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Original article:

Clinical characteristics of AY.4 infections are similar to B.1.617.2 infections: A preliminary study

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Abstract:

Background: The SARS-CoV-2 Delta variant (B.1.617.2) was first detected in India in late 2020 and soon became the predominant lineage owing to its high transmissibility. Over time, the virus has acquired mutations and has evolved into many new sub-lineages. AY.4 is one such sub-lineage that grew in frequency globally. Therefore, we aimed to compare the severity of infection due to Delta sub-lineages to Delta infections in Pune, Maharashtra, India.

Material and Methods: Whole-genome sequencing and analysis of 255 SARS-CoV-2 positive samples, collected between 1st August to 1st September 2021, by BJ Government Medical College, Pune, was carried out at the Indian Institute of Science Education and Research (IISER), Pune and the Council of Scientific and Industrial Research–Institute of Genomics and Integrative Biology (CSIR-IGIB), New Delhi. Individual-level data on these patients were collected from ICMR COVID-19 Data Portal. Additional information regarding the presence of any symptoms, comorbidities, hospitalization, international travel history within 14 days and vaccination status was collected by telephonic interview with each patient by the BJGMC Sequencing Team.

Results: Of the 255 samples sequenced, 161 (63.13%) had VOC B.1.617.2, 90 (35.29%) had AY.*. The AY.4 (51.1%) was the predominant Delta sub-lineage, followed by AY.12 (22.2%) and AY.16 (15.6%). A total of 201 patients were contacted telephonically, of which 30 (14.93%) cases were asymptomatic, and 171 (85.07%) cases were symptomatic. Of the symptomatic cases, 58.21% had Delta, 11.94% had AY.4 and 14.93% were infected with other Delta sub-lineages. Oxygen therapy by mask/nasal prongs was required in 13.18% of Delta cases, and one (0.78%) case required mechanical ventilation. In contrast, only 3.03% and 5.13% cases of AY.4 and other Delta sub-lineages, respectively, needed oxygen therapy. Of 49 individuals vaccinated with two vaccine doses, 71.43% were infected with Delta, 20.41% with other Delta-sub-lineages and 8.16% with AY.4. There was no evidence of a significant difference in hospital attendance, disease severity, oxygen therapy requirement, hospitalization days, vaccination status, and disease outcome among patients infected with Delta and AY.* Delta sub-lineages.

Conclusion: The preliminary results suggest that the disease severity in patients with Delta sub-lineages is not markedly different from those infected with Delta variant. Therefore, currently, it shares the same public health concern as those of Delta.

1. Introduction

The SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. It has one of the largest RNA viral genomes, approximately 30 kb in length.[1] Since its emergence in December 2019, the virus has evolved and has acquired mutations associated with increased transmissibility and immune escape. These new variants require constant monitoring as they directly impact public health and the healthcare delivery system globally. To understand the impact of the variants, the World Health Organization (WHO) has classified them into Variants of Concern (VOC), Variants of Interest (VOI) and Variants Under Monitoring (VUM) based on transmissibility, virulence and effectiveness of public health and social measures.[2]

The Delta variant (B.1.617.2), a B.1.617 sub-lineage, was first identified in India in late 2020[3] and was detected in England in March 2021.[4] It was escalated to a Variant of Concern (VOC) on 6th May 2021 in the United Kingdom (UK)[4] and on 11th May 2021 by WHO.[2] Among the sequences submitted to publicly available datasets, the current global genetic epidemiology of SARS-CoV-2 is characterized by the predominance of the Delta variant. Between 08th October to 7th December 2021, 8,97,886 sequences have been uploaded on GISAID, a Global Initiative on Sharing Avian Flu Data, of which 99.8% were Delta. In most countries, the prevalence of other variants is declining as the Delta variant has outcompeted other variants, including other VOCs like Alpha, Beta and Gamma.[5]

