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# Genomic characterization unravelling the causative role of SARS-CoV-2 Delta variant of lineage B.1.617.2 in 2nd wave of COVID-19 pandemic in Chhattisgarh, India

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## ABSTRACT

COVID-19 pandemic 2nd wave catastrophic effect in the state of Chhattisgarh, India, from where no exclusive genomic data yet published, has prompted us to undertake this study to unearth the causative variant. Whole-genome sequencing of SARS-CoV-2 isolated from COVID-19 infected nine vaccinated healthcare workers (HCW), thirty mild/moderate, seventeen severe, and twenty-seven deceased patients, was performed. The significant predominance of the SARS-CoV-2 variant of concern (VOC), Delta (lineage B.1.617.2) identified in sixty-four (77.1%) cases in contrast to B.1 and its sublineage in eleven (13.2%), variant under monitoring (VUM), Kappa (lineage B.1.617.1) in five (6.0%) and another VOC Alpha (lineage B.1.1.7) in three (3.6%) cases respectively ( $p < 0.05$ ,  $\chi^2 = 162.49$ ). 88.8% vaccine breakthrough, 60% mild/moderate, 94.4% severe and 81.5% dead patients were infected by Delta. Kappa presents exclusively in mild/moderate, Alpha in vaccine breakthrough, mild/moderate, and dead patient and B.1 and its sublineages in mild, severe, and dead patient categories. Delta variant spike mutation of T19R, G142D, E156G, L452R, and deletion (F157 and R158) helps in escaping antibody response, T478K and D614G enhance viral affinity with ACE2 receptor while P681R and D950N result in higher replication and transmissibility by cleaving S1/S2 at furin site. We conclude that Delta variant predominant role along with co-occurrence of Kappa, Alpha, and B.1 variant during COVID-19 2nd wave pandemic in Chhattisgarh may pose a potential threat of future outbreak through hybrid variant evolution. Thus, intensive genomic surveillance for monitoring variant evolution and a more efficacious vaccine against the Delta and Alpha variants are required.

## 1. Introduction

COVID-19 disease pandemic caused by a novel coronavirus (nCoV) named SARS-CoV-2 has so far posed the biggest public health challenge of the 21st century across the globe since its emergence in December 2019 from Wuhan, China. India, the second most affected country after the United States of America (USA), although managed first wave reasonably well, was unfortunately hit hard by 2nd wave of COVID-19 pandemic. 2nd wave of the pandemic that started around March 1, 2021 has accounted for eighteen million laboratory-confirmed cases and

two hundred five thousand death at average daily death of over 2000 till 11th June 2021 [1]. The highest single-day number of cases rose to four hundred fourteen thousand on 6th May 2021. The worst affected states reported were Maharashtra, Karnataka, Kerala, Delhi, Andhra Pradesh, Telangana, Madhya Pradesh, and Chhattisgarh. This second wave was largely uncontrollable and unmanageable as it nearly crippled the nation's healthcare system and led to an unprecedented rise in COVID cases and deaths. The severe shortage of hospitals beds, oxygen supply, and medicine have haunted humanity as overwhelmed crematoria were seen working round the clock to keep up with the pace of dead bodies.

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Patients presented more with the clinical features of difficulty in breathing, fever, headache, myalgia, acute respiratory distress syndrome (ARDS), pneumonia, severe lung damage, gastrointestinal symptoms, and multiorgan failure. National guidelines have classified the patients into mild (normal saturation), moderate (pneumonia with no severe disease sign), and severe (severe pneumonia) [2]. Strict implementation of early diagnosis, treatment, contact tracing, lockdown, masking, and restricting social gathering has eventually brought down 2nd wave disastrous effect under control by mid of June 2021.

The emergence of different variants of SARS-CoV-2 has been reported as the plausible explanation of 2nd wave [3,4]. As per WHO information latest on 25th November 2021, SARS-CoV-2 variants along with their country of origin, namely Alpha (B.1.1.7), United Kingdom, Beta (B.1.351), South Africa, Gamma (P.1), Brazil, and Delta (B.1.617.2), India have been classified as VOC while Kappa (B.1.617.1) variant was reclassified from earlier Variant of Interest (VOI) on 4th April 2021 to Variant under Monitoring (VUM) on 20th September 2021 (Fig. 1) [5]. The various other lineages along with their country of origin have been recognized under a group named 'Alerts for Further Monitoring' [5].

The causative nCoV variant of pandemic 2nd wave from various states of India has been reported [3,4]. Delta variant lineage B.1.617.2 outpacing B.1.1.7 in Delhi and B.1.617 identification from Maharashtra have been reported [3,4].

Chhattisgarh, one of the largest central states of India, was also severely affected by COVID-19 2nd wave, with majorly affected cities were Raipur (capital city of Chhattisgarh), Bhilai and Durg. Around 662,325 people were found infected, including 9275 deaths [1]. So, the need for whole-genome sequencing of SARS-CoV-2 isolated from COVID-19 patients categories of vaccine breakthrough, mild/moderate, severe and deceased from these districts of Chhattisgarh was utmost felt to unravel the variant/s responsible for this menace.

Accordingly, the present study was undertaken to genetically characterize SARS-CoV-2 strains obtained from COVID-19 patients in Chhattisgarh by sequencing the whole genome to identify accumulated viral mutation/s, strains, and lineages. The information, thus generated, would be utilized for formulating policies for effective management of the future course of the pandemic.

## 2. Materials and methods

### 2.1. Samples collection

All India Institute of Medical Sciences (AIIMS), Raipur, and its Viral Research and Diagnostic Laboratory (VRDL) are the state nodal tertiary care treatment and diagnostic center for SARS-CoV-2 since the emergence of COVID-19 in the state of Chhattisgarh in March 2020. A randomly selected eighty-three (83) laboratory-confirmed cases of COVID-19 by real-time PCR showing Ct value of less than 25 for E and RdRP gene of SARS-CoV-2 were included in the study. These cases comprised fifty-three males (63.8%) and thirty (36.2%) females. Their collective mean age was 47.5 yrs being 45.9 and 50.2 years for males and females respectively (Supplementary Table). The majority of these cases were reported from the districts of Durg, Bhilai, and Raipur of Chhattisgarh, between 1st March to 5th May 2021 (Fig. 2). These cases comprised of vaccine breakthrough HCW (n = 09, 10.8%), mild/moderate symptomatic (n = 30, 36.14%), severe (n = 17, 20.4%) and deceased patients (n = 27, 32.7%). The viral RNA from all these cases were isolated from the collected combined clinical specimen of nasopharyngeal (NPS) and oropharyngeal swab (OPS) from every patient before anti-COVID treatment by following earlier described method [6]. All dead patients had a history of providing their specimen on the first day of hospitalization. Unfortunately, these patients were reported to succumb to COVID-19 during treatment. The institutional ethical approval obtained wide approval number 1453/IEC-AIIMSRPR/2021.

