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Original Article

Public Health Section

Seroprevalence of Immunoglobulin G Levels (IgG) against Varicella in Healthy Indian Adults: A Cross-sectional Study

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ABSTRACT

Introduction: The key role of vaccines is to prevent infections caused by viruses and bacteria. Many adults remain unaware and unvaccinated. Varicella-Zoster Virus (VZV) is a pathogenic human herpes virus that causes varicella (chickenpox) infections in both children and adults. Only a fraction of the population in India receives the varicella vaccination through private practitioners, as it is not part of the national immunisation programme.

Aim: To determine the levels of protective antibodies against varicella among different age groups of healthy adults.

Materials and Methods: A cross-sectional study was conducted from July 2019 to September 2020 in the Department of Internal Medicine and the Blood Donation Centre of the Department of Transfusion Medicine at PGIMER, Chandigarh, Punjab, India. The study involved healthy adults aged 18 years and above attending blood donation camps or those accompanying patients in the Internal Medicine Outpatient Department (OPD). They were divided into four groups: Group A: Adults aged 18-30 years, Group B: Adults aged 31-45 years, Group C: Adults aged 46-60 years, Group D: Adults aged >60 years. Variables like age, sex, socioeconomic status, history of VZV vaccination, history of natural varicella infection in the past and VZV IgG levels tested with a commercially available Enzyme-linked Immunosorbent Assay (ELISA) kit were analysed. The dependent variables were classified as Varicella IgG positive (>12 U/mL), equivocal (8-12 U/mL) and negative (<8 U/mL). Data collected were analysed using Statistical Package for the Social Sciences (SPSS) version 21.0. The Chi-square test (χ) and Fisher's-exact test were used for proportions, while the Spearman's correlation test was used to assess the correlation between age and Immunoglobulin (IgG) levels. A p-value of <0.05 was considered significant.

Results: Out of 300 participants, 244 (81.3%) were male and the mean±SD age was 44.26±15.50 years. A total of 88.7% had protective levels of varicella IgG >12 U/mL. Lower socioeconomic status and younger age groups were associated with negative or equivocal (<12 U/mL) IgG levels. A positive correlation between age (in years) and VZV IgG (in U/mL) was found in the present study.

Conclusion: Overall, protective immunity among adults against varicella was found to be 88.7%. Serosurveillance surveys in the community and healthcare facilities should be implemented for vaccine-preventable diseases so that vaccination can be offered to the susceptible population, thereby preventing outbreaks.

Keywords: Chickenpox, Immunity, Immunoglobulins, Vaccination

INTRODUCTION

Vaccines are very important and play a key role in preventing infections caused by viruses and bacteria. Many adults remain unvaccinated despite the availability of vaccines because they are either unaware of the necessity of adult vaccinations or have misinformation about vaccines and the diseases they are intended to prevent. Therefore, the issue of adult vaccinations needs to be addressed immediately [1].

As a pathogenic human herpes virus, VZV first causes varicella (chickenpox), after which it develops latently in peripheral ganglia. The virus may reactivate decades later to cause herpes zoster, either on its own or in response to several triggers [2]. Varicella is highly contagious, with an incubation period of 14-16 days after exposure and is transmitted through airborne droplets as well as through direct contact with skin lesions [3]. The development of a live attenuated vaccine to prevent varicella was accomplished by Takahashi in 1974. The availability of the live attenuated varicella vaccine directly led to the development of a live vaccine to prevent zoster [4].

The global annual varicella disease burden includes 4.2 million severe complications leading to hospitalisation and 4,200 deaths [5]. In India, varicella continues to be a burden on society, with the majority of cases not being reported. Limited data are available regarding the incidence of the infection and the prevalence of protective titres in our country. Published literature is limited to reports of various outbreaks of varicella in different parts of the country [6,7]. However, there is a possibility that a significant number of adolescents and adults remain susceptible to varicella, as the age of acquiring primary infection may be increasing due to the shifting age of primary infection with varicella.

Varicella results in a skin vesicular rash that forms small, itchy blisters that scab over. It is accompanied by fever, cough, headache, body aches, nausea, vomiting and abdominal pain. Complications include pneumonia, brain inflammation and bacterial skin infections. The disease is often more severe in adults than in children [5,8]. In older subjects, the disease is more severe and prolonged, with a mortality rate 15-25 times higher than that in children. Varicella pneumonitis is the most common and severe complication in adults [9].

