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Clinico-epidemiological investigation on Varicella Zoster Virus indicates multiple clade circulation in Maharashtra state, India

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Abstract

Background: Varicella Zoster Virus (VZV) is consistently in circulation and shows an increase in disease burden during the spring season. Due to a wide range of clinical presentation from a vesicular rash to bleeding or neurological complications, it makes the clinical diagnosis difficult. The present study aims to understand whether the same strain of virus is responsible for the increase in the seasonal outbreaks occurring in different parts of the country with reference to the samples from Maharashtra, Rajasthan and Gujarat states of India.

Materials and methods: This study reports the clinico-epidemiological and laboratory findings of suspected Varicella cases. To understand the circulating clade few representative real-time Polymerase Chain Reaction (PCR) positive were analyzed by conventional PCR and partial Open Reading Frame (ORF) 22, partial ORF 38 and partial ORF 54 were sequenced to identify single nucleotide polymorphisms responsible for clade determination. Further partial glycoprotein B gene was sequenced, and a phylogenetic tree was generated.

Results: A total of 50 cases from Maharashtra (Mumbai district) and referred clinical samples of Rajasthan (Barmer district; $n = 12$) and Gujarat States (Gandhi Nagar, Surat districts; $n = 17$) were tested for the presence of VZV. Vesicular rash with fever was a common clinical presentation with 82% cases having contact history with VZV positive cases, suggesting higher secondary attack rate. The vesicular fluid of all 50 cases from Mumbai revealed the presence of VZV by real-time PCR. Urine, serum and throat swab samples showed positivity by real-time PCR. Healthcare provider's samples from Rajasthan showed 36.4% [4/11] positivity. Clinical samples from Gujarat had positivity of 41.2% [7/17].

Conclusions: This study analyses the clade based circulation of VZV in three states in India and suggests different clades circulating in Maharashtra state. Health education amongst the general population is suggested to reduce the secondary cases by early diagnosis, effective isolation policies and vaccination to reduce the burden of disease.

Keywords: Epidemiology, Infectious disease, Public health, virology

1. Introduction

Varicella Zoster Virus (VZV) is known for causing two highly infectious diseases – Varicella (chickenpox) and Herpes Zoster (Shingles). VZV is a highly contagious agent, and until now humans are the only host of the virus. The infectivity rate of the virus is more than 85–90 % and signifies that VZV spreads with ease amongst the contacts thereby increasing the burden of disease burden (Singh et al., 2011). The virus mainly sheds from nasopharyngeal secretions, skin vesicular and pustular lesions. Viremia and viruria are prominent features of VZV infection. A constant presence and circulation of VZV in the community along with the increase in a number of cases during the months of January to May were recorded. Spurts of infection are recorded in these months as this period is in tandem with maximum fluctuations in environmental factors like temperature and humidity (Singh et al., 2011).

Though Varicella is typically known as a childhood disease, a higher incidence of infection has been noted among young adults in tropical countries like India. The prevalence of VZV in children between the age group of 1–4 years is about 16% while 5 to 14-year-old children showed 54% and 15–25 years old individuals showed 72 % seroprevalence (Lee, 1998).

The incubation period of VZV infection usually ranges from 1 to 3 weeks. Maximum contagiousity is observed from 3 days of onset of rash till scabbing (Heininger and Seward, 2006). Varicella has a varied spectrum of clinical presentations ranging from high-grade fever with vesicular rash, sore throat, cough, diarrhea

and bleeding manifestations in severe cases. The disease is usually benign in nature with an overall case fatality of less than 1% (Singh et al., 2011). Fatality is usually observed in patients with bleeding or respiratory complications, and may often be confused with other viral hemorrhagic fever or respiratory viral infections. There is always the risk of Congenital Varicella Syndrome (CVS) if the antenatal mother is affected with VZV in the third trimester of pregnancy. Congenital Varicella Syndrome presents as scarring lesions (cicatrical) and is associated with the neurological and ophthalmic deficit. Limb shortening and hypotonia are persistent features of CVS (Singh et al., 2011; Gnann, 2002; Ojah et al., 2016; Lee, 1998).