The diversity within the Delta lineage is being monitored through lineages and individual mutations. New sub-lineages are regularly identified and designated using the Phylogenetic Assignment of Named Global Outbreak LINeages (PANGOLIN, also known as Pango) nomenclature. With the availability of new sequencing data, the classification of Delta lineage by Pango nomenclature is constantly changing for fine-scale tracking of the virus. These subclades within Delta are called AY.* lineages that show significant geographical clustering, and AY.4 lineage is one designated PANGO lineage within Delta VOC that saw a relative expansion over the Delta variant in recent times.[6]

Because of the rising concern about the spread of AY.4 and other Delta sub-lineages in the country and little existing knowledge about the severity of the disease caused by these Delta sub-lineages, the current study was undertaken to compare the disease characteristics of Delta sub-lineages with the Delta variant. The findings of this study will help to understand the clinical characteristics of AY.4 infections and thereby help guide the public health response to variants circulating in India.

2. Material and Methods

This cohort study was conducted in the Department of Microbiology, Byramjee Jeejeebhoy Government Medical College (BJGMC), Pune, Maharashtra. The study was a part of BJGMC-Pune Knowledge Center (PKC) MOU, and the Directorate of Medical Education and Research (DMER)-CSIR-IGIB MOU for sequencing of SARS-CoV-2 in the state of Maharashtra.

This study falls within the research activities approved by the Institutional Ethics Committee of BJGMC, Pune, Maharashtra, India.

2.1. Sample Acquisition

Freshly extracted viral RNA with a cycle threshold (Ct) value less than 25 available in the Biorepository maintained at BJGMC, Pune as well as samples collected and transported to BJGMC, Pune, from various RT-PCR testing laboratories across Pune, maintaining a cold chain at 2–8^{O}C. The samples were stored appropriately at -80^{O}C until further processing. The RNA samples were sent to the Indian Institute of Science Education and

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Research (IISER), Pune and the Council of Scientific and Industrial Research–Institute of Genomics and Integrative Biology (CSIR-IGIB), New Delhi, for SARS-CoV-2 whole genome sequencing and its analysis.

2.2. Data Collection

As COVID-19 is a notifiable disease, the Indian Council of Medical Research (ICMR), New Delhi, India, maintains data of all individuals tested for COVID-19 on the ICMR COVID-19 Data Portal, a centralised data entry portal for COVID-19 testing in India. A set of individual-level data corresponding to the samples received, including age, gender, area of residence, contact number, occupation and date of specimen collection and testing, were obtained from the ICMR data portal. Additional information regarding the presence of any symptoms, comorbidities, hospitalisation, international travel history within 14 days and vaccination status was collected by telephonic interview with each patient. This information was used to grade the clinical severity of the patients using the World Health Organization (WHO) clinical progression ordinal scale, which measures the severity of illness from 0 (not infected) to 10 (death). A WHO ordinal scale of 6 or higher is defined as a severe disease requiring positive pressure respiratory support.[7] Categorisation of comorbidities was done based on the presence of no conditions, one condition, or at least two conditions. This categorisation has been defined in the International Severe Acute Respiratory and Emerging Infection Consortium 4C Mortality Score, using a modified Charlson index.[8]

2.3. Library preparation, Next-Generation Sequencing and lineage analysis

Libraries for Covid-19 were prepared using the defined protocol for Illumina COVIDseq RUO test kits (Illumina Inc, USA). After annealing random hexamers, the complementary DNA (cDNA) synthesis reaction was carried out on extracted RNA from COVID-19 positive samples. It was followed by SARS-CoV-2 genome enrichment using a multiplex PCR protocol employing two primer pools- COVIDseq Primer Pool 1 and COVIDseq Primer Pool 2, containing a total of 98 amplicons to amplify the SARS-CoV-2 virus-specific sequences and 11 amplicons for amplifying human RNA. The PCR amplified products were further processed for tagmentation, and adapter ligation using 8 IDT for Illumina PCR Indexes Sets 1-4, each containing 96 indices. Following enrichment and clean-up of tagmented amplicons as per recommended protocols, 96 sample batches were pooled together, with each batch containing a positive control (CPC HT) and one no template control (NTC). Pooled libraries were quantified using the Qubit 4.0 fluorometer (Invitrogen), and the Illumina Pooling calculator was used to combine all the pools to a final concentration of 4nM. For sequencing, the final pooled library was denatured, neutralized, and diluted to a final concentration of 150 pM (CSIR-IGIB, Delhi protocol)/ 1.4 pM (IISER, Pune protocol) and paired-end sequencing was performed using the Illumina NovaSeq 6000 (CSIR-IGIB, New Delhi)/ NextSeq 550 Sequencing (IISER, Pune) Platform. Sequenced Read files were demultiplexed and converted to FASTQ using the FASTQ Generation Tool from Illumina Basespace. Reads were aligned to the reference Covid-19 genome (Wuhan-Hu-1, GenBank accession number MN908947.3), and variant calling was performed using the DRAGEN COVID Lineage application followed by lineage and clade analysis using Pangolin and Nextclade software.

