncontrolled open-label study, the principles of randomization and blinding were not applicable. The sample size calculation was based on the literature available and previous safety study conducted.<sup>15</sup>

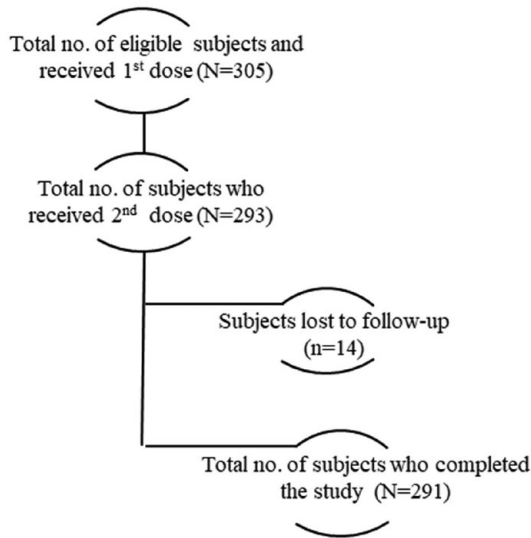
*Analysis of immunogenicity data.* The immunogenicity analysis was performed on the per protocol population (i.e. all enrolled subjects who completed the study as per the procedures mandated by protocol). Levels of VZV IgG antibody were summarized as GMT. The GMT or log-transformed GMT values were compared at various time points for detecting any statistically significant difference by repeated measure ANOVA test. Mann–Whitney *U* test was used for computation of the level of significance. A *p* value  $< 0.05$  was considered statistically significant. Comparison of the seroconversion rates at different time points was performed using Mc Nemar’s test for detecting any statistically significant difference.

The BT infection rate occurring post vaccination (from 42 days after first and second dose of vaccine to the end of the study, i.e. up to 12 months) was computed as a percentage. Data analysis was carried out by using the SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp) statistical software.

*Statistical analysis of safety data.* Safety evaluation was performed on the intention to treat population (i.e. any subject who received one dose of the vaccine). The AE incidence rate (both serious and non-serious AEs) was calculated as the percentage of the vaccinated subjects who developed either local and/or systemic AEs.

## **Results**

*Subject Disposition.* A total of 305 subjects meeting the inclusion/exclusion criteria were enrolled in the study. Among 305 subjects, 158 (51.80%) were boys. The mean age was 4.42 ( $\pm 2.64$ ) years. In total, 70.8% ( $n = 217$ ) subjects were seronegative with a baseline titer value  $< 10$  mIU/ml.



**Figure 1.** Subject disposition.

Overall, 14 subjects were lost to follow-up between Day 28 (Visit 2) and end of the study. Of these, 12 subjects did not receive a second dose of vaccine.

Thus, 291 subjects completed the study. The discontinuations were mainly due to voluntary withdrawal. The baseline demographic and laboratory parameters in both groups were well matched.

Figure 1 represents the subject disposition and the visiting schedule.

### *Evaluation of immunogenicity*

**Endpoint.** The analysis of immunogenicity and safety was performed for all 305 subjects enrolled into the study. The immunogenicity was also separately analyzed for subjects who were baseline seronegative (217 subjects) and seropositive (88 subjects). The GMTs were comparable in both populations. We are emphasizing on data in seronegative subjects as seropositivity may have an interference with the natural antibodies resulting in variations in antibody titers. At baseline, the average GMT was <10 IU/ml and 78.85 IU/ml in seronegative and seropositive subjects, respectively. At Day 28 (Visit 2), post first-dose of vaccination, eight subjects dropped out. Therefore, 297 subjects were considered for

analysis. GMTs at visit 2 were reported as 71.67 IU/ml and 243.6 IU/ml in seronegative and seropositive subjects, respectively. At Day 112 (Visit 4) post first-dose, a further six subjects dropped out. Thus, 291 subjects were considered for analysis.

The GMT at this stage was reported as 760.87 IU/ml and 774.89 IU/ml in seronegative and seropositive subjects, respectively. The analysis of GMT and 95% CI in all seronegative and seropositive subjects are presented in Table 1. The extent of the rise of VZV IgG antibody from baseline values to 112 days post-vaccination was evaluated for the two-fold, three-fold, and four-fold rise in study subjects completing the study. The comparison of extent of rise of VZV IgG antibody in all subjects, seronegative and seropositive is presented in Figure 2.