### 2.2. Whole genome sequencing of SARS-CoV-2

The isolated RNA from all the eighty-three cases was used for their cDNA and library preparation using QIAseq SARS-CoV-2 Primer Panel and QIAseq FX DNA Library Unique Dual Index (UDI) Kit from Qiagen GmbH, Germany. The enriched multiplexed amplicons were purified through AMPure XP bead (Beckman Coulter) and quantified using a Qubit dsDNA High Sensitivity Assay Kit (Invitrogen, USA) on Qubit 4 fluorometer (Invitrogen, USA). The fragmentation was performed to reduce median fragment size to approximately 250bp, and it was followed by end repairing. Sequencing adapters were then added to ligate to both ends of the DNA fragmented amplicons. The AMPure XP bead purification was done to remove excess of dNTPs, salts, free adapters and

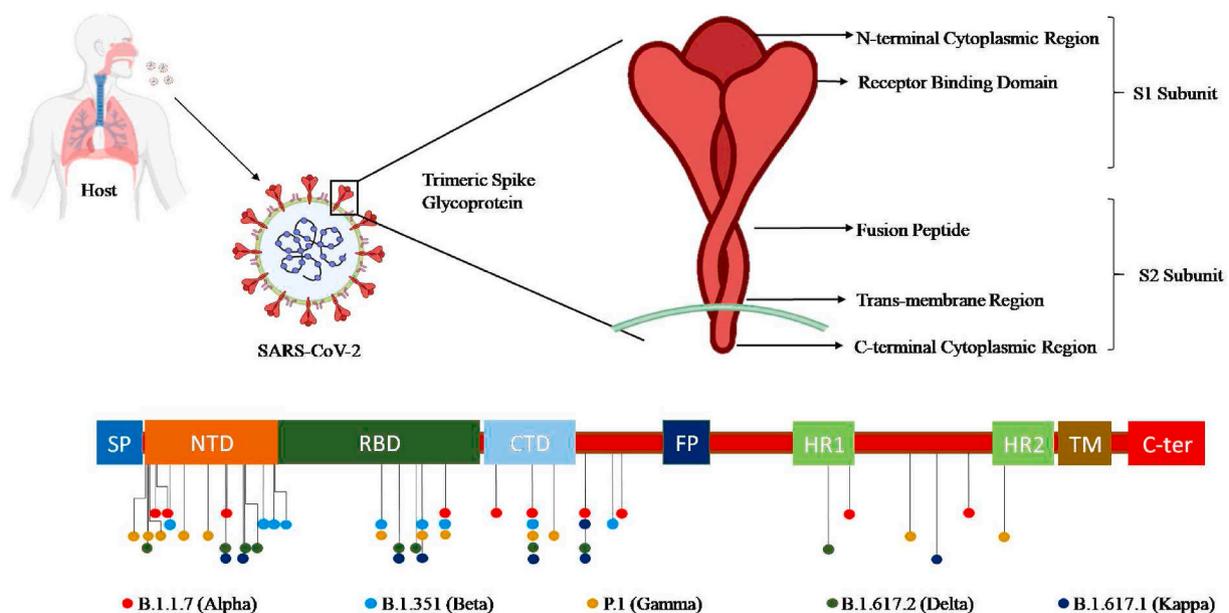
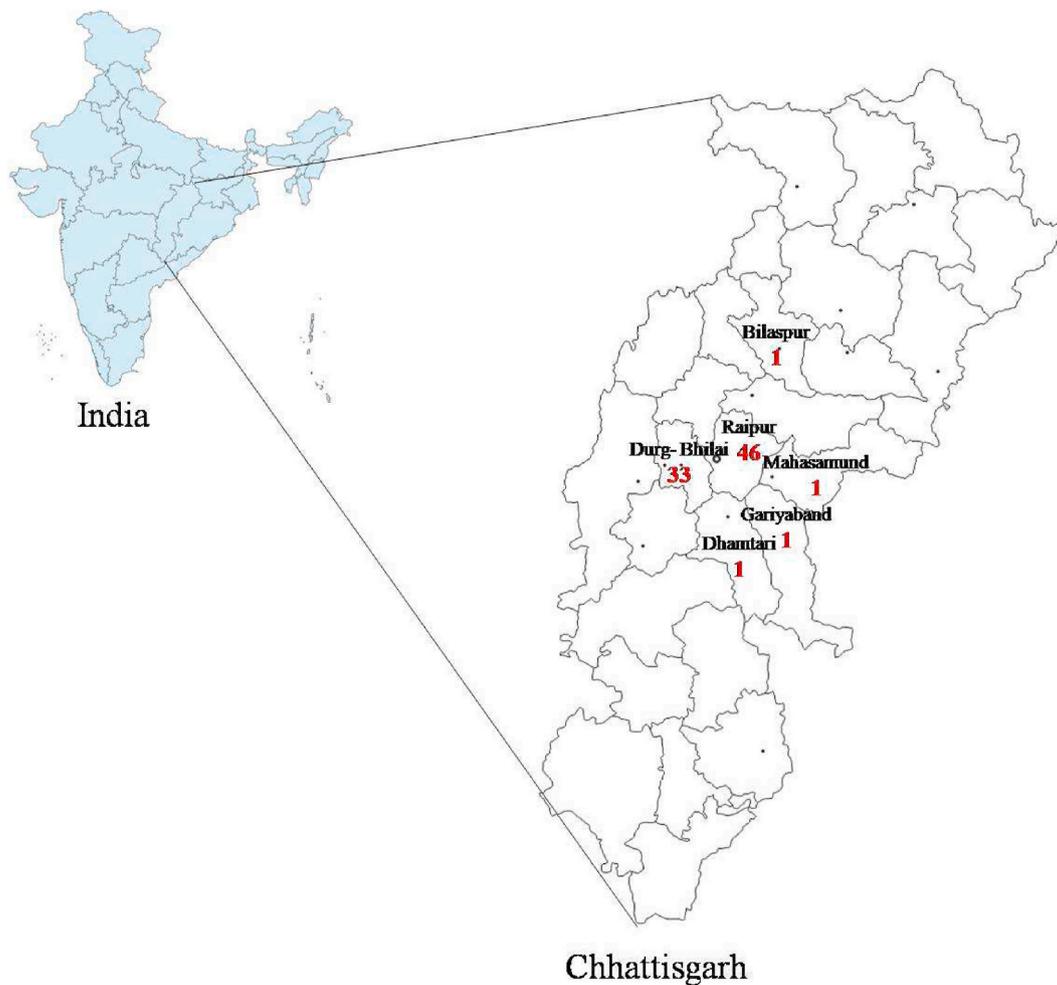


Fig. 1. WHO Classified VOC Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1) and Delta (B.1.617.2) and VUM Kappa (B.1.617.1) showing specific signature mutational pattern.



**Fig. 2.** Schematic representation of a central state of Chhattisgarh in India showing worst effected district during 2nd wave and number of cases included in the study.

enzymes. The purified ligated fragmented DNA amplicons were finally amplified to enrich the library with anti-adaptor primers and then purified using AMPure XP beads. Library obtained were then checked for quality by Bioanalyzer 2100, Agilent, US using Agilent High Sensitivity DNA Chip kit, Germany. The fragmented library amplicons are quantified as described above. Thereafter, library was diluted to normalize and then pooled. Finally, the pooled library were sequenced in the MiniSeq platform of Illumina, US, using MiniSeq Mid Output Reagent Cartridge from Illumina (300 cycles) in  $150 \times 2$  PE read and FastQ only mode using MiniSeq local run manager.