2.4. Statistical Analysis

All demographic and clinical data were recorded in Microsoft Excel. The data were analyzed using the JMP statistical software, version 13.0.0. Univariable comparisons of categorical variables were made using χ^2 or Fisher's exact tests. The analysis of the difference in Ct values of the three groups was made using one-way ANOVA test.

3. Results

Between 1st August 2021 to 1st September 2021, there were 29,708 cases positive for SARS-CoV-2 virus in Pune.[9] A total of 255 RT-PCR positive RNA samples from this period were included for SARS-CoV-2 whole-genome sequencing.

Of the 255 samples sequenced, 161 (63.13%) had VOC B.1.617.2, 90 (35.29%) had AY.* Delta sub-lineages and 4 (1.57%) samples failed to generate an adequate genome for analysis. The AY.4 (51.1%) was the predominant Delta sub-lineage, followed by AY.12 (22.2%) and AY.16 (15.6%) (**Table 1**).

Table 1. Variant Distribution among the samples sequenced between 1st August 2021 to 1st September 2021

Variant distribution among the sequenced samples		**Total Count**	**Percentage**
Delta	B.1.617.2	161	63.13%
Delta sub-lineages		90	35.29%
AY.*	**Counts (%)**		
AY.4	46 (51.11%)		
AY.12	20 (22.22%)		
AY.16	14 (15.56%)		
AY.5	3 (3.33%)		
AY.10	3 (3.33%)		
AY.7	1 (1.11%)		
AY.7.1	1 (1.11%)		
AY.11	1 (1.11%)		
AY.25	1 (1.11%)		
Unclassified		4	1.57%
Total		**255**	**100**

3.1. Demographic characteristics of the study population

The mean age of the study population was 37.59 ± 17.96 years. Of the 255 cases, 161 (63.14%) were male, and 94 (36.86%) were female. The males and the age group 19 to 59 years were predominantly affected by Delta and sub-lineages. There was no significant difference in the proportion of Delta and AY.* cases in urban and rural Pune (p=0.1446) (**Table 2**).

Table 2. Demographic characteristics of the study population with respect to SARS-CoV-2 variants

	NUMBER OF CASES INFECTED WITH DELTA VARIANT	NUMBER OF CASES INFECTED WITH AY.4 DELTA SUB-LINEAGE	NUMBER OF CASES INFECTED WITH OTHER AY.* DELTA SUB-LINEAGES	TOTAL COUNT (%)	*p* VALUE
1. Gender-wise distribution					**0.8006**
MALE	100 (62.11%)	31 (67.39%)	30 (62.50%)	161 (63.14%)	
FEMALE	61 (37.89%)	15 (32.61%)	18 (37.50%)	94 (36.86%)	
2. Age-wise distribution (in years)					**0.8507**
1 to 18	18 (11.18%)	05 (10.87%)	04 (8.51%)	27 (10.63%)	
19 to 59	114 (70.81%)	35 (76.09%)	33 (70.21%)	182 (71.65%)	
60 and above	29 (18.01%)	06 (13.04%)	10 (21.28%)	45 (17.72%)	
3. Area of Residence					**0.1446**
RURAL	75 (46.58%)	22 (47.83%)	30 (62.50%)	127 (49.8%)	
URBAN	86 (53.42%)	24 (52.17%)	18 (37.50%)	128 (50.2%)	
4. Ct Value Analysis					**0.00499***
Mean Ct Value	24.20	22.76	25.48		