Based on sequence variations, VZV has been classified into five clades amongst which clades 4 and 5 have been reported from India so far (Schmidt-Chanasit and Sauerbrei, 2011). Earlier studies indicated that classification of VZV DNA was based on restriction fragment length polymorphism (RFLP) analysis. The RFLP markers usually considered in VZV clade identification, vaccine selection and epidemiological studies constitute polymorphisms of open reading frames (ORF) 38 (*PstI*), 54 (*BglII*) and 62 (*SmaI*) (Schmidt-Chanasit and Sauerbrei, 2011; Loparev et al., 2000; LaRussa et al., 1992; Sauerbrei et al., 2003). VZV genotyping is usually performed on the basis of sequencing to screen for single nucleotide polymorphisms (SNP) in different ORFs of VZV genome (Faga et al., 2001; Wagenaar et al., 2003). Loparev analyzed entire sequences of the five VZV glycoprotein (g) genes gH, gI, gL, gB, gE and IE62 gene, performed the combination of ORF 22-based genotyping and analyzed either ORF 21 or ORF 50 to verify the presence of the five confirmed clades E1, E2, J, M1, M2 and the two provisional clades M3 and M4 (Loparev et al., 2004, 2007). In addition, VZV had been phylogenetically analyzed based on full-genome sequencing (Peters et al., 2006) and by clustering the strains into the four major clades (Wagenaar et al., 2003). However, sequence based approach is not feasible for all clinical specimens to be genotyped, as VZV strains are difficult to propagate in cell cultures and the amount of viral DNA is limited.

In the current study, we have used partial ORF 22 (4 single nucleotide polymorphism sites), partial ORF 38 (*PstI* restriction digestion site) and partial ORF 54 (*BglII* restriction digestion site) as defined by Loparev for classification of VZV (Loparev et al., 2009). These ORFs were used for identifying circulating clades in different states and in understanding the association of different clades with clinical profile of the disease.

This study highlights the consistent presence of circulating VZV strains in three states of India, and, different clinical presentations of VZV, as an important criterion for differential diagnosis from other viral hemorrhagic fevers. Moreover, atypical clinical cases of VZV are also discussed in this study from Rajasthan and Gujarat states, India. The study also aims to understand the magnitude and

clinico-epidemiological details of increase of Varicella cases in Mumbai district, Maharashtra State, India.

2. Material and methods

2.1. Ethical consent

Written informed consent was obtained before collection of the clinical specimen from VZV cases. The Institute's Human Ethics Committee at the Indian Council of Medical Research (ICMR) - National Institute of Virology (NIV), Pune had reviewed and approved the investigations (IHEC Number-NIV/IEC/2017/D-86). The patients who were less than 18 years, informed consent of parents/guardian was obtained. Permissions were obtained from the hospital authority to allow patients to complete the semi-structured questionnaires designed using Epi-info software version 7.2.1.0. The detailed clinico-epidemiological questions were administered by face-to-face interview. Due care was taken in explaining details of health education and modes of transmission of this disease to the patients. All the respondents cooperated well with the investigators in completing the questionnaire. All the samples were registered in the central registry of the institute and allotted a personally non-identifiable institutional number as per International Ethical Guidelines for Epidemiological Studies.

2.2. Study location

The investigation was initiated in April 2017, due to increase in number of cases in Mumbai district of Maharashtra state. Fifty acute cases (all were males) of clinically suspected Varicella (fever with vesicular rash) were enrolled for investigation. It was hospital-based surveillance of clinically suspected Varicella cases visiting an Infectious Disease Hospital, Mumbai for treatment. The fifty cases which were enrolled in the study were admitted cases and those who had given written informed consent during the said period of investigation.

Clinical samples of suspected VZV referred to ICMR-NIV, Pune from Rajasthan state (Barmer district) and Gujarat state (Gandhi Nagar and Surat districts) were included in the study. The clinical specimens of Rajasthan and Gujarat were referred to ICMR-NIV, Pune being the apex laboratory for testing of Viral Hemorrhagic Fever in India and those samples turned out to be VZV cases. Hence clinical specimens of 17 cases from Gujarat and 12 cases from Rajasthan which were referred were included as they have a different clinical presentation. It was interesting to note that the fatal case from Rajasthan showed hemorrhagic manifestations and nosocomial infections noted among the healthcare workers attending the case. Since an extreme manifestation of disease was observed in the fatal case from Rajasthan state,

we selected referred clinical samples from the neighboring state (Gujarat) to understand disease profile in these two states.