### 2.3. Mutational and phylogenetic analysis

Individual consensus sequences of eighty-three nCoV isolates obtained using QIAGEN CLC Genomics Workbench version 21.0.4 were submitted in Global Initiative on Sharing Avian Influenza Data (GISAID) (Supplementary Table). The subsequent mapping and alignment of these sequences against the SARS-CoV-2 Wuhan variant reference sequence (GenBank ID NC\_045512) were done to analyze mutational changes. The phylogenetic tree was constructed by the Neighbour-joining (NJ) method using MEGA-X version 10.2.

### 2.4. Structure modelling and evaluation

The 3D modeling of spike glycoprotein of the Wuhan reference prototype and our predominant identified lineage of Alpha, Delta and Kappa was performed using the SWISS-MODEL server and PyMOL to

study the effect of the specific amino acid (AA) mutational changes [7].

### 3. Results

The whole-genome sequence analysis of SARS-CoV-2 from all eighty-three cases showed major nonsynonymous mutational AA changes primarily in viral receptor spike gene in comparison with reference Wuhan prototype (Table 1). Spike sequence alignment and phylogenetic analysis showed that sixty-four (77.1%) sequences belonged to VOC Delta variant of lineage B.1.617.2 and clade G/478 K.V1 (Figs. 3 and 4). Among these, forty-seven (47, 56.6%) sequences exhibiting eight nonsynonymous mutations (T19R, G142D, E156G, L452R, T478K, D614G, P681R and D950 N) and two deletions of F157del and R158del have shown two mutational group of with (twenty seven sequences) and without (twenty sequences) G142D mutation (Table 1). Seventeen sequences (20.4%) harbored six nonsynonymous changes of T19R, L452R, T478K, D614G, P681R, and D950 N and no deletions. Five (6.0%) sequences resembled Kappa variant (lineage B.1.617.1/clade G/452 R.V3) and exhibited changes of L452R, E484Q, D614G, and P681R. Three (3.6%) sequences belonged to the Alpha variant (lineage B.1.1.7/clade GR/501Y.V1) and showed a mutational pattern of N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, and three deletions of H69, V70, and Y144. Importantly, eleven (13.2%) sequences belonged to B.1 lineage, and on its sub-lineages analysis, two sequences belonged to B.1 whereas others nine belonged to B.1 sublineage (02- B.1.216, 01- B.1.1.306, 01- B.1.1.326, 01- B.1.36.10, 01- B.1.36.17 and 03- B.1.36.29). These sequences were relatively found with fewer variations

**Table 1**  
Mutational pattern and GISAID allotted lineage of the eighty three sequences observed on alignment of spike region against reference Wuhan sequence.

Lineage	Mutation	Accession number	Clinical status			
B.1.1.7 (3)	H69del, V70del, Y144del, N501Y, A570D, D614G, P681R, T716I, S982A, D1118H	EPI_ISL_1731751	Vaccinated (1)			
		EPI_ISL_2098713	Dead (1)			
		EPI_ISL_1914586	Mild (1)			
	B.1.617.2 (64)	L452R, T478K, D614G, P681R	EPI_ISL_2307104	Dead (2)		
			EPI_ISL_2620739	Mild (1)		
			EPI_ISL_1914588	Severe (2)		
		L452R, T478K, D614G, P681R, D950 N	EPI_ISL_2307110, EPI_ISL_2307123, EPI_ISL_2307126, EPI_ISL_2307134, EPI_ISL_2620742	EPI_ISL_2307114	Dead (5)	
				EPI_ISL_2307114	Severe (4)	
				EPI_ISL_2307121, EPI_ISL_2307125, EPI_ISL_1914596, EPI_ISL_2098720, EPI_ISL_1914597, EPI_ISL_1969753	Mild (3)	
			T19R, E156G, F157del, R158del, L452R, T478K, D614G, P681R, D950 N	EPI_ISL_1731755, EPI_ISL_2307136, EPI_ISL_1914592, EPI_ISL_2307117, EPI_ISL_2307120	EPI_ISL_1914584	Vaccinated (2)
					EPI_ISL_1914593, EPI_ISL_1914595, EPI_ISL_2098714, EPI_ISL_2098716-19, EPI_ISL_2098721	Dead (3)
					EPI_ISL_2307105, EPI_ISL_2307112-13, EPI_ISL_2307115, EPI_ISL_2307124, EPI_ISL_2307128	Mild (9)
T19R, G142D, E156G, F157del, R158del, L452R, T478K, D614G, P681R, D950 N				EPI_ISL_1731752-54, EPI_ISL_2307132-33, EPI_ISL_2307135, EPI_ISL_2307107, EPI_ISL_2620740, EPI_ISL_2620741, EPI_ISL_2307102, EPI_ISL_2307109, EPI_ISL_2307116, EPI_ISL_2307118, EPI_ISL_2307119, EPI_ISL_2307122, EPI_ISL_2307129, EPI_ISL_2307130, EPI_ISL_2307131	EPI_ISL_1914591, EPI_ISL_1914598, EPI_ISL_2098715, EPI_ISL_2098722, EPI_ISL_2307108, EPI_ISL_2620738, EPI_ISL_2098710-11, EPI_ISL_2307127	Severe (6)
					EPI_ISL_1914585, EPI_ISL_1914587, EPI_ISL_1969752, EPI_ISL_1914583	Vaccinated (6)
					EPI_ISL_1914583	Dead (12)
	B.1.617.1 (5)			L452R, E484Q, D614G, P681R	EPI_ISL_1914585, EPI_ISL_1914587, EPI_ISL_1969752, EPI_ISL_1914583	Mild (5)
					EPI_ISL_1914591, EPI_ISL_1914598, EPI_ISL_2098715, EPI_ISL_2098722, EPI_ISL_2307108, EPI_ISL_2620738, EPI_ISL_2098710-11, EPI_ISL_2307127	Mild (3)
					EPI_ISL_1914585, EPI_ISL_1914587, EPI_ISL_1969752, EPI_ISL_1914583	Mild (1)
		E154K, L452R, E484Q, D614G, P681R, Q1071H		EPI_ISL_1731757	G142D, E154K, L452R, E484Q, D614G, P681R, Q1071H	Mild (1)

**Table 1 (continued)**

Lineage	Mutation	Accession number	Clinical status
B.1 (2)	D614G	EPI_ISL_2307103	Dead (1)
	D614G, P681R, D950 N	EPI_ISL_2307106	Dead (1)
B.1.1.216 (3)	D614G	EPI_ISL_1731756	Dead (1)
		EPI_ISL_1914579	Mild (1)
B.1.1.306 (1)	D614G	EPI_ISL_1914589	Mild (1)
B.1.1.326 (1)	D614G	EPI_ISL_2098711	Severe (1)
B.1.36.10 (1)	D614G	EPI_ISL_1914590	Mild (1)
B.1.36.17 (1)	N440K, D614G	EPI_ISL_1914580	Mild (1)
B.1.36.29 (2)	N440K, D614G	EPI_ISL_2098712	Dead (1)
		EPI_ISL_1914581, EPI_ISL_1914582	Mild (2)

as D614G was dominantly found in all sequences, and only four sequences showed additional changes of N440K while one sequence exhibited two more changes of P681R and D950 N (Table 1). So our result showed that the Delta variant of lineage B.1.617.2 has significantly outpaced all other variants of Alpha (B.1.1.7), Kappa (B.1.617.1), and B.1 other variant with its predominant occurrence of 77.1% while other lineages were observed in variable distribution frequency of 23% ( $p < 0.05$ ,  $\chi^2 = 162.49$ ) (Fig. 4).