3.2 Clinical characteristics of the study population

Of the 255 patients, 201 (78.82%) could be contacted telephonically to get more information about the presence of any symptoms, comorbidities, hospitalisation, international travel history within 14 days and vaccination status. None of the patients gave a history of international travel within 14 days of specimen collection. Community-acquired SARS-CoV-2 infection was seen in 198 (98.51%) patients and hospital-acquired SARS-CoV-2 infection in 03 (1.49%) cases. All three cases of hospital-acquired infection were health care workers working in COVID care centres, of which two were infected with Delta, and one had AY.4 infection.

At the time of specimen collection, 30 (14.93%) cases were asymptomatic, and 171 (85.07%) cases were symptomatic. Of the 171 symptomatic cases, 58.21% had Delta, 11.94% had AY.4 and 14.93% were infected with other Delta sub-lineages. On assessing the disease severity, 13.18% of Delta cases required oxygen therapy by mask/nasal prongs, and one (0.78%) case required mechanical ventilation. In contrast, only 3.03% and 5.13% cases of AY.4 and other Delta sub-lineages, respectively, needed oxygen therapy. Of the 101 vaccinated individuals, 49 (48.51%) were vaccinated with two doses of vaccine, of which 71.43% were infected with Delta variant, 20.41% with other Delta-sub-lineages and 8.16% with AY.4 variant. The characteristics of patients according to the presence of comorbid conditions, whether family members were infected, type of quarantine facility, the severity of disease and vaccination status are shown in **Table 3**. There was no evidence of a difference in either hospital attendance, disease severity, oxygen therapy requirement, hospitalisation days, vaccination status, and disease outcome among patients infected with Delta and AY.* Delta sub-lineages. **Table 4** shows the severity of the disease (in terms of symptomatic disease and oxygen therapy requirement) by vaccination status. No significant difference was detected in symptom status and oxygen requirement between those vaccinated and those not vaccinated.

Table 3. Clinical characteristics of the study population with respect to SARS-CoV-2 variants

	DELTA VARIANT INFECTION	**AY.4 DELTA SUB-LINEAGE INFECTION**	**OTHER AY.* DELTA SUB-LINEAGE INFECTION**	**TOTAL COUNT** (%)	***p* VALUE**
1. Symptom status at the time of sample collection					**0.0126***
Asymptomatic	12 (9.30%)	9 (27.27%)	9 (23.08%)	30 (14.93%)	
Symptomatic	117 (90.70%)	24 (72.73%)	30 (76.92%)	171 (85.07%)	
2. Comorbidity Score					**0.4740**
0 (No Comorbidity)	117 (90.70%)	30 (90.91%)	33 (84.62%)	180 (89.55%)	
1 (Presence of one condition)	08 (6.20%)	03 (9.09%)	05 (12.82%)	16 (7.96%)	
>2 (Presence of two or more conditions)	04 (3.10%)	0 (0%)	01 (2.56%)	05 (2.49%)	
3. If family members were infected					**0.2656**
Yes	63 (48.84%)	11 (33.33%)	17 (43.59%)	91 (45.27%)	
No	66 (51.16%)	22 (66.67%)	22 (56.41%)	110 (54.73%)	