Only a fraction of the population receives varicella vaccination in India, primarily through private practitioners, as it is not part of the National Immunisation Programme of the country [6,10]. Puri S et al., reported coverage as low as 2.8% in children under five in Chandigarh, with the majority (91.9%) immunised in the private sector [11].

To date, to the best of authors knowledge, there has been limited data regarding the prevalence of protective antibodies to the Varicella Zoster virus in adults [12,13], especially in the geriatric population [14-16]. The present study was conducted to determine the levels of protective antibodies (IgG) against varicella among different age groups of healthy adults using commercially available ELISA kits.

MATERIALS AND METHODS

A cross-sectional study was conducted from July 2019 to September 2020 in the Outpatient Department (OPD) of Internal Medicine and the Blood Donation Centre of the Department of Transfusion Medicine at PGIMER, Chandigarh, Punjab, India. The

study involved healthy adults aged 18 years and above attending blood donation camps or the blood donation centre at PGIMER, as well as apparently healthy attendants accompanying patients in the Internal Medicine OPD. Ethical approval was obtained from the Institutional Ethics committee (IEC No. 001946) before the commencement of the study.

Inclusion criteria: The study was conducted among healthy adults aged 18 years and above attending blood donation camps or the blood donation centre at PGIMER, as well as apparently healthy attendants accompanying patients in the Internal Medicine OPD.

Exclusion criteria: Individuals who refused to participate, those with a history of blood, plasma transfusion, or immunoglobulin administration within the last three months, those on prolonged (>2 weeks) steroid therapy or <4 weeks after cessation of steroid therapy, pregnant individuals and those with other acute infections were excluded from the study.

Sample size: Estimating an anti-varicella seroprevalence of 86.9% among adults [17] (with an accuracy of 4%, a confidence level of 95% and adding a non-response rate of 10%), the sample population size was determined to be 300 sera.

Study Procedure

A total of 300 participants who fulfilled the above criteria were enrolled in the study through convenient sampling. For the purpose of the study, the enrolled participants were divided into four groups based on the following age criteria:

1. Group A - Adults aged 18-30 years (n=75)
2. Group B - Adults aged 31-45 years (n=75)
3. Group C - Adults aged 46-60 years (n=77)
4. Group D - Adults aged >60 years (n=73)

Variables like age, sex, religion, address, family type, socioeconomic status (measured by the modified Kuppuswamy scale) [18], history of VZV vaccination, history of natural varicella infection in the past and VZV IgG levels tested with a commercially available ELISA kit (Demeditec Diagnostics GmbH, Germany) were analysed. The dependent variables were classified as Varicella IgG positive (>11 U/mL), equivocal (9-11 U/mL) and negative (<9 U/mL). The ELISA kit was produced by Demeditec Diagnostics GmbH, Germany [19].

STATISTICAL ANALYSIS

The collected data were entered and analysed using SPSS (IBM) version 21.0. Data summarisation was carried out using descriptive statistics such as mean, median, standard deviation and percentages. The χ^2 test or Fisher's-exact test was used for categorical variables, while the Spearman's correlation test was employed to assess the correlation between age and IgG levels. A p-value of <0.05 was considered statistically significant.

RESULTS

Of the 300 individuals invited to participate, all 300 agreed to take part. As shown in [Table/Fig-1], 81.3% of the participants were male and 18.7% were female. The mean±SD age of the participants was 44.26±15.50 years, with a median {Interquartile Range (IQR)} age of 45.50±30.75-60 years and the age ranged from 18 to 78 years. As indicated in [Table/Fig-1], there was no significant association between age group, gender, residence, religion and type of family with varicella IgG levels. The maximum number of participants with negative or equivocal IgG levels were observed to have a lower socioeconomic status compared to those with positive IgG levels and this finding was significant. Approximately 26.7% of young adults in Group A (18-30 years), 9.4% of adults in Group B (31-45 years), 3.9% in Group C (46-60 years) and 5.5% in the elderly Group D were found to be susceptible to varicella infection. Out of the total 300 participants, 88.7% had protective levels of varicella IgG >11 U/mL, as shown in [Table/Fig-2]. A significant susceptibility to varicella infection among the younger age groups (Groups A and B) compared to the older age groups (Groups C and D) is indicated in [Table/Fig-3].