Notable mutational variations were noticed among the four groups of vaccine breakthrough HCW, symptomatic mild/moderate, severe, and dead patients (Figs. 5 and 6). In the first category of the vaccinated health care worker, eight were found infected with Delta variant while one was infected with Alpha nCoV variant (Figs. 5A and 6). The Delta variant in all eight cases harbored the nonsynonymous mutations of T19R, E156G, L452R, T478K, D614G, P681R, D950 N, and two deletions of F157del and R158del, respectively. Among them, six vaccinated cases showed one more G142D AA change, while the other two vaccinated cases did not exhibit this mutation (Fig. 4A). The one case of Alpha nCoV variant was found with mutations as described above (Figs. 5A and 6).

Among mild/moderate symptomatic cases, eighteen cases were found infected by Delta, five by Kappa, one by Alpha and six by nCoV B.1 variant having various sublineages respectively (Fig. 6). The sublineages revealed two infected with B.1.36.29, while one case each was infected with sublineage of B.1.1.216, B.1.36.17, B.1.36.10, and B.1.1.306 (Table 1). The Delta variant in eighteen cases showed similar mutational pattern as described above except four cases without harbouring two deletions (F157del and R158del) and one nonsynonymous mutation of E156G, thirteen cases without G142D and one case without T19R mutation were observed (Table 1, Fig. 5B). The important observation included exclusive finding of Kappa variant with its earlier described characteristic mutational pattern only in the mild/moderate symptomatic cases (Fig. 5B). The one case of Alpha variant exhibited similar mutational changes as observed in Alpha variant infected vaccinated patients. In the rest six cases of different B.1 sublineages, the mutation of D614G was found in all cases, while N440K was found in three cases (Fig. 5B).

Among seventeen severe symptomatic cases, sixteen cases were found infected with Delta variant while only one case by B.1.1.326 variant (Figs. 5C and 6). Delta variant exhibited L452R, T478K, D614G, P681R, and D950 N mutations in all seventeen cases, while the frequency of other mutations varied as T19R and G142D were detected in fourteen and four cases respectively. E156G and two deletions (F157del and R158del) were observed in ten patients infected by the Delta variant. One case infected by B.1.1.326 harbored a sole mutation of D614G (Fig. 5C).

In twenty-seven deceased patients, the majority of twenty-two cases were found succumbed to the Delta variant (Figs. 5D and 6). In other five

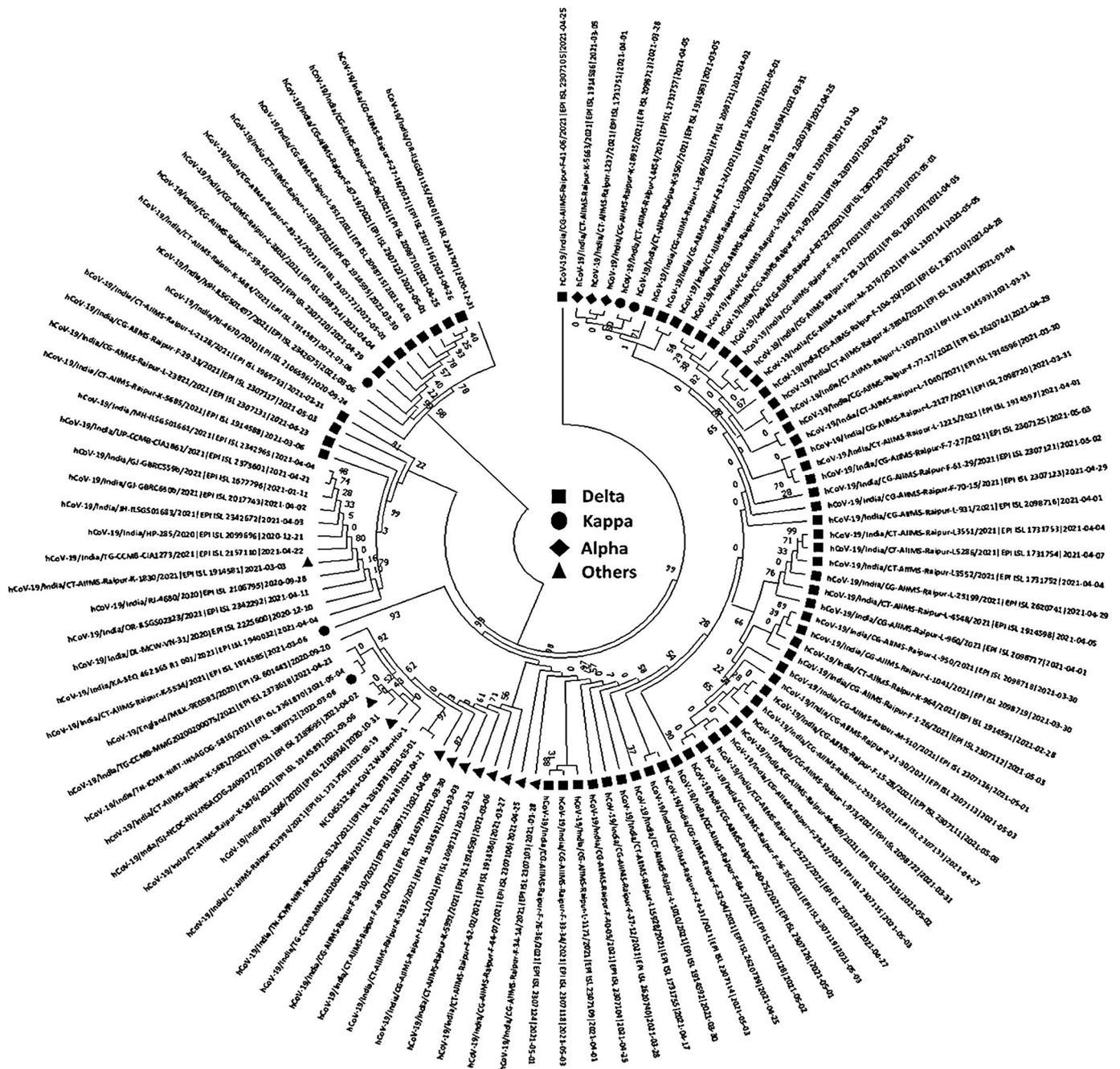


Fig. 3. Phylogenetic distribution of eighty-three sequences of SARS-CoV-2, belonging to various lineages, in comparison with reference sequence of Wuhan strain.

cases, one case was found infected with Alpha variant while the remaining four were found infected with B.1 lineage of variable sub-lineage (B.1-two cases, B.1.1.216-one case and B.1.36.29-one case) (Table 1). The mutational analysis found that the Delta variant exhibited all designated mutational AA changes except G142D in twelve, T19R in twenty, and E156G and two deletions (F157del and R158del) were observed in fifteen cases respectively (Fig. 5D). The Alpha variant in one case has shown the same mutation as described for other patients categories earlier. The remaining four cases infected with B.1 lineage have shown the sole common mutation of D614G (Fig. 5D).