2.3. Sample collection

Clinical specimens viz. serum, urine, throat swab, vesicle fluid, crust (if present) were collected from enrolled cases ($n = 50$) from Mumbai district. Follow up samples were also taken at different post onset day (POD) to see the detection of positivity of VZV by real-time PCR.

Blood sample (3–5 ml) collected by vein puncture taking all aseptic precautions by vein puncture and after clotting serum was stored at $-20\text{ }^{\circ}\text{C}$. Throat swab collected using a polyester swab and was immediately transferred to Viral Transport Media. Vesicular fluid from the lesions was collected using a sterile needle and sterile polyester swab. A sterile needle was used to un-roof the top of the vesicle. A sterile swab was then used to swab the base of the lesion applying enough pressure to collect epithelial cells without causing bleeding and collect vesicular fluid. It was necessary to collect infected epithelial cells from the base of the lesion because they usually contain a significant amount of virus. Swabs were then placed individually into separate microfuge tubes to avoid contamination and were kept dry. Crust/swabs, if available was lifted off the skin using a glass slide and transferred directly in microfuge tubes. Urine samples were collected in a screw-capped leak-proof container. All the clinical samples were transported in dry ice to ICMR-NIV, Pune for further investigations.

2.4. Data entry and statistical analysis

The patient's clinical data were registered in Microsoft Excel version 2010 and analysis was performed using Epi-info software version 7.2.1.0. Statistical tools used were a mean and standard deviation, proportions and percentages.

2.5. Quantitative real-time PCR, PCR, and sequencing

Real-time PCR and PCR was performed on the clinical samples to detect the presence of VZV DNA (Weidmann et al., 2003). DNA from samples was extracted by using Qiagen complete nucleic acid extraction kit (QiaAmp total nucleic acid extraction kit, Cat No/ID: 52906). The real-time PCR reactions were set up using Invitrogen qPCR kit (Invitrogen, USA; Cat. number: 18080044). The samples found positive by real-time PCR were sequenced for ORF 22, ORF 38, ORF 54 (to identify the clade), ORF 63 (as this is the largest sequence of VZV available from India, Accession number: JN315707.3) and gB protein (the smallest envelope glycoprotein). This also allowed in determining the clustering of sequences from samples.

Sequencing was performed using Big Dye Terminator cycle sequencing technology and ABI3130xl genetic analyzer (Applied Biosystems California, USA).

Primers used for amplification and sequencing of these regions is described in Table 1. The sequences obtained were assembled using Sequencher 5.0 and deposited in the GenBank. The accession numbers obtained from GenBank for deposited sequences are as follows: Glycoprotein B [MF503692–MF503711], ORF-22 [MF503712–MF503738], ORF-38 [MF503739–MF503771], ORF-54 [MF503772–MF503806] and ORF-63 [MF503807–MF503829]. Apart from the

Table 1. List of primers used for amplification and sequencing.