Characteristics		No. of participants, n (%)		Total, n (%)	p-value
		Positive	Negative+ Equivocal		
Age	18-30 years	55 (73.3)	20 (26.7)	75	0.782
	31-45 years	68 (90.6)	7 (9.4)	75	
	46-60 years	74 (96.1)	3 (3.9)	77	
	>60 years	69 (94.5)	4 (5.5)	73	
Gender	Male	216 (88.5)	28 (11.5)	244 (81.3)	0.912
	Female	50 (89.2)	6 (10.8)	56 (18.7)	
Residence	Rural	129 (91.4)	12 (8.6)	141 (47.0)	0.146
	Urban	137 (86.1)	22 (13.9)	159 (53.0)	
Religion	Hindu	187 (89.4)	22 (10.6)	209 (69.7)	0.344
	Sikh	71 (88.7)	9 (11.3)	80 (26.7)	
	Muslim	6 (66.7)	3 (33.3)	9 (3.0)	
	Christian	1 (100)	0	1 (0.3)	
	Jainism	1 (100)	0	1 (0.3)	
Socioeconomic status*	Upper	22 (95.6)	1 (4.4)	23 (7.7)	0.043
	Middle	165 (91.1)	16 (8.9)	181 (60.3)	
	Lower	79 (82.2)	17 (17.8)	96 (32.0)	
Family	Nuclear	85 (90.4)	9 (9.6)	94 (31.3)	0.516
	Joint	181 (87.8)	25 (12.2)	206 (68.7)	
Total		266 (88.7)	34 (11.3)	300 (100)	

[Table/Fig-1]: Socio-demographic characteristics in the study population (N=300). *modified Kuppuswamy's scale

S. No.	Varicella IgG level	No. of participants	Percentage (%)
1.	Positive	266	88.7
2.	Negative	28	9.3
3.	Equivocal	6	2.0
4.	Total	300	100

[Table/Fig-2]: Distribution of the patients by varicella IgG level (N=300).

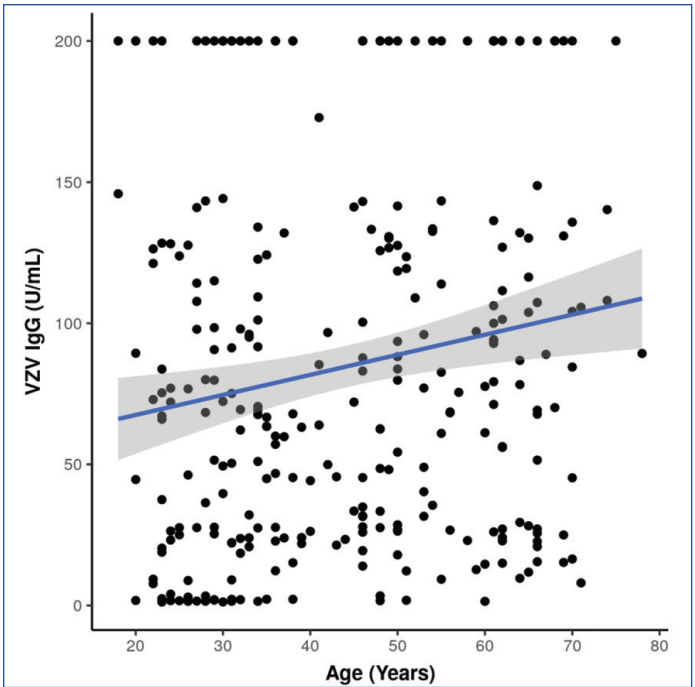
Age group in years	No. of participants, n (%)		Total, n (%)	p-value
	Positive	Negative+Equivocal		
Group-A and B (≤45 years)	123 (46.2)	27 (79.4)	150 (50)	<0.001
Group-C and D (>45 years)	143 (53.8)	7 (20.6)	150 (50)	

[Table/Fig-3]: Association between age group and varicella IgG level (N=300).

Similarly, as shown in [Table/Fig-4], there was no significant association between varicella IgG levels and either the presence of past varicella infection or a history of varicella vaccination. As shown in [Table/Fig-5,6], a non parametric test (Spearman's Correlation) was used to explore the correlation between the two variables, as atleast one of the variables was not normally distributed. It was found that there was a weak positive correlation between age (years) and VZV IgG (U/mL), which was statistically significant (rho=0.18, p=0.001). For every one-unit increase in age (years), the VZV IgG (U/mL) increased by 0.71 units.