Overall, only D614 G mutation was found in all cases with 100% occurrence among eighty-three sequences. L452R, T478K, and P681 H/R were seen in 69(83.1%), 65(78.3%), and 73(87.9%) sequences, respectively (Fig. 5E). D950 N was detected in 64 cases with a frequency of 77.1%. The rest of the other prominent mutations, namely T19R,

G142D were observed in 59 (92.2%) and 28 (33.7%) sequences, whereas E156G and two deletions (F157del and R158del), were detected in forty-seven (73.4%) sequences respectively (Fig. 5E).

The whole-genome analysis also showed mutations in specific genes/regions other than spike either among all the lineages or restricted exclusively to Delta and/or Kappa variant (Table 2). Notably, all variants showed P323L mutation in nonstructural protein (NSP) 12 encoding RNA dependent RNA polymerase (RdRP) gene, and Delta variant exclusively showed I82T mutation in membrane protein-encoding gene.

The 3D modeling of spike glycoprotein has depicted positional changes in AA residue of SARS-CoV-2 variant of VOC and VUM in comparison to the Wuhan prototype (Fig. 7A–D). L452R, T478K, N501Y and A570D mutations were located in spike protein receptor binding domain (RBD). N-terminal domain (NTD) region of these sequences harbored T19R, G142D, and E156G, respectively along with deletions of

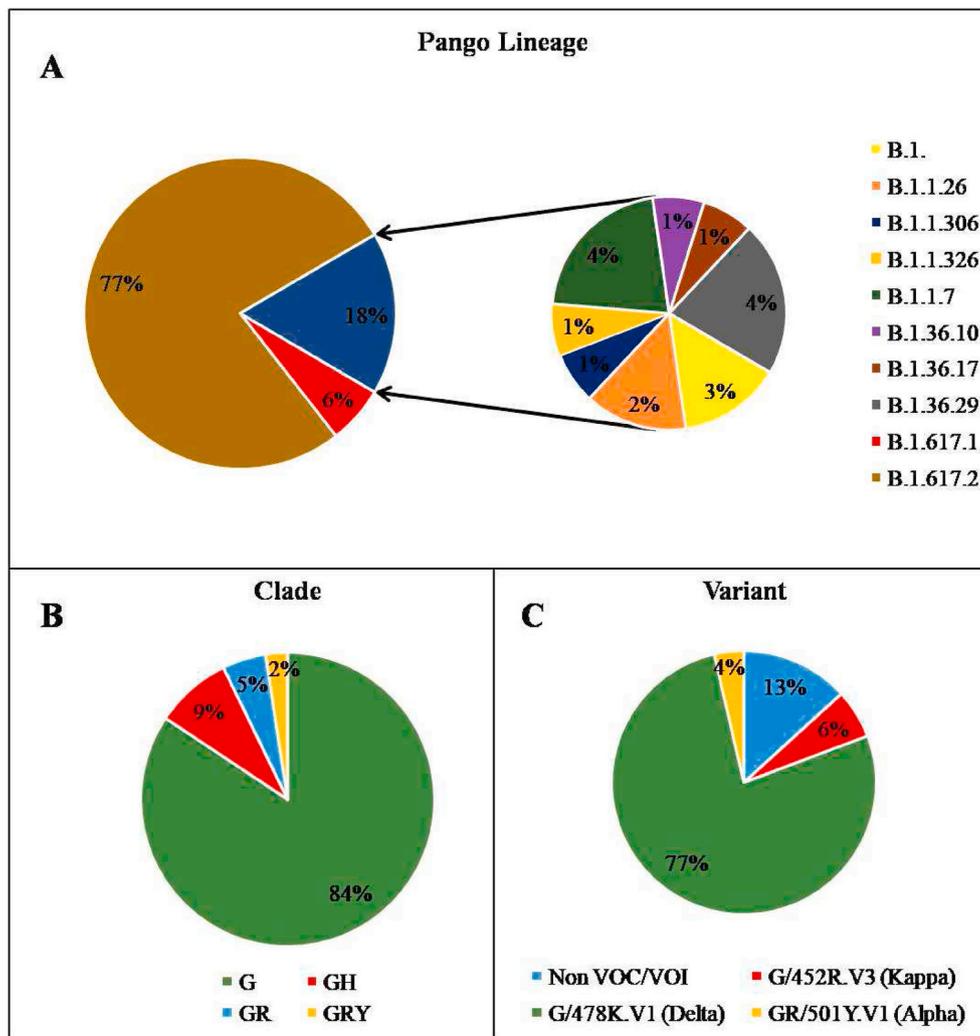


Fig. 4. The representation of the eighty-three genomes sequenced from Chhattisgarh state, in terms of- (A) Pango Lineage, (B) Clades and (C) Variants. All percentage figures in decimal are represented to near round off percentage.

H69, V70, Y144, F157 and R158del. Notable mutation D614G was identified distally to the furin cleavage site. Mutations of P681 R/H, D950 N, T716I, S982A, Q1071H, and D1118H were located in proximity to the S1/S2 cleavage site. VOC B.1.1.7 (n = 3) and B.1.617.2 (n = 64) exhibited substitution of same codon residue by different AA namely P681H and P681R (Fig. 7 E, F). The P681R mutation in B.1.617.2 appears to disrupt the cleavage site by changing the Proline, a non-polar AA (imino acid group), to Arginine, a polar positively charged AA (guanidino group) (Fig. 7E and F). Similarly, P681H mutation in B.1.1.7 also changed the furin cleavage site by substituting Proline to Histidine, a polar AA (imidazole group) (Fig. 7E and F). Both substituted Arginine and Histidine AA has a basic side chain with which they tend to bind protons; gaining a positive charge in the process to eventually result in the improved rate of membrane fusion, virus entry, and higher transmissibility [8,9].

#### 4. Discussion

The phylogenetic analysis identified our eighty-three sequences belonged to 10 different PangoLIN lineages, all within clade G. These lineages were dominated by Delta and followed by B.1 and its sub-lineages, Kappa and Alpha. Therefore Delta variant, B.1.617.2 of clade G/478 K.V1 with 77.1% presence was the predominant lineage that has outpaced all other co-circulatory lineages to become the chief causative variant responsible for COVID-19 2nd wave in Chhattisgarh. INSACOG,

a national multi-agency consortium of Genome Sequencing Laboratories set up by the Government of India on 8th July 2021 has also reported the presence of VOC B.1.617.2 from 174 districts in 35 states and union territories in India, with the highest numbers reported from Maharashtra, Delhi, Punjab, Telangana, West Bengal, and Gujarat. B.1.617 was detected initially from Maharashtra and from there it appeared to spread to other states [3]. High transmission of B.1.617.2 lineage has outpaced the earlier circulating lineage of B.1.1.7 between January to February 2021 to infect larger number of people at Delhi during COVID-19 2nd wave [4]. SARS-CoV-2 of other Pango lineage were observed in lesser proportion in Chhattisgarh. Kappa B.1.617.1 (G/452 R.V3) and Alpha B.1.1.7 lineage (GR/501Y.V1) were observed in 6 and 3.6% cases, respectively. Furthermore, the co-occurrence of B.1 and its sub lineage in 13.2% of the sequences has indicated that the multiple lineages in circulation underwent convergent evolution during the COVID-19 2nd wave at Chhattisgarh.