The seroconversion rate in seronegative subjects considering a cut off value  $\geq 10$  IU/ml of anti-varicella IgG antibody was 93.3% post first-dose of vaccination and 100% post two-doses. Subjects who had antibody titer >100 IU/ml at baseline ( $n = 38$ ) did not achieve a 4-fold rise at 28 days but seroconverted at 112 days post-vaccination.

### *Comparison between different age groups*

We compared the GMT titers and seroprotection rates among different age groups: 12–18 months, 18–60 months, and above 60 months. GMT increased from post first-dose of vaccination to post second-dose by approximately nine times in both age groups 12–18 months and 18–60 months.

This increase was five times in the age group above 60 months. A 100% seroprotection rate was reported post first-dose of vaccination in the age-group 12–18 months, 99.43% in 18–60 months and 99.02% in age group of above 60 months. The seroprotection rate post second-dose of vaccination in all age-groups was 100%. The vaccine prevented varicella infection in 100% of subjects in both 18–60 months and above 60 months age groups. However, there was one BT infection reported in age group 12–18 months and thus vaccine prevented varicella infection in 96.15% subjects in this group. The details of comparison

**Table 1.** Geometric mean titration (GMT) of anti-VZV IgG antibody values for all 305 subjects, seronegative subjects (GMT < 10 IU/ml), seropositive subjects (GMT > 10 IU/ml).

Category	Pre vaccination (baseline)	Post first-dose vaccination	Post second-dose vaccination
For all 305 subjects			
Evaluated subjects (N)	305	297	291
GMT	18.14	101.84	764.84
IU/ml (95% CI)	(20.7–15.05)	(115.47–89.17)	(782.71–747.09)
<i>p</i> value (baseline versus post dose)	-	<0.001	<0.001
For seronegative subjects			
Evaluated subjects (N)	217	212	208
GMT	10	71.67	760.87
IU/ml (95% CI)		(64.01–80.04)	(738.88–783.03)
<i>p</i> value (baseline versus dose)	-	<0.001	<0.001
For seropositive subjects			
Evaluated subjects (N)	88	85	83
GMT	78.85	243.60	774.89
IU/ml (95% CI)	(47.44–111.71)	(182.84–320.99)	(746.07–804.83)
<i>p</i> value (baseline versus dose)	-	<0.001	<0.001
CI, confidence interval; VZV, varicella-zoster virus.			

of immunogenicity between all age groups are shown in Table 2.

#### *Assessment of immunogenicity in household exposure cases*

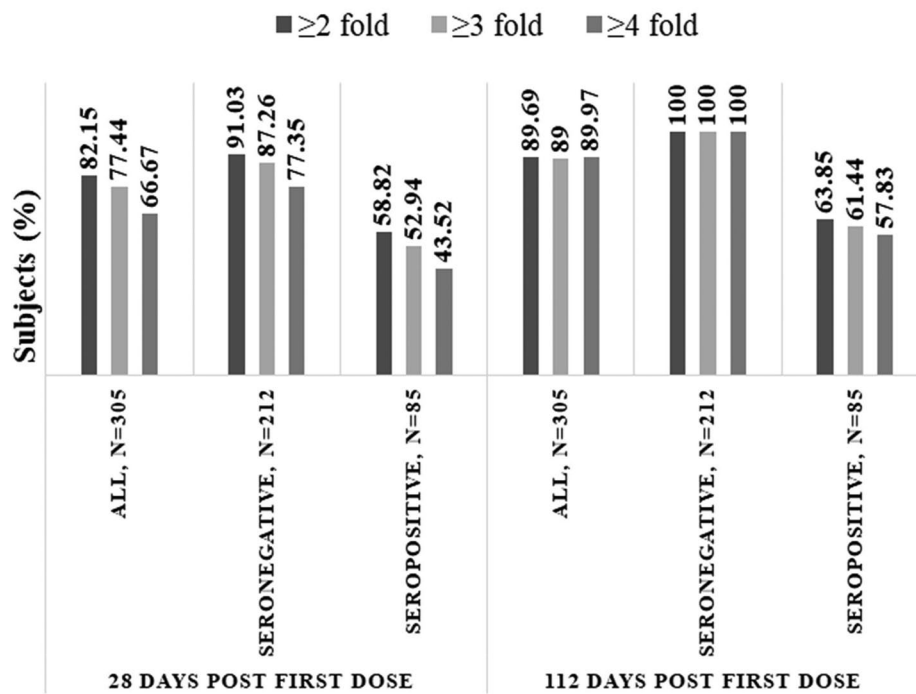
A total of 15 subjects reported exposure to varicella infection during the study either through a household contact or a family member, while one subject (6.66%) developed a BT infection. The subject had a mild form of infection with less than 50 vesicles. The vaccine prevented breakthrough in 94% subjects exposed to varicella infection ( $n = 15$ ). Two subjects of the exposed study population had a significantly higher titer at baseline and the subject who developed BT infection also was seroprotected at baseline. The mean duration of exposure from the first dose of vaccination was  $90.53 \pm 38.86$  days. Table 3 shows the GMT of subjects exposed to varicella