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4. Type of Quarantine					**0.8087**
Home Quarantine	77 (59.69%)	19 (57.58%)	21 (53.85%)	117 (58.21%)	
Institutional Quarantine (in hospitals)	52 (40.31%)	14 (42.42%)	18 (46.15%)	84 (41.79%)	
5. WHO Clinical Progression Ordinal Scale					**0.0309***
1 (Asymptomatic, RNA detected)	12 (9.30%)	9 (27.27%)	9 (23.08%)	30 (14.93%)	
2 (Symptomatic, Independent)	65 (50.39%)	11 (33.33%)	14 (35.90%)	90 (44.78%)	
4 (Hospitalized, No Oxygen Requirement)	34 (26.36%)	12 (36.36%)	13 (33.33%)	59 (29.35%)	
5 (Hospitalized, Oxygen by mask/ nasal prongs)	17 (13.18%)	1 (3.03%)	2 (5.13%)	20 (9.95%)	
7 (Intubation and mechanical ventilation)	1 (0.78%)	0 (0%)	0 (0%)	1 (0.50%)	
10 (Death)	0 (0%)	0 (0%)	1 (2.56%)	1 (0.50%)	
6. Hospitalisation Days					**0.4673**
1-5 days	6 (4.65%)	2 (6.06%)	1 (2.56%)	9 (4.48%)	
6-10 days	28 (21.71%)	7 (21.21%)	6 (15.38%)	41 (20.40%)	
11-14 days	14 (6.06%)	2 (6.06%)	9 (23.08%)	25 (12.44%)	
>14 days	4 (3.10%)	3 (9.09%)	2 (5.13%)	9 (4.48%)	
No admission needed	77 (59.69%)	19 (57.58%)	21 (53.85%)	117 (58.21%)	
7. Outcome of disease					**0.1920**
Recovered	129 (100%)	33 (100%)	38 (97.44%)	200 (99.50%)	
Dead	0 (0%)	0 (0%)	01 (2.56%)	01 (0.50%)	
8. Vaccination Status					**0.6465**
Unvaccinated	62 (38.51%)	16 (34.78%)	22 (45.83%)	100 (49.75%)	
Vaccinated	67 (41.61%)	17 (36.96%)	17 (35.42%)	101 (39.61%)	

Cases with no history	32 (19.88%)	13 (28.26%)	09 (18.75%)	54 (21.18%)	
9. Number of vaccine doses					**0.1676**
Not Vaccinated	62 (48.06%)	16 (48.48%)	22 (56.41%)	100 (49.75%)	
Vaccinated					
1 Dose	32 (24.81%)	13 (39.39%)	07 (17.95%)	52 (25.87%)	
2 Doses	35 (27.13%)	04 (12.12%)	10 (25.64%)	49 (24.38%)	

Table 4. Symptom status and Oxygen requirement among vaccinated and unvaccinated cases

Vaccination Status	**Symptom status of patients**		***p*= 0.8181**
	Symptomatic Disease	**Asymptomatic Disease**	**Total Count (%)**
Fully vaccinated with two doses of vaccine	41 (83.67%)	08 (16.33%)	49 (24.38%)
Vaccinated with one dose/ Unvaccinated	130 (85.53%)	22 (14.47%)	152 (75.62%)

Vaccination Status	**Oxygen therapy requirement of patients**		***p*= 0.8007**
	Number of patients requiring oxygen therapy	**Number of patients NOT requiring oxygen therapy**	**Total Count (%)**
Fully vaccinated with two doses of vaccine	06 (12.24%)	43 (87.76%)	49 (24.38%)
Vaccinated with one dose/ Unvaccinated	17 (11.18%)	135 (88.82%)	152 (75.62%)

4. Discussion

In the ongoing SARS-CoV-2 pandemic, the emergence of new VOC requires rapid genomic, epidemiological, and clinical characterization to guide public health responses. The wider use of whole-genome sequencing and advanced bioinformatic tools helps to investigate the emerging strains and variants in near real-time. The potential of these variants to increase disease transmission, become resistant to treatment and vaccines and alter disease severity must be rapidly assessed. [10]

Delta (B.1.617.2) was the dominant SARS-CoV-2 variant followed by AY.4 sub-lineage in the present study. Of all the sequences submitted on GISAID, the apparent cumulative prevalence of the Delta variant worldwide is 3%, and in India, it is 23%.[11] Till date, the Delta variant has spread to more than 148 countries and 56 states of USA, replacing all previously circulating VOCs.[12] Similarly, as of 17th December 2021, there are 7,52,298 sequences identified as AY.4 Delta sub-lineage globally, with a worldwide global prevalence of 13%.[13] Recently, India has also seen a relative expansion of AY.4 lineage, compared to B.1.617.2, as per the INSACOG weekly bulletin dated 4th October 2021.[14] However, the prevalence of AY.4 lineage is less than 0.5% in the country. It has been detected in at least 92 countries and 54 states of USA with the highest levels in the United Kingdom (47% of sequences).[13]