Analysis of individual patients groups revealed 88.8% vaccine breakthrough, 60% mild/moderate, 94.1% severe, and 81.4% dead patients were infected by Delta variant. Epidemiological and genomic data of the eighty-three cases revealed that males were twice more infected than females. The majority of the infected cases were adults with a collective mean age of 47.49 yrs. Since Delta variants was detected in all different patient groups, no specific mutation inclination pattern or change was seen in severe or dead cases. So, it appeared that Delta variant characteristic signature mutations enhancing virus

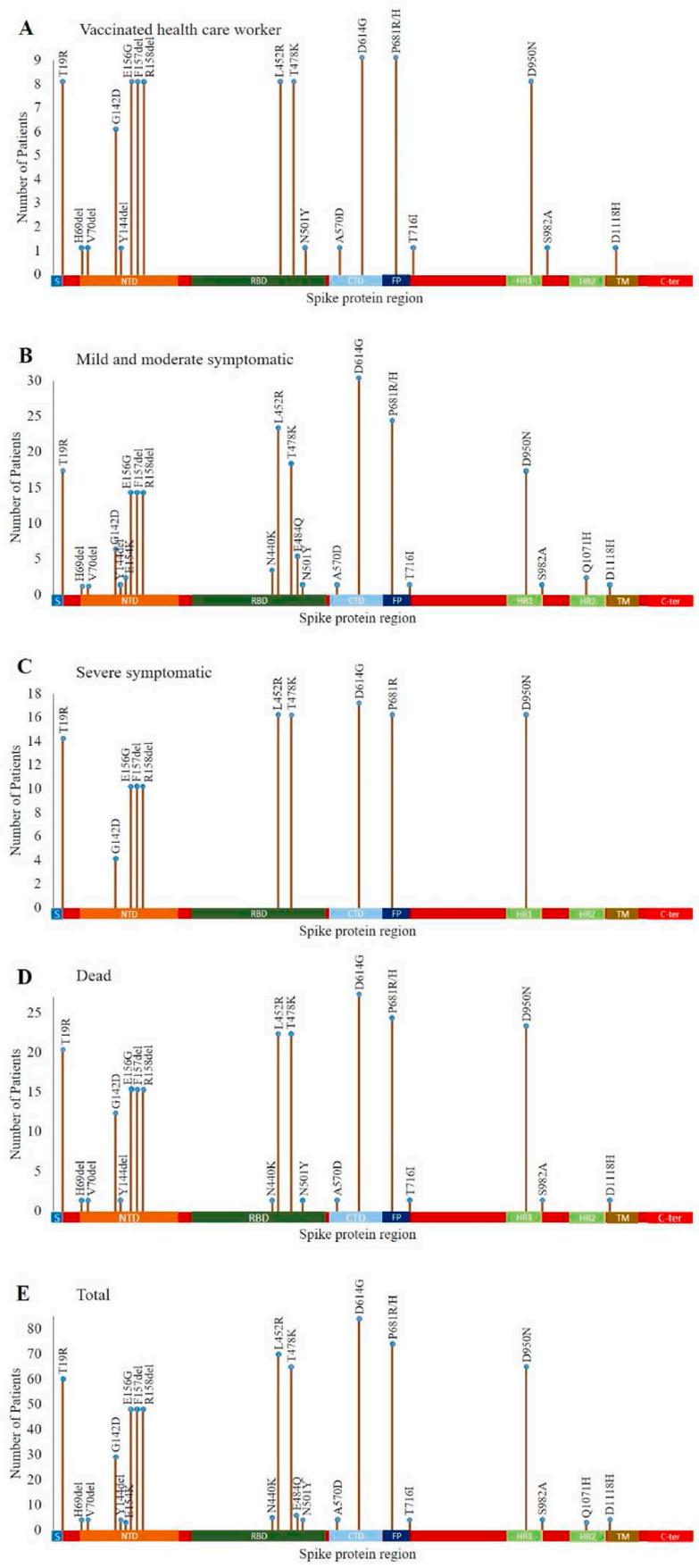


Fig. 5. Schematic representation of the mutational patterns in the four studied groups. A) Vaccinated health care workers (B) Symptomatic cases with mild/moderate disease (C) Symptomatic cases with severe disease (D) Dead patients and (E) Total.

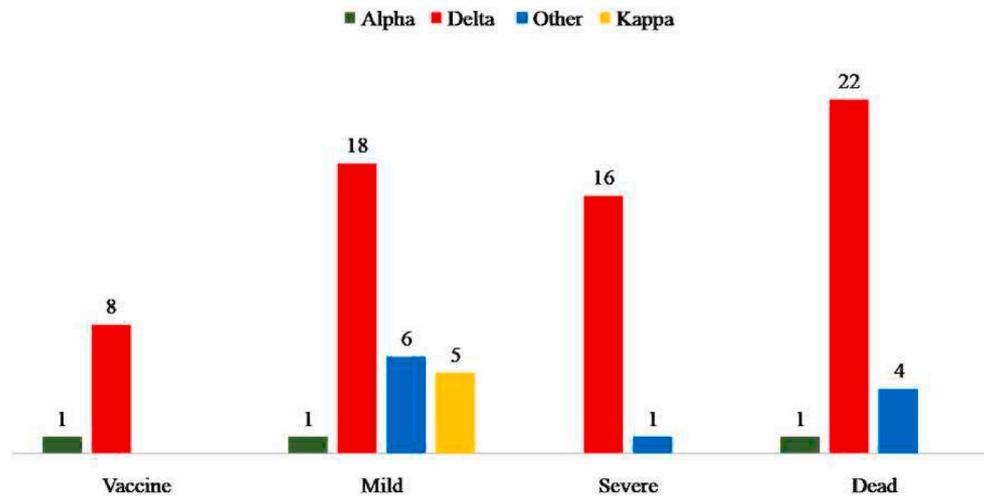


Fig. 6. Infection frequency of SARS-CoV-2 viral lineage in four category of vaccinated, mild and moderate, severe and dead patients.

Table 2

Various vital mutational pattern across whole genome of SARS-CoV-2 isolated from eighty three studied.