infection during the study and Table 4 shows the extent of rise of antibody titer of subjects exposed to varicella infection.

#### *Breakthrough infection (BT)*

Only one subject had a BT infection a day after the first dose of vaccination, and this was mild in nature with <20 lesions for a short duration of 7 days. The subject recovered fully without administration of any concomitant medication. The contact person was his father who had a severe form of varicella infection with >200 lesions.

The BT infection in the subject appeared 67 days after receiving the first dose of vaccination. The titer at baseline in this subject was 13.91 IU/ml, which increased to 147.8 IU/ml post first-dose of vaccination, implying the immunogenicity



**Figure 2.** Extent of rise of varicella (VZV) IgG antibody from baseline values to post 28 days and 112 days of first dose of vaccination for all, seronegative and seropositive subjects.

**Table 2.** Comparison of immunogenicity between all age groups.

Age Groups (months)	Category	GMT (IU/ml)	Seroprotection (%)	Breakthrough (%)
12–18 ( <i>n</i> = 26)	Baseline	10.43		
	Post first-dose vaccination	*90.75	100	3.8
	Post second-dose vaccination	*808.98	100	(one BT infection)
18–60 ( <i>n</i> = 176)	Baseline	12.37		
	Post vaccination post first-dose	*87.52	99.43	0
	Post vaccination post second-dose	*774.04	100	
>60 ( <i>n</i> = 103)	Baseline	40.097		
	Post vaccination post first-dose	*135.80	99.02	0
	Post vaccination post second-dose	*739.02	100	

BT, breakthrough; GMT, geometric mean titers.  
\**p* value baseline versus post first-dose and second-dose <0.001.

**Table 3.** Geometric mean titration (GMT) of anti-VZV IgG antibodies in subjects (15) exposed to varicella infection during the study.

Category	GMT (IU/ml)	95% CI	p value (baseline versus post dose)
Pre-vaccination (baseline)	18.27	7.56–44.17	
Post first-dose vaccination	95.60	50.53–180.87	<0.001
Post second-dose vaccination	755.72	676.19–844.62	<0.001

CI, confidence interval, VZV, varicella-zoster virus.

**Table 4.** Extent of rise of anti-VZV IgG antibody titer of subjects exposed to varicella infection.

Fold Rise	28 Days post First dose (n = 14)	Percentage (%)	112 days post first dose (n = 13)	Percentage (%)
≥ two-fold rise	12	85.71	11	84.61
≥ three-fold rise	11	78.57	11	84.61
≥ four-fold rise	10	71.42	11	84.61

VZV, varicella-zoster virus.

of varicella vaccine in an actual clinical scenario when the subject is exposed to varicella infection.

### Safety Evaluation:

#### Reporting of Adverse Events (AE) and Serious Adverse Events (SAE) (Table 5)

Post visit 1 – Post administration of first dose of vaccination, there were 34 subjects (11.14%) who developed AEs, of these 9 subjects (2.93%) were observed with pain only at the vaccination site. A total of 20 (6.55%) subjects reported pain, redness, swelling, and tenderness at the vaccination site; 7 (2.29%) developed fever and 5 (1.97%) reported malaise. One subject (0.3%) had a cough and cold and one subject (0.32%) reported diarrhea.

Post visit 3 – Post administration of second dose of vaccination, there were 16 subjects who (5.24%) developed AEs; of these, one subject (0.34%) had only pain at the vaccination site. A total of 14 (4.77%) subjects reported pain, redness, swelling, and tenderness at the vaccination site; 3 (1.02%) developed fever, 1 (0.34%) had diarrhea and 1 (0.34%) developed myalgia and nausea.

Five SAEs were reported in this study. However, none of these were related to the study vaccine.

