

Original Research Article

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Genomic Distribution of Sars-Cov-2 Variants - A Tertiary Care Hospital Experience

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A B S T R A C T

By 2019 December saw a deadly virus starting to spread across Wuhan city in China and started to spread across the globe by 2020 and was named as SARS-CoV-2. The Covid-19 variant spreading in India, namely the Delta variant is found to be more contagious and has been classified as 'being of concern' by the World Health Organization. RT-PCR positive samples from a tertiary care hospital were subjected to sequencing after fulfilling the criteria as per the standard protocol. Out of 70 samples sequenced, B.1.617.2, AY.4, AY.6, AY.12 & Delta-like variants were detected. The Delta variant B.1.617.2 was found to be the predominant strain. The sub lineage of Delta variant strain like AY.6 (1.4%), AY.4 (12 %), AY.12 (14%) were also detected. Delta-like variant was found in 20% of the samples sequenced. The results of sequencing might help to understand the characteristics of the strains prevalent during second wave of SARS-CoV-2 and would be helpful to tackle further infection of COVID.

Keywords

SARS-CoV-2,
Delta variant,
Sub lineage

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Introduction

Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. COVID-19 is a novel coronavirus with an outbreak of unusual viral pneumonia in Wuhan, China, and caused the ongoing pandemic. COVID-19 belongs to Betacoronavirus genera based on its phylogenetic relationships and genomic

structures. Human Betacoronaviruses (SARS-CoV-2, SARS-CoV, and MERS-CoV) have many similarities, but differences are seen in their genomic and phenotypic structures. COVID-19 is single-stranded (positive-sense) RNA related to a nucleoprotein within a capsid comprised of matrix protein.(1) The Global data shows that 2,29,88,719 cases of covid19 have been reported with a mortality of 47,13,43. So far, Tamil Nadu had reported

26.5 Lakhs cases of SARS-CoV-2 and 35,400 deaths. Tamil Nadu tops fourth in SARS-CoV-2 infections in India, next to Maharashtra, Kerala & Karnataka.(2) The morbidity and mortality was very high in Tamil Nadu during the second wave that began in April than the first wave.

The most frequent signs and symptoms in both waves were fever, dyspnea, pneumonia, and cough, with cardiovascular diseases, type 2 DM, and chronic neurological diseases as major comorbidities. Emergence of important mutations and deletions are being reported frequently within SARS-CoV-2. These mutations have led to different variants of COVID 19 strain. One of these is named the delta variant. As per CDC this delta variant is considered as “variant of concern” as it is highly transmissible from one person to another. As of July 2021, delta is considered the foremost contagious sort of the SARS-CoV-2 coronavirus thus far. Delta variant of SARS-CoV-2, the virus that causes COVID-19, is now in many countries and people travelling internationally are likely to encounter it. Vaccination for COVID 19 can prevent from delta variant and infections do occur sometimes but the morbidity and mortality is seen very less after vaccination.

The Viral Research and Diagnostic Laboratory (VRDL) at Madras Medical College (MMC) was started in March 2020 and 17,11,166 (Seventeen lakhs eleven thousand one hundred and sixty six) COVID samples have been tested at Viral Research and Diagnostic Laboratory (VRDL), Madras Medical College (MMC) by RTPCR. In view of increasing Delta variants, VRDL, MMC has been included as ‘Sentinel centre’ in Tamil Nadu for selection of samples for Sequencing. The results of sequencing might help to understand the characteristics of the second wave and the behaviour of SARS-CoV-2 to tackle further infection of COVID.

Materials and Methods

Sample Collection

Samples were collected in viral transport medium from nasopharyngeal & oropharyngeal sites according to ICMR guidelines.

RNA Extraction

200 μ l of sample from VTM was transferred to deep well blocks containing lysis buffer & proteinase K mixture and RNA extraction is carried out in HELINI RNA/DNA automated extraction system. The eluted RNA is then used for RTPCR testing.

RTPCR Testing

RTPCR is done using ROCHE Light Cycler96 (ROCHE diagnostic system, Basel, Switzerland) & BIORAD CFX96 (BIORAD Laboratories INC, USA) with LABGUN Exofast RTPCR kits (SIEMENS) supplied by TNMSC.