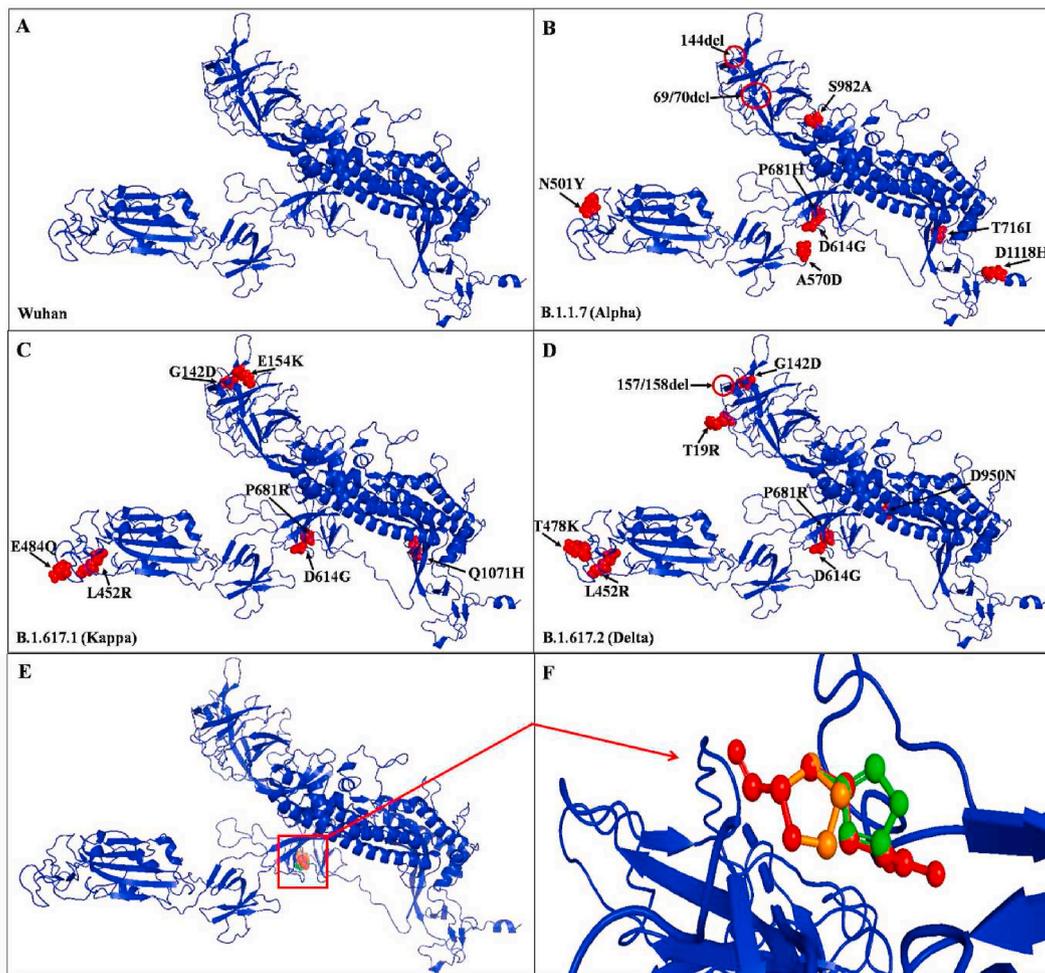
Genes	Mutational codon	No. of mutated variants	Affected variant
Membrane Protein	I82S	3	Kappa
	I82T	64	Delta
Nucleocapsid Protein	D377Y	10	Delta
	D63G	48	Delta, Kappa
	R203 M	65	Delta, Kappa
	R385K	70	All variants
NS3	S26L	63	Delta, Kappa
NS7a	L116F	48	Delta
	T120I	47	Delta
	V82A	47	Delta, Kappa
NSP12 (RdRp)	G671S	67	All variants
	P323L	83	All
NSP13	G206C	3	Kappa
	M429I	5	Kappa
	P77L	62	Delta
NSP14	P46L	50	Delta
NSP15	K259R	4	Kappa
	S261A	2	Kappa
NSP2	P129L	63	Delta, Kappa
NSP3	A890D	3	Alpha
	P822L	47	Delta
	T749I	2	Kappa
NSP4	A446V	65	Delta, B.1
NSP6	G107del	3	Alpha
	S106del	2	Alpha
	T77A	6	Kappa, Delta
	V149A	64	Delta and B.2

transmissibility and pathogenicity and various other factors plausibly could explain severity or death in infected patients. Probable factors includes more lethal and highly transmissible Delta variant, a higher proportion of patients with underlying diseases, delay in diagnosis and hospitalization, unavailability of oxygen beds, and antiviral remdesivir. In the absence of any substantial inhibition, Delta variant rampantly colonized and damaged the lung. The exaggerated immune response mediated viral entry in other organs had further worsened the clinical conditions leading to severity and death due to ARDS or multiple organ failure. Unfortunately, the lack of complete follow-up clinical data did not allow us to correlate which factors aggravated different nCoV variants to cause severity and death.

In vaccination breakthrough cases showing mild infection, eight cases were infected by VOC B.1.617.2, while one was infected with B.1.1.7 lineage. This indicated that vaccine although not giving cent

percent efficacy yet the fact of these patients showing only mild flu like symptom and not requiring hospitalization suggest that vaccination reduces the severity of the disease and should be carried out at war foot scale to vaccinate at least 70% population to ensure herd immunity. Further, vaccine developers also need to work on improving the efficacy of their present vaccination especially against Delta and Alpha variant. An earlier study substantiated our finding of vaccine breakthrough cases, reporting 86.69% presence of Delta variant in infected vaccinated cases [10]. While Delta variant was observed in all four different patients categories, Kappa variant presence was only noticed in mild cases whereas Alpha variant in vaccine breakthrough, mild and dead cases. B.1 and its various sublineages were also detected in mild, severe, and dead cases. These findings may suggest that Delta, Alpha, B.1 and its sublineage are capable of causing severe infection and death in case of delayed diagnosis/treatment.

The nonsynonymous mutations observed in the spike gene were analyzed for their biological significance. Various mutations in spike protein of B.1.617.2 has inflicted the enhanced pathogenicity and transmissibility. Mutation of T19R, G142D, T478K, D950 N, and two deletions of F157 and R158 were exclusively seen in the Delta variant, while other mutations namely L452R, D614G, and P681R in Delta variant was also found in other lineages reported in our study. T19R, G142D, E156G, and two deletions of F157 and R158 located within NTD have altered a recognized monoclonal antibody (mAb) recognition site N1 and N3 loop to helps the virus in escaping antibody response [4,11]. The two significant deletions of F157 and R158 and one substitution E156G at NTD antigenic supersite in Delta variant have also been reported as ‘surge-associated mutations’ to increase infectivity by 10 fold due to mechanism of evading neutralizing antibodies [12,13]. L452R, T478K occurred in the RBD region of the spike protein. L452R has already been earlier reported for escape from antibody immune response as these mutations reduces the antibody binding to mutation variants compared to wild type [3]. An earlier study also reports that L452R mutation had abolished the neutralizing effect of 14 mAb out of around 35 RBD mAb [14]. L452R mutation was further reported to help in virus escape from cellular immunity induced with human leukocyte antigen (HLA)-24 to eventually result in increased viral replication and infectivity [15]. It was reported earlier that in T478K, mutated Lysine reduced the gap to the extent of 8.3 Å between spike and ACE2 receptor in comparison to wild prototype which with threonine measured as 10 Å to eventually enhance the viral affinity with ACE2 resulting in increased viral infectivity [4]. Remarkably, L452R and T478K observed at the critical RBD in Delta variant have specifically been responsible for enhancing ACE2 binding [16,17]. D614G mutation has been reported earlier as the first mutation adapted by the variants evolved from