Characteristics		No. of participants, n (%)		Total, n (%)	p-value
		Positive	Negative+Equivocal		
History of past varicella infection	Present	8 (100)	0	8 (2.7)	0.645
	Absent	258 (88.6)	34 (11.4)	292 (97.3)	
History of varicella vaccination	Present	10 (100)	0	10 (3.3)	0.610
	Absent	256 (88.2)	34 (11.8)	290 (96.7)	

[Table/Fig-4]: Association between past varicella infections and vaccination with IgG levels (N=300).



[Table/Fig-5]: Correlation between age (years) and VZV IgG (U/mL) (N=300). The above scatterplot depicts the correlation between age (years) and VZV IgG (U/mL). Individual points represent individual cases. The blue trendline represents the general trend of correlation between the two variables. The shaded grey area represents the 95% confidence interval of this trendline

Correlation	Spearman's correlation coefficient	p-value
Age (Years) vs VZV IgG (U/mL)	0.18	0.001

[Table/Fig-6]: Correlation between age (years) and VZV IgG (U/mL) (N=300).

Conversely, for every one-unit increase in VZV IgG (U/mL), the age (years) increased by 0.04 units.

DISCUSSION

In the current study, ELISA was used to determine protective titres of IgG against varicella. Titre of ≥12 U/mL was considered protective for varicella. More than 85% of the study participants were found to harbour protective levels of VZV IgG antibodies. It was observed that there was variation in the VZV IgG protective titres among the various groups, with Group A (18-30 years) and Group B (31-45 years) harbouring protective titres of 73.3% and 90.7%, respectively, compared to Group C (46-60 years) and Group D (over 60 years), which had protective titres of 96.1% and 94.5%, respectively. Thus, the authors observed a varying increase in VZV IgG protective levels with increasing age. Although the natural history of varicella and vaccination was found in a low percentage among study participants, it is a known fact that subclinical and mild infections trigger the immune system to generate protective antibodies. This may explain the persistence of VZV protection with increasing age.

Out of the 300 recruited participants, male predominance was observed. Various reasons may account for this male predominance, including the finding that males were more willing to provide blood samples to be tested for IgG antibodies against varicella. Moreover, healthy male family members often accompany patients for treatment at the hospital, while in India, females typically manage household responsibilities, making it more difficult for them to leave the house for extended periods. Additionally, Indian society is characterised by deep gender inequality, as discussed by Ram U et al., [20].

Amongst the study participants, 47% and 53% belonged to rural and urban areas, respectively. The study Institute provides affordable healthcare services to all, resulting in a nearly equal proportion of patients and, consequently, healthy accompanying attendants (as in the present study) from rural areas. Das J et al., observed in their study that among urban households, 70% of all hospital visits were made to private-sector providers, while the remaining 30% were to public healthcare centres/hospitals [21]. Thus, the results of the present study are largely in concordance with the published literature, which indicates that the majority of patients in public hospitals come from rural backgrounds [21].

The majority of the study participants belonged to the middle class (60.3%), as compared to those from upper socioeconomic status (7.7%). Individuals from upper socioeconomic backgrounds typically visit private sector hospitals, while those from low and middle classes tend to visit public hospitals more frequently due to financial constraints. Moreover, public healthcare is free for those below the poverty line. Most of the participants were Hindus (69.7%), followed by Sikhs (26.7%), with very few representatives from other religious communities. The predominant catchment area of PGIMER is from North/North Western India, where these two communities predominate. Additionally, due to Coronavirus Disease-2019 (COVID-19) pandemic restrictions, interstate travel was limited during 2020, which may have affected these observations.

In the present study, 11.3% of participants were found to be susceptible to varicella, a figure almost similar to that reported in the literature from India by Lokeshwar MR et al., where VZV susceptibility was found to be between 11.9% and 8.9% among their study populations aged 21-30 years and 31-40 years, respectively [22]. Venkitaraman AR and John TJ reported a seroprevalence of 72%, which is slightly lower than the findings of the current study, but their study was conducted 40 years ago [23]. Thongmee T et al., O'Grady KA et al., Ziaeian M et al., and Gorny AW et al., reported similar seroprevalence results, indicating a higher level of seroprevalence and lower susceptibility to varicella [14,16,17,24]. Immunity is known to develop due to mild or asymptomatic varicella infections in childhood and adulthood, which explains the lower percentage of susceptibility to varicella in these studies [22]. Intergroup variation in terms of susceptibility to varicella infection was observed in the present study.