The VOC B.1.617.2 and the AY.4 sub-lineage share almost all characteristics mutations at different genomic regions of the virus (**Table 5**). **Figure 1** illustrates the various mutations shared by B.1.617.2 and AY.4 sub-lineage. Based on the prevalence of mutations (ratio of the count of sequences containing a given set of mutations to the total sequences of a particular lineage), the mutations are highlighted from a dark purple (100%) to white (0%).[15] Several unique mutations- T19R, G142D, T95I, E156G, del 157/158- are located in the N-terminal domain of the spike protein. Mutations L452R and T478K are located at the Receptor-binding Domain (RBD) of the spike protein. These mutations at NTD and RBD play a vital role in the increased infectivity of the Delta variant by affecting the viral binding to ACE2 receptors in the host cells. The mutations in the neutralizing antibody epitopes of the RBD also help the virus to escape from neutralizing antibodies, thus responsible for the immune-escape property of the virus.[16]

The defining mutation in AY.4 lineage is the A2529V mutation in the ORF1a gene, which affects the Non-Structural Protein 3 (Nsp3). Nsp3 is the largest of all SARS-CoV-2 Nsps and plays a vital role in the viral replication and transcription process. It encodes for papain-like protease, which helps in processing viral polypeptides during viral replication.[17] However, the effect of A2529V mutation on viral replication is unknown and requires further study.

Table 5: Characteristic mutations seen in more than 75% of the sequences within the lineages (Based on sequences submitted on GISAID) [15]

Genomic Region	B.1.617.2	AY.4 Delta sub-lineage
ORF 1a	A1306S*, P2046L*, P2287S*, A2529V*, V2930L*, T3255I*, T3646A*	A1306S, P2046L, P2287S, **A2529V**, V2930L, T3255I, T3646A
ORF 1b	P314L, G2662S, P1000L, A1918V*	P314L, G2662S, P1000L, A1918V
S	T19R, T95I**, G142D, E156G, del 157/158, L452R, T478K, D614G, P681R, D950N	T19R, T95I, G142D, E156G, del 157/158, L452R, T478K, D614G, P681R, D950N
ORF 3a	S26L	S26L
M	I82T	I82T
ORF 7a	V82A, T120I	V82A, T120I
ORF 7b	T40I *	T40I

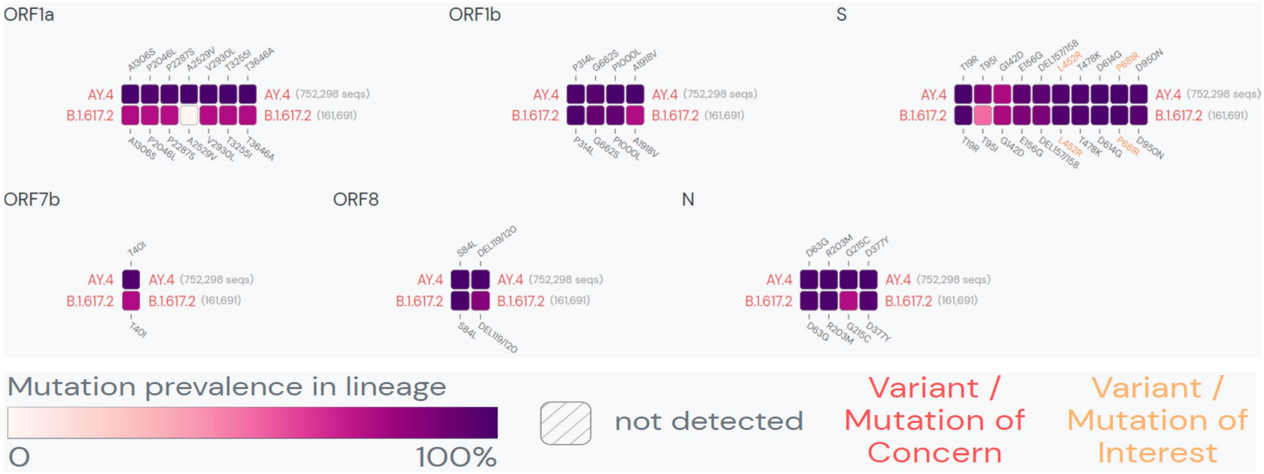
ORF 8	S84L, del 119/120	S84L, del 119/120
N	D63G, R203M, D377Y, G215C*	D63G, R203M, G215C, D377Y