**Fig. 7.** Spike glycoprotein representation of our sequences of SARS-CoV-2 of Alpha, Kappa and Delta lineage. (A) Wuhan spike glycoprotein prototype in blue colour. The amino acid residue substitution and deletion site are marked in red colour and circle respectively in three lineages. (B) B.1.1.7 Alpha VOC (C) B.1.617.1. Kappa VUM (D) B.1.617.2 Delta VOC and (E, F) Overlap substitutions shown in spike protein P681 H/R; Proline (orange), Histidine (red) and Arginine (green).

original Wuhan nCoV [4]. It has been identified distally to the furin cleavage site to enhance viral cell entry efficiency by increased affinity to ACE2 cell receptors [4]. This may lead to increased viral loads in the upper respiratory tract COVID-19. D614G mutation earlier reported producing an allosteric conformational change in the furin cleavage site to result in RBD opening [18]. The detection of D614G in all our reported lineages has strongly suggested that the emergence of all variants of SARS-CoV-2 occurred after the worldwide adaptation and rise of the D614G mutants. The two mutations of P681R, D950 N were found close to the S1/S2 cleavage site to enhance viral replication and transmissibility [19]. P681R was seen in Delta, whereas P681H was observed in Alpha variant. Although both mutations increase the basicity of the S1–S2 poly-basic stretch at the furin cleavage site, P681R has inflicted more advantage to the virus by cleaving more efficiently S1/S2 cleavage at the furin site leading to the enhanced entry of the virus into host cells. This may augment the rate of viral membrane fusion and internalization into the host cell to result in higher replication and transmissibility, facilitating Delta enhanced fitness over Alpha variant [20]. Both L452R and P681R were reported to also aid in escaping the virus from specific monoclonal antibody responses [14,21–23]. In totality, these non-synonymous mutations in B.1.617.2 were the appropriate reason for its higher statistical significance occurrence in patients with all sorts of clinical features from mild to moderate, severe, and dead.

Kappa variant B.1.617.1 was only detected in five mild cases. The Kappa variant harbored only four mutations of L452R, E484Q, D614G, and P681R (Fig. 5B). The combined mutations of L452R and E484Q

have already been earlier reported for escape from antibody immune response [3,24]. However, the lack of other mutations in Kappa variant has restricted its infectivity lower than Delta variant.

Alpha variant B.1.1.7 was found in one case each from vaccine breakthrough, mild and dead patient category showing nonsynonymous mutations of N501Y, A570D, D614G, P681H, T716I, S982A, D1118H and three deletions of H69del, V70del, and Y144del. N501Y, A570D, and three deletions are characteristic signature mutations of the Alpha variant. N501Y and A570D at critical RBD enhance viral interaction with ACE2 human receptor [4]. Further, P681H in Alpha disrupts the furin cleavage site by changing the Proline to improve the rate of membrane fusion, virus entry, and higher transmissibility, albeit less efficiently than Delta variant manifesting P681R [20]. During 2020 year-end, subsequent to the first predominant mutation of D614G in the prototype variant, many countries have started noticing the first VOC B.1.1.7 variant after it was first reported from London. Many studies reiterated that UK variants might confer increased transmissibility and reduced susceptibility to neutralizing antibodies [25,26].

B.1 and its sublineages showing the persistent presence of D614G in their all sublineages of B.1, B.1.1.216, B.1.36.10, B.1.1.306, B.1.1.326, B.1.36.29 and B.1.36.17 confirms its paramount importance in increasing virion spike density and infectivity [27]. Only B.1.36.29 and B.1.36.17 also showed N440K mutation, which was reported earlier as prone to immune escape. N440K mutation, since first detected from the state of Andhra Pradesh, has been reported in around six percent of the nCoV sequenced across India [28].

Mutational analysis of genes/regions other than spike has revealed the mutational changes seen either in all lineages or restricted to the Delta and/or kappa variant. All lineages have shown P323L mutation in the interface domain of the RdRP region. P323L leads to stability of the tertiary protein structure of the virus to help in virus successful survival in different geographical conditions [29]. Although mutation in membrane gene relatively uncommon, I82T exclusive presence in Delta variant helps in viral host cell attachment, heparin sulfate proteoglycan, protein assembly in conjunction with nucleocapsid(N) and envelope (E) genes, and enhanced glucose transport to provide more rigidity and stability resulting in increased transmissibility and pathogenicity [30].

The limitation of the study included less number of studied cases, especially the vaccine breakthrough cases. There can be several reasons for this, including the acquisition of protective immune response in vaccinated people, continued adherence to COVID appropriate behavior, limited data on breakthrough cases, and poor prospective follow-up of completely vaccinated people for acquiring SARS-nCoV-2 infection. Nonetheless, we believe that even nine vaccinated infected patients have provided essential baseline information that Delta and Alpha variants could infect even vaccinated individuals. Thus there is a need for improvement in the vaccine efficacy against these Alpha and Delta nCoV variants.

We conclude that although SARS-CoV-2 Delta variant harboring consortium of various nonsynonymous mutations was predominantly responsible for an unprecedented surge in the cases during COVID-19 pandemic 2nd wave in Chhattisgarh. The simultaneous existence of various other lineages has fuelled the disastrous effect of COVID-19. The different lineage variant's simultaneous co-occurrence always poses a threat of virus could undergo further evolution to result in hybrid variants having increased transmissibility and infectivity. Hence, the vaccination also need to ramp up in both its improvement of effectiveness against all lineages and to cover at least 70 to 80% of population by the end of 2021 to prevent any further waves of pandemic or at least to thwart the disastrous effect in terms of reducing morbidity and mortality associated with it. Continuous monitoring of the emergence of new SARS-CoV-2 variants and identifying it's mutational changes and their role in alteration of spike protein, and subsequent effect of these changes in the transmissibility and pathogenicity of the virus is essentially required and is one of the pillars of formulating future public health strategies for combating COVID-19 disease.

#### CRedit authorship contribution statement

**Pushpendra Singh:** Formal analysis, Investigation, Methodology, Writing – original draft. **Kuldeep Sharma:** Methodology, Formal analysis, Data curation. **Priyanka Singh:** Resources. **Anudita Bhargava:** Writing – review & editing, Validation, Resources. **Sanjay Singh Negi:** Conceptualization, Project administration, Supervision, Visualization, Writing – review & editing. **Pratibha Sharma:** Writing – review & editing, Writing – original draft. **Mayuri Bhise:** Software. **Manish Kumar Tripathi:** Visualization, Validation, Software, Methodology. **Atul Jindal:** Resources, Writing – review & editing. **Nitin M. Nagarkar:** Resources.

#### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Dr. Sanjay Singh Negi reports financial support was provided by All India Institute of Medical Sciences (AIIMS), Raipur. Dr. Sanjay Singh Negi reports a relationship with All India Institute of Medical Sciences (AIIMS), Raipur that includes:. Dr. Sanjay Singh Negi has patent pending to NA. NA.

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