Region	Primer Name and sequence	Primer Position	Product Size	Reference	
<i>Bgl</i> I site (ORF 54)	VZV-54BgII_F; 5'CGTAATGCATAACAGGCCAACAC3'	95005–95027	496bp	Loparev et al., 2007	
	VZV-54BgII_R; 5'GAAACCTGGCGTCAAACATTACA3'	95501–95479			
ORF 22 (Region containing 4 SNP sites)	VZV_p22R1_F; 5'GGG TTT TGT ATG AGC GTT GG3'	37837–37856	546bp		
	VZV_p22R1_R; 5'CCC CCG AGG TTC GTA ATA TC3'	38383–38356			
<i>Pst</i> I site (ORF 38)	VZV_ORF38_F; 5'AATGGTCCGTATT AAATCTTGAGCAAC3'	69121–69148	550bp		
	VZV_ORF38_R; 5'CAAGTATAATTGTTAGTCGGCGCG3'	69648–69671			
ORF 63	VZV_ORF63_F; 5'GCCGCTAGCCATATGATGTTTT GCACCTCACCGCTA3'	110703–110724	960bp	Primers designed during this study	
	VZV_ORF63_R; 5'GCCGGATCCCTACACGCCA TCGGGGGGGCGGTATAT3'	115539–111514			
Glycoprotein B	VZV_gB_F1; 5'ATGTCCCCTTGTGGCTATTATT CAAAG3'	56819–56845	2772bp		
	VZV_gB_R1; 5'TTACACCCCGTTA CATTCTCGGT3'	59614–59591			
	VZV_gB_IntR2; 5'GAAACGGGAATTGGTACCCT ATCC3'	57420–57397			Sequencing Primers
	VZV_gB_IntF2; 5'GATGTTATCGTTAGCA CGGCGTGG3'	57335–57358			
	VZV_gB_IntR3; 5'CAACCTCACGCCACTTGACA AGC3'	57950–57927			
	VZV_gB_IntF3; 5'AGTCACGCCTCAT TTAACGGTTGG3'	57868–57890			
	VZV_gB_IntR4; 5'TAACGTCGCCGAG AATACGAGC3'	58553–58532			
	VZV_gB_IntF4; 5'GCGGACTATTTCCAATTAACCCA3'	58470–58492			
	VZV_gB_IntR5; 5'GTGGTAAATCCGTGTACGGT GG3'	59160–59139			
	VZV_gB_IntF5; 5'CGTTGGACATGTGGTTCTTGG3'	590090–59118			

sequences retrieved from this study, representative sequences belonging to different clades were downloaded from the GenBank database of National Center for Biotechnology Information (NCBI). The retrieved sequences encompasses VZV-Dumas (clade-I), Oka Varicella vaccine virus (V-Oka), commercially produced VariVax strain (clade-II), HJO strain from Germany (clade-III), DR strain from Morocco, US (clade-IV) and CA123 and 413/2000 (clade-V) for sequence analysis as analyzed by Tamura (Tamura et al., 2013). A phylogenetic tree was generated for the envelope protein gB for a stretch of 2688 bps (region: 57008-59555). Alignment and tree construction was performed using MEGA V6.0 (Tamura et al., 2013). A phylogenetic tree was created with Maximum Likelihood method using the best substitution model with pair-wise deletion of the missing data. A bootstrap value of 1000 replication was used to generate the phylogenetic tree. An unrooted tree was obtained during the analysis as no out-group was considered during the study. The obtained sequences were also analyzed using the ORF-22 method as described by Loparev (Loparev et al., 2007).

3. Results

3.1. The clinical profile of acute cases of suspected Varicella (n = 50) from Infectious Disease Hospital Mumbai

Vesicular rash was found amongst all cases (100%) (Fig. 1). Common clinical features included high-grade fever (88%, 44/50), headache (52%, 26/50), body ache (58%, 29/50) with malaise and dry cough (36%, 18/50). Other symptoms included nausea (22%, 11/50), vomiting (4%, 2/50) and abdominal pain (4%, 2/50). Thirty-four cases (68%) were from the age group of 18–25 years signifying delay in age onset presentation of Varicella (though being a childhood disease) (Table 2). History

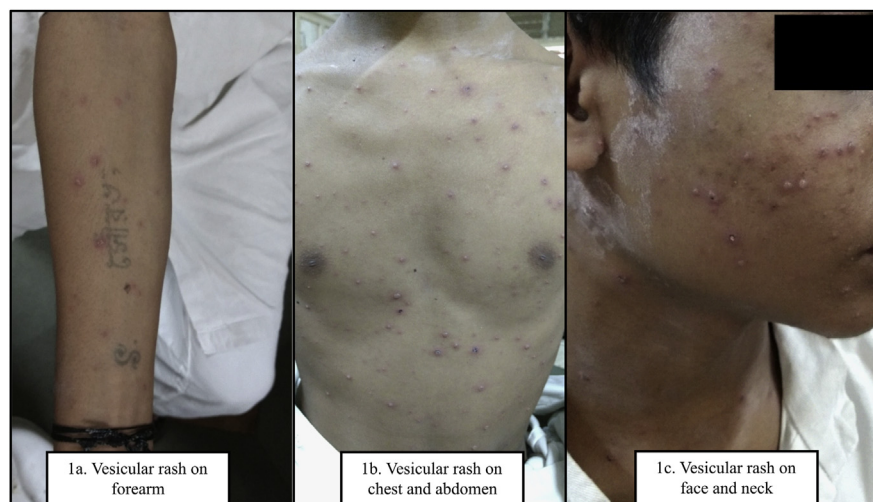


Fig. 1. Typical vesicular lesion of varicella seen among patients.