### Discussion

The current study reported that the two-dose schedule of varicella vaccine given 3 months apart was well tolerated and immunogenic. The seroprotection rate post second-dose (112 days) of vaccine in all the age-groups was 100%. In this study, we observed high levels (nine times) of GMT from post first-dose vaccination to post second-dose in both age groups 12–18 months and 18–60 months. It increased to five times in the age group above 60 months from post first-dose vaccination to post second-dose. This is an interesting observation as GMT has an inverse correlation with body mass index (BMI). As BMI increases with age, GMT decreases with increasing age and BMI.<sup>21</sup> However, we observed higher levels in the younger age group in whom the immunity against VZV infection is most needed. The immunity in the younger age group is important, especially in India where a high incidence of VZV is recorded in the children of the younger age group. A single case of BT infection was observed a day after the first dose of vaccination that was mild in nature



**Table 5.** Evaluation of adverse events.

Adverse event	Post visit 1 [n (%)]	Post visit 3
Pain	9 (2.9)	1 (0.3)
Redness/swelling	20 (6.5)	14 (4.7)
Fever	7 (2.29)	3 (1)
Malaise	5 (1.97)	1 (0.3)
Cough and cold	1 (0.3)	1 (0.3)
Diarrhea	1 (0.3)	1 (0.3)

with <20 lesions for 7 days. The AEs reported were mild in nature and none of the SAE reported was related to the study drug. Our results are consistent with the study done by Shapiro *et al.*, where the effectiveness of a single dose of the varicella vaccine was 86.0% (95% CI: 44.5%–99%;  $p=0.124$ ) and that two doses were 98.3% (95% CI: 83.5%–100%;  $p<001$ ). Shapiro *et al.* recommended two doses of varicella vaccine and observed that in first 2.5 years after introduction of the second dose, the odds of developing varicella for children who received two doses of the varicella vaccine were 95% lower than for those who had received a single dose [matched odds ratio for double dose *versus* single dose: 0.053 (95% CI: 0.002–0.320;  $p<001$ )].<sup>22</sup> The study also concluded that the immune response was better at the age of 2 years in comparison with 3 or 4 years. The authors concluded that a two-dose vaccine schedule not only prevents BT infection, but could also decrease latent infection with WT-VZV and thus lower the subsequent risk of developing zoster. Kuter *et al.* in their 10-year follow-up study of children who received either one or two doses of varicella vaccine suggested that the second dose may be more important in increasing the proportion of children achieving protective antibody titer than in developing high levels of varicella antibody.<sup>20</sup> Vaccine dosing schedule and appropriate age for vaccination are the two most important factors for optimal response to a vaccine. Vaccines are generally recommended in the youngest age group at risk of experiencing the disease for which vaccine effectiveness (VE) and safety are demonstrated. The recommended schedule is designed on spacing between the subsequent doses. A “minimum interval” is the shortest time between

two doses of a vaccine series in which an adequate response to the second dose can be expected.<sup>23</sup> In India, as per the IAP immunization schedule (2018), the minimum age for receiving the varicella vaccine is recommended at 12 months, and the first dose of varicella vaccine is recommended at the age of 15 months and second dose at 4–6 years.<sup>18</sup> As per the recommendations, the risk of BT varicella is considered to be lower if given 15 months onwards, the second dose might be administered before 4 years of age, provided at least 3 months have elapsed since the first dose. For children aged 12 months through to 12 years, the recommended minimum interval between doses is 3 months. However, if the second dose was administered at least 4 weeks after the first dose, it can be accepted as valid.<sup>18</sup> Redesigning the immunization schedule for varicella with an early administration of the vaccine, and with a 3 month interval between the two doses, might be a potential option for a country like India where a high prevalence of varicella outbreaks among children of preschool-age (1–4 years of age) and school-age (5–9 years of age) are reported.<sup>2,24</sup>