Varicella seroprevalence as reported by various studies is presented in [Table/Fig-7] [14-17, 22-24]. Lokeshwar MR et al., in their study published in 2000, included samples from four different cities: Mumbai, Bangalore, Calcutta and Ahmedabad [22]. A total of 1,546 volunteers aged from birth to 40 years were included in their study. The overall seroprevalence of VZV antibodies in their study was found to be 68.2%. The age-related seroprevalence rates were 29%

S. No.	Studies and place of study	Sample size	Year of study	Seroprevalence of VZV IgG
1.	Thongmee T et al., [14] Chonburi province, Thailand	Total participants=950; 333 participants (<20 years); 617 participants (20 years and above)	2024	Overall is 64.8% <20 years is 30.9% and in >20 years is 83.1%
2.	Liyanage NP et al., [15], Colombo, Srilanka	Total participants=913; 523 participants (<20 years); 390 participants (20 years and above)	2007	Overall is 36.2% <20 years is 21.4% and >20 years is 56.1%
3.	O'Grady KA et al., [16] Darwin, Australia	298 women (15 years and above)	2000	92%
4.	Ziaeian M et al., [17] Shiraz, Iran	444 adults (16 years and above)	2010	86%
5.	Lokeshwar MR et al., [22] India	410 participants (from 21 to 30 years) 258 participants (from 31 to 40 years)	2000	88.1% 21-30 years 91.1% 31-40 years
6.	Venkitaraman AR and John TJ, [23], Vellore, India	24 participants (from 15 to 25 years)	1984	72%
7.	Gorny AW et al., [24] Singapore	6701 adults participants	2014	91.7%
8.	Current study	300 adult participants	2020	88.7%

[Table/Fig-7]: Comparison of the present study findings with other studies [14-17, 22-24].

(1-5 years), 51.1% (5-10 years), 71.7% (11-15 years), 79.8% (16-20 years), 88.1% (21-30 years) and 91.1% (31-40 years). Venkitaraman AR et al., in 1984, from Vellore, Tamil Nadu, also showed that the VZV antibody seroprevalence rate was around 15% in individuals under 5 years of age and this protection gradually rose to a maximum of 72% in young adults aged 15 to 25 years [23].

Varicella vaccination is still not part of the national immunisation schedule in India. However, the Indian Academy of Paediatrics recommends the use of varicella vaccination in a two-dose schedule, starting from 15 months of age. The second dose may be administered three months after the first but is usually given at 4-6 years. Only a fraction of the population receives varicella vaccination in India, primarily through private practitioners. Puri S et al., reported coverage as low as 2.8% among children under five in Chandigarh, with the majority (91.9%) of them being immunised in the private sector [11]. In this study, subjects with low protective antibody levels against varicella were informed about their susceptibility to the virus and were counselled regarding receiving two doses of the varicella vaccine, three months apart. These recommendations were made according to World Health Organisation (WHO)/Indian Academy of Paediatrics (IAP) recommendations for countries with a high number of susceptible populations after the age of 9-12 years, as shown in our study (i.e., 26%). WHO suggests a two-dose vaccination schedule for such populations [5].

Limitation(s)

The current study is a single-centre study; the sample was enrolled through convenience sampling and may not represent the broader population. Most of the patients were unaware of their vaccination status, which could lead to possible recall bias. Consequently, they were by default considered to have a negative history for the purposes of the study. A multicentre study with a larger sample size and simultaneous estimation across various age groups can be planned to provide more generalisable results.

CONCLUSION(S)

Overall protective immunity among adults against varicella is found to be over 80%. However, protection levels for varicella are inadequate among adults, making it necessary to increase prevention activities, including vaccination, particularly for young adults in low socioeconomic families. Serosurveillance surveys in the community and healthcare facilities should be implemented for vaccine-preventable diseases so that vaccination can be offered to the susceptible population and outbreaks can be prevented. Healthy adults are harder to reach through the public health system, making vaccination of this age group more challenging. Better public understanding of the seriousness of vaccine-preventable diseases and the benefits of vaccination is essential. Studies have shown that literacy status and socioeconomic profile are important determinants associated with adult immunisation. Health education programmes can help increase public understanding of the need for and benefits of adult immunisation.

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