Table 2. Clinico-epidemiological profile of VZV cases investigated from Mumbai, Maharashtra, India (n = 50).

Clinical Profile (n = 50)	N	Percentage (%)
Vesicular Rash	50	100
Fever	44	88
Cough	18	36
Headache	26	52
Bodyache	29	58
Nausea	11	22
Vomiting	2	4
Pain in abdomen	2	4
Age wise distribution (n = 50)		
<18 years	4	8 %
18–25 years	34	68 %
26–35 years	9	18 %
>35 years	3	6 %
History of addiction (n = 50)		
Alcohol	5	10 %
Tobacco chewing	22	44 %
Smoking	1	2 %
Others	0	0
No addiction	21	42 %
History of contact with similar cases (n = 50)		
Yes	41	82 %
No	9	18 %
Occupational History		
Jari workers	44	88 %
Labors in gold shop	4	8 %
Labors at construction sites	1	2 %
Others	1	2 %

of tobacco chewing (44%, 22/50), alcoholism (10%, 5/50) and smoking (2%, 1/50) were also elicited from patients. One patient had a history of Type 2 Diabetes Mellitus and Tuberculosis. No other immune-compromised conditions were noted. All 50 cases were residing in the slum areas of Mumbai. Forty-one cases (82%) showed positive contact history with a known case of clinically diagnosed chickenpox (friends/room-mates/co-workers) signifying high secondary attack rate amongst Varicella cases. Majority of cases (90%) were working as Jari workers (making aesthetically decorative clothing) and as labor in a gold shop and were migrant from West Bengal and Orissa. None of these 50 cases were immunized previously with Varicella vaccine nor had Varicella in the past, and no case fatality was reported. As the patients were admitted in the isolation ward of the hospital and

adequate safety measures like vaccination of healthcare workers were taken, no cases were reported among the healthcare workers attending the patients. Clinical samples (serum, urine, throat swab, vesicular fluid, and crust) were tested for the presence of VZV with real-time PCR.

3.2. Laboratory findings of VZV cases from Mumbai, Maharashtra state

Vesicular fluids of all 50 suspected VZV samples were tested and found to be positive for VZV by real-time PCR. As per the feasibility of collection and attrition, 22 patients were followed up for the presence of VZV DNA by real-time PCR in serum, urine, throat swab, vesicle fluids, and crust (if available). Fig. 2 depicted VZV positivity of clinical samples by real-time PCR. It was observed that vesicle fluid was persistently positive for real-time PCR till 9th POD, which suggests that vesicle fluid is a perfect clinical specimen for diagnosis of VZV infection. Positivity by real-time PCR at 2nd 3rd and 4th POD was also found in clinical samples like serum, throat swab and urine.

3.3. Laboratory findings of VZV cases referred from Rajasthan and Gujarat states

Clinical sample of 35-year-old patient with a history of abdominal pain, fever, nausea, bleeding from nose and rash all over body was referred by civil hospital Barmer district, Rajasthan to civil hospital Jodhpur, Rajasthan [this case was later