Though the safety and efficacy of the two-dose varicella vaccine schedule with second dose administered 3 months after the first dose is established, there are some concerns regarding the early dosing of the second dose. Some researchers suspect that a second dose given too early after the previous dose may reduce the response to that dose; however, administering a dose a few days earlier than the minimum interval or age is unlikely to have a substantially negative effect on the immune response to that dose.<sup>25</sup> In case of live attenuated varicella vaccine, a lower dose of varicella virus is administered to susceptible people, and passively acquired antibodies may interfere with the response to low-dose varicella vaccine for up to a year depending on what product is given.<sup>23</sup> However, studies conducted with a two-dose schedule of varicella vaccine refute this. Previous studies reported that GMT of the anti-varicella antibodies had an incremental increase after the second dose. It was also concluded that a second dose significantly enhances the resistance to VZV due to the sharp increase in GMT of the antibody after administering a two dose vaccine.<sup>26,27</sup> Kuter *et al.* observed that 100% of children who received two injections of the vaccine 3 months apart developed gp ELISA titers  $\geq 5$  units/ml.<sup>20</sup> Our study also showed similar results. The extent of

the rise of anti-VZV IgG antibody titers post 28 days of the first dose, with two-fold, three-fold and four-fold rises were 93.39%, 90.56%, and 80.66% respectively. 100% of the subjects achieved a 4-fold rise post second-dose. Another concern is that as per the latest immunization schedules, infants by the age of 2 years are already scheduled for multiple vaccines. Hence, immunizing them with the same vaccine strains for another dose, with other vaccines at the same time, or at short intervals of a few months or days might cause adverse short-term effects on the developing infant immune system that reflect in an increased susceptibility to heterologous infection. However, in one of the recent studies conducted to compare the time lapse of varicella vaccine with other vaccines, no risks for BT varicella were reported when varicella vaccine was given within 30 days after DTP, Hib, OPV, IPV, or hepatitis B vaccines.<sup>28,29</sup> There is limited information available for BT varicella in persons who have received two doses of varicella vaccine. However, it appears to occur less frequently and the disease may be even milder among people vaccinated with two doses of varicella vaccine compared with persons who have received a single dose of varicella vaccine.<sup>25</sup> We observed a mild BT infection in a single child that resolved within 7 days.

We also assessed the immunogenicity in 15 subjects having varicella virus exposure through a household contact or a family member; only one subject developed a BT with a mild form of the infection (<50 vesicles). As there were a substantial number of subjects exposed to varicella infection through household contacts, and considering the risk percentage of 0.06 (proportion calculation of a single BT infection out of the 15 cases exposed to natural infection), the vaccine prevented breakthrough in 94% of the exposed population. Our findings are comparable with one of the clinical trials conducted in the USA comparing one and two doses of varicella vaccine over 10 years. The two doses of vaccine prevented varicella in 98.3% subjects with community exposure and 96.4% subjects with household exposure (higher than after one dose).<sup>20</sup> It explains that the incidence of BT was higher with a one-dose schedule as compared with a two-dose due to an incomplete vaccination schedule. VZV vaccines are generally safe and well tolerated; the adverse events reported with all VZV vaccines are usually either mild or

moderate in nature. The global safety committee in their report in 2013 listed herpes zoster, pain, and rash as the three most frequent terms for SAEs with VZV vaccine.<sup>30</sup> Rare complications with VZV Oka strain vaccine that have been reported include pneumonia, hepatitis, herpes zoster (HZ) meningitis, recurrent HZ, severe rash, and secondary transmission. However, these complications occur mainly in patients who are immunocompromised or who have other serious medical conditions that are undiagnosed at the time of vaccination. Overall, the varicella vaccine was well tolerated throughout the study period. No immediate adverse reactions were reported after vaccination. The AEs reported were mild in nature and solicited. SAE were reported in five patients that were not related to the study vaccine. All the patients recovered fully. The two-dose schedule of varicella vaccine is safe and immunogenic when given 3 months apart. A high antibody response was observed in all baseline seronegative subjects, more so in the younger age group. Only a single case of BT infection was reported post 67 days of first dose which was mild in nature. Though, this was a single arm, single-center study conducted in smaller population; being a real-world study, it generated important observations. Future large-multicentric studies will help to substantiate the important findings of this study.

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### Author contributions

Dr. Monjori Mitra was the chief investigator of the study and was involved in the development of study design and execution of the study. Dr. Jaydeep Chowdhury was the co-investigator of the study and was involved in study design and patient care. Dr. Surupa Basu, Dr. Partha Pratim Halder and Dr. Mallar Mukherjee contributed to research methodology, assessing patient sample and patient care. Dr. Archana Karadkhele was involved in the development of study design, formulating the study, co-coordinating with the patient site, statistical methods, and study report preparation. Dr. Gaurav Puppalwar and Dr. Rishi Jain helped in reviewing, statistical methods, manuscript preparation, and revision. All the authors reviewed and approved the manuscript before submission.

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