ORF: Open Reading Frame, S: Spike, M: Membrane, N: Nucleocapsid

**Mutations are seen in 73-74% of sequences available on GISAID for the lineage. The characteristic mutations may change with the increase in the number of available sequences.*

***mutation is seen in 49% of sequences.*

Figure 1: Mutation prevalence across Delta variant and AY.4 Delta sub-lineage (Based on sequences submitted on GISAID) [15]



The Ct value was taken as a surrogate to assess if patients with Delta sub-lineage infection had increased viral load than those with Delta variant infection. Patients with AY.4 infection had a lower mean Ct value (22.76) compared to those with Delta (24.20) and other sub-lineage (25.48) infections. However, this slight difference in Ct value might be due to sampling of patients at different stages of infection. Also, the criteria for selecting samples for whole-genome sequencing might restrict generalizing this finding as only samples with a Ct value less than 25 were included in the study as they are most likely to be successfully sequenced.[18]

The patient's clinical condition during illness was graded as per the WHO Clinical Progression Ordinal Scale based on the history obtained. Patients with Delta variant infection were hospitalized more often and were more likely to receive oxygen therapy than patients with Delta sub-lineages. This finding is consistent with studies showing that the risk of hospitalization, ICU admission and death increases in patients with Delta infection.[18,19] There was no difference in the number of hospitalization days between the three groups. However, the hospital admissions might be influenced by the heterogenous health-care-seeking behaviour of patients and change in hospital admission policy due to local hospital burden over time and area.[18] All cases in the study had recovered from the infection, with a single case of death with AY.5 infection. Thus, there was no clear pattern suggesting more severe disease in patients with Delta sub-lineage compared to Delta variant.

Many studies have reported a reduction in effective protection against severe disease and the neutralizing ability of vaccines and immune serum against the Delta variant. They indicate a 10-20% reduction in vaccine effectiveness or 2-5-fold reduction in neutralization with AstraZeneca-Vaxzevria/Covishield, Bharat-Covaxin and Pfizer BioNTech-Comirnaty vaccines. Similarly, with the Moderna-mRNA-1273 vaccine, there is less than a 10% reduction in vaccine effectiveness and a 10-20% reduction in neutralization.[19] In the present study,

vaccine-breakthrough infections after two vaccine doses were commonly seen with Delta variant compared to its sub-lineages. However, there was no significant difference in numbers between the groups. Also, there was no significant difference in disease severity among those vaccinated and those not vaccinated. Further study is needed on a greater sample size to clarify if the hospitalization risks and the disease severity differ in vaccinated individuals infected with the Delta variant compared with its sub-lineages.

5. Conclusion

This study provides essential preliminary evidence that the disease severity in patients with Delta sub-lineages is not markedly different from those infected with Delta variant. Currently, there is no evidence of added clinical or public health concerns of these sub-lineages. Good quality clinical data with whole-genome sequencing of SARS-CoV-2 is imperative in concluding whether the emergence of new variants is associated with adverse clinical outcomes. These findings will be fundamental for proper resource planning and policy decisions to mitigate the impact of these variants in India and other countries as the virus will continue to evolve.

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Authors' Contribution:

Authors RD, RK, SJ and SJ designed the study. Authors SK, PK, NS, RS, DN, MP, AR, AG and KK from IISER, Pune and RB, BJ, VS, MI, AS, MD, KP, VS and SS from CSIR-IGIB, New Delhi participated in SARS-CoV-2 genome sequencing. BJGMC sequencing team collected the clinical data of positive patients. RD and RK analysed the data and wrote the manuscript. The manuscript was reviewed and finalized by all authors.

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