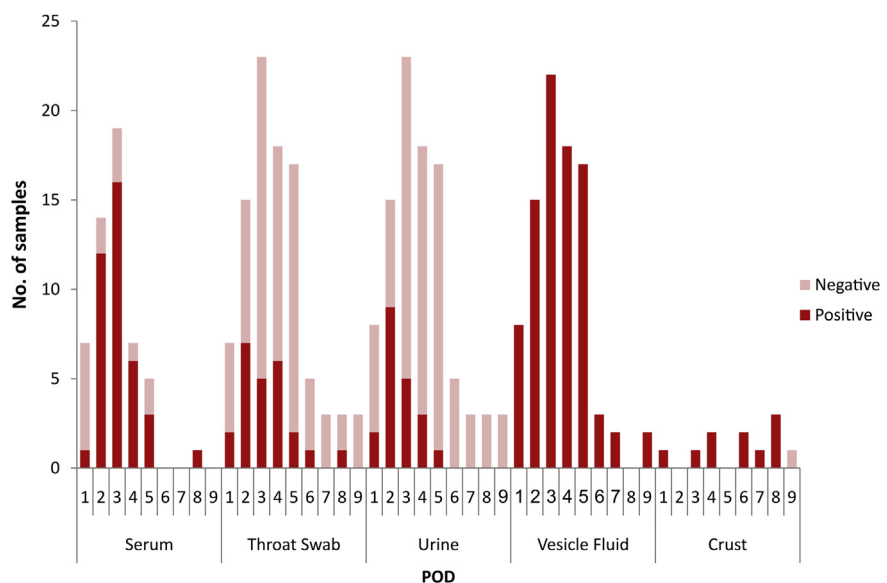


Fig. 2. VZV positivity of serum, throat swab, urine, Vesicular fluid, and crust clinical specimens by Real-time PCR at different POD interval.

shifted to tertiary care hospital in New Delhi]. The patient died despite all efforts of revival within three days of onset of illness due to disseminated intravascular coagulation and internal bleeding. The autopsy samples were showing vesicular bullous rashes and liver showing multiple petechial spots. The clinical and autopsy samples were referred to ICMR-NIV, Pune in February 2017 for the diagnosis of viral hemorrhagic fever. The sample was found to be positive for VZV by real-time PCR and negative for other viral hemorrhagic fevers like Crimean Congo Hemorrhagic Fever (CCHF), Dengue, Chikungunya and Kyasanur Forest Disease (KFD). Four out of eleven contact healthcare personnel who were involved in the patient care of the deceased case from Rajasthan state also showed symptoms of fever with vesicular rash and their samples were tested and found positive for VZV by real-time PCR.

Clinical samples ($n = 17$) of suspected VZV cases from Gujarat state [Gandhi Nagar district ($n = 5$) and Surat district ($n = 12$)] with a clinical history of fever with vesicular rash were referred to ICMR-NIV, Pune for diagnosis and identification of viral etiological agent. All the samples ($n = 17$) were positive for VZV by real-time PCR.

Based on results of PCR based sequencing, samples from two contact cases of the 35-year-old deceased patient from Rajasthan state and samples from Maharashtra state were sequenced to identify VZV clade and variation in viral sequence if any. A total of the approximately 4.5kb sequence was obtained from partial ORF 22 (346bp), ORF38 (445bp), ORF54 (388bp), ORF63 (700bp) and Glycoprotein gB (2688bp).

Based on the analysis of SNPs from ORF22 of all sequences obtained during this study, twenty sequences belonged to M1 genotype, which corresponded with clade 5, while three sequences belonged to M2 genotype corresponding to clade 4 (Table 3). All the 23 sequences obtained were $BglI^+PstI^+$ and corresponded to the Asian strains of VZV. The three sequences belonging to M2 genotype (Accession numbers: MF503717, MF503719, MF503731) were from Mumbai district of Maharashtra. Based on phylogenetic analysis of the gB protein, thirteen sequences belonging to the M1 genotype were clustered with clade 4/5 and three sequences belonging to M2 genotype were clustered with clade 1/3. Based on Clustal W based alignment of ORF 63 region, sequences (Accession numbers: MF503814, MF503816, MF503813, MF503829, MF503826 and MF503828) differed in a single nucleotide position at 110877, 1102328, 1102328, 1102340, 1102340 and 1102340 respectively.

The samples of clinical cases from Rajasthan, which showed hemorrhagic manifestation and nosocomial infections, demonstrated the presence of already circulating strain (Clade 4 and 5) (Schmidt-Chanasit and Sauerbrei, 2011). Hence, no correlation with disease severity or virulence was observed by the presence of any particular circulating strain.

Table 3. Single nucleotide polymorphism of partial ORF 22, *PstI* site (partial ORF 38) and *BglI* site (partial ORF 54) based analysis of VZV sequences.

Accession numbers for ORF-22	Genotype	Clade	37902	38019	38055	38081	38177	38229	<i>PstI</i> (ORF 38)	<i>BglI</i> (ORF 54)
MF503712	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503713	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503714	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503715	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503716	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
* MF503717	M2	1/3	A	G	C	C	A	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503718	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
* MF503719	M2	1/3	A	G	C	C	A	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503720	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503721	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503722	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503723	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503724	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503725	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503726	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503727	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503728	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503729	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503730	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
* MF503731	M2	1/3	A	G	C	C	A	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503732	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503733	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺
MF503734	M1	4/5	A	G	T	C	G	A	<i>PstI</i> ⁺	<i>BglI</i> ⁺

* Samples showing mutation in sequence in comparison to other circulating sequences.

4. Discussion

The present study focuses on the epidemiological and clinical presentation of VZV cases investigated in Mumbai, Maharashtra state. This study also focuses on the trends of VZV seen in India in three states with different clinical presentations thus making differential clinical diagnosis difficult and critical. The need for the universal use of personal protective equipment by healthcare providers along with Varicella vaccination to prevent the infection amongst the healthcare providers has been highlighted based on observations in the fatal case of Rajasthan state which resulted in secondary infections in 11 healthcare providers including doctors, nurses and paramedical staff (Burgess et al., 1999). During an outbreak situation, it becomes difficult to manage with the shortage of beds and available healthcare staff.

Adherence to strict hospital infection control guidelines and hand washing becomes important to reduce the nosocomial spread. It is also necessary to understand the immune status of the healthcare workers before allocating them to care for Varicella infected patients. Immunization and antibody titers against VZV should be known before allowing them to work in Varicella wards. Understanding the importance of cohorting (in 2–3 cohorts) Varicella patients so that other general patients will not be infected is necessary (Aitken and Jeffries, 2001). Varicella is usually seen in winter and spring seasons, but it is interesting to note that there has been a rise in cases even in the months from February to April. Overcrowding and poor ventilation in slums and dwelling places in urban cities specifically increase the secondary attack rate (Heininger and Seward, 2006; Lee, 1998; Verma et al., 2011). VZV patients are usually treated with Acyclovir a potent antiviral to reduce the complications and hasten the healing of lesions (Wallace et al., 1992). Our study showed positivity by real-time PCR in follow up of twenty-two clinical samples like serum, throat swab, and urine. Similar findings were reported by Shinji Kido et al., 1991 and Jessica Leung et al., 2010.

Usually, cases of VZV are confirmed based on clinical presentations. Molecular detection methodologies are rarely needed for confirmation of VZV diagnosis. Therefore, only a few studies describing the VZV strain circulation pattern in India are available (Kaushik et al., 2008; Biswas et al., 2011; Chow et al., 2013). The studies were carried out to understand whether the strains circulating in three different states differed from the vaccine strain (Oka strain, Japan). It was found upon restriction digestion analysis that the Indian strains were $BgII^+PstI^+$ (wild type) as compared to the vaccine-derived Oka strain, which was $BgII^+PstI^-$, thus specifically segregating these strains. Change in position of two nucleotides in the gB protein (Positions 57302: C → A, 57956: T → C) resulted in differential segregation of three sequences (Accession numbers: MF503696, MF503702, and MF503708) into clade 1/3 as observed in Fig. 3. A detailed sequence-based analysis using a larger number of VZV sequences needs to be done to understand the significance of these nucleotides in the gB protein. This study had demonstrated the circulation of multiple clade (1, 3, 4 and 5) from different clinical samples of VZV cases. The study was based on limited clinical samples from three states and hence the findings could not be generalized to the general populations in the three states. This study is a step towards identification of new circulating clades (1 and 3) in the Maharashtra, which has not been reported till date.

Universal Immunization Program (UIP) in India does not include the vaccination against Varicella. But the increase in the cases in adulthood with high secondary rate and complications is suggestive of the need of inclusion of Varicella vaccination in UIP schedule. Two doses of Varicella vaccine have more than 85% efficacy in preventing Varicella infection and more than 95% in preventing complications (Heininger and Seward, 2006; Lee, 1998; Verma et al., 2011). Isolation of 7–10

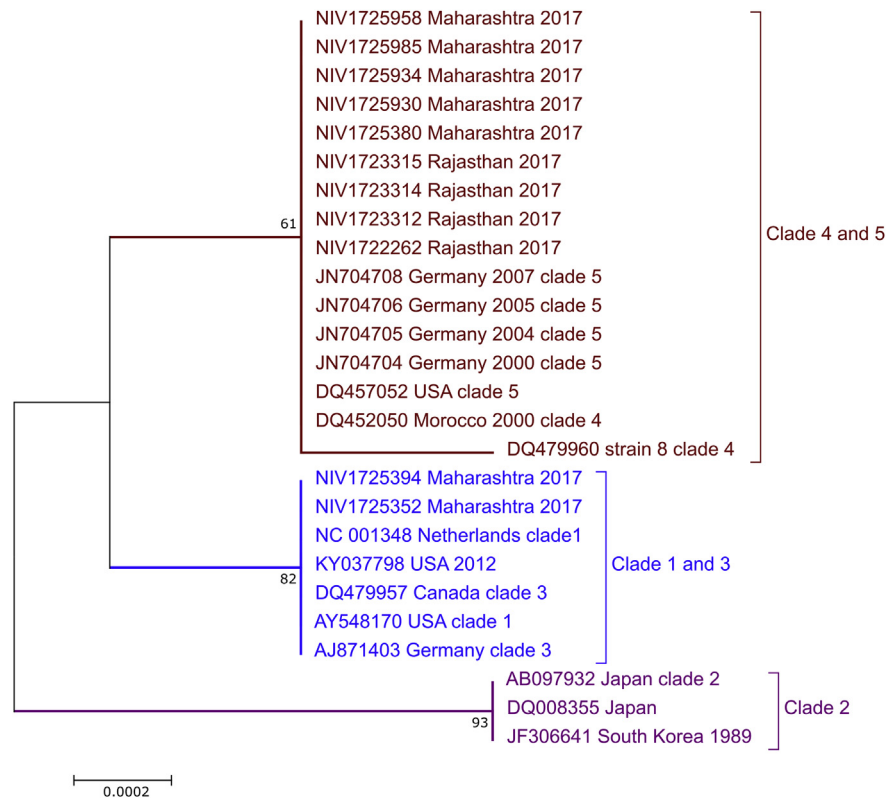


Fig. 3. Phylogenetic tree of representative VZV gB sequences retrieved from the study along with reference VZV sequences downloaded from GenBank.

days for all the cases of Varicella increases the hospital stay and also imposes an economic burden on the patients including loss of wages. Health education activities need to be strengthened to make the population aware of the disease and focus should be on early diagnosis and isolation reducing the secondary attack rate and burden of disease.

In the current study, we sequenced fragments of the VZV genome that had helped in identifying new clades (clade 1 and 3) circulating in Maharashtra state. Results indicate that virus sequences obtained from the majority of the samples, irrespective of the state from which they were obtained, belonged to genotype M1 based on single nucleotide polymorphism analysis while three sequences belonged to genotype M2. Phylogenetic tree based on 16 representative gB glycoprotein sequences showed that the virus strains formed two clusters: 13 sequences in a cluster of clade 4/5 and three sequences in a cluster of clade 1/3 (Fig. 3).

Declarations

Author contribution statement

Rima R. Sahay: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Pragya D. Yadav: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Triparna Majumdar, Swapnil Patil, Prasad Sarkale, Anita M. Shete, Savita Patil: Performed the experiments.

Gouri Chaubal: Performed the experiments; Wrote the paper.

Vinay R. Dange: Contributed reagents, materials, analysis tools or data.

Dimpal A. Nyayanit: Analyzed and interpreted the data; Wrote the paper.

Jayanthi Shastri: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Devendra T. Mourya: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

Data associated with this study has been deposited at GenBank data base of National Center for Biotechnology Information (NCBI) under the accession numbers MF503712–MF503734 for ORF-22 MF503814, MF503816, MF503813, MF503829, MF503826 and MF503828 for ORF-63.

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