Selection of Samples for Sequencing

The samples which were positive by RTPCR and fulfilled the sequencing criteria were selected for sequencing and sent to INSTEM lab, Bangalore & CDFD lab, Hyderabad through SPHL, Chennai.

The basis for selection of samples for sequencing were- Community cluster, Family cluster, Positive samples from children, infection among young adults with severe lung infection, deceased patient without any comorbidities, suspected re-infection cases and Vaccine breakthrough cases.

15 samples with Ct value less than 25 were selected and sent bimonthly for sequencing from April to July 2021.

Results and Discussion

SARS-COV2 Delta Variant (B.1.617.2, AY.1, AY.2, AY.3, AY.4, AY.5, AY.6, AY.7, AY.8, AY.9, AY.10, AY.11 and AY.12) or Indian variant was first detected in India in late 2020. It was declared as a “variant of concern” in June, 2021 and is the cause for Second Wave of India (3)

As such, the Delta variant (B.1.617.2) has given birth to several sub-lineages called ‘Delta Plus’ variants that bear most of its characteristic mutations but are different in other ways. One of these sub-lineages, AY.12, has all the characteristic Delta mutations except one.

7, 08,985 Samples were tested during the second wave at VRDL, MMC from April 2021 to July 2021. A total of 55,276 samples were found to be positive for Covid -19 by RTPCR. 70 samples which fulfilled the criteria for sequencing were selected and sent in 6 cycles to the sequencing laboratories, as per standard protocol. And the results are tabulated as shown below.

Out of 7,08,985 Samples tested during the second COVID wave at VRDL, MMC from April 2021 to July 2021, a total of 55,276 samples were found to be positive for Covid -19 by RTPCR. 70 samples which fulfilled the criteria for sequencing were selected among the positive samples and sent in 6 cycles to the sequencing laboratories, as per standard protocol. The samples were transported to SPHL lab, Chennai and sent to INSTEM lab, Bangalore & CDFD lab, Hyderabad for testing.

Out of 70 samples, males were more susceptible to the mutant strain (54%) and females were found to be 46%. More samples were from young adults with severe lung infection (27.1%) followed by community

clusters (21.4%), family cluster (15.7%), post vaccination (17.1%), positive children (10%) and suspected re-infection (8.5%)

Delta variant, B.1.617.2 was found to be the predominant variant (51%) in the sequenced samples.. The sub lineages of Delta variant strain like AY.6 (1.4%), AY.4 (12 %), AY.12 (14%) were also detected. Delta –like variant was found in 20% of the samples sequenced.

A study done at Maharastra (4) in June 2021, showed 26% of B.1.617.2 among positive samples by sequencing, compared to our study which showed 30 % positivity.

Prevalence of Delta (B.1.617.2) variant was found in 8 cases (17%) in post vaccinated patients in our study, in contrast to a study(5) done by Thangaraj, *et al.*, in August 2021, which states that there was no difference between the vaccinated and unvaccinated groups in the sequenced samples. Male predominance of 58.9% was found in the study by Thangaraj, *et al.*, compared to 52.8% in our study.

Multiple SARS-CoV-2 variants are circulating globally. One of these is the B.1.617 lineage, detected in India earlier this year during the second wave of SARS-CoV-2. Early evidence suggests that its sub-lineage B.1.617.2, known as the Delta variant, is more transmissible than contemporary lineages.

This study focuses on detecting the common variants found in RTPCR positive cases during the period of April to July, 2021.

The results of sequencing done on these samples gives an idea of the common mutated strains of SARS-CoV-2. Further studies with a larger sample size and clinical correlation will help in detecting the mutated variants associated with severity of the disease and the need of vaccination to control the pandemic.

Table.1 Category of Samples Selected for Sequencing

S.No	Category of Sample for Sequencing	Total No. of Samples Selected
1	Community cluster	15
2	Family cluster	11
3	Positive samples from children	7
4	Infection among young adults with severe lung infection	19
5	Deceased patient without any co-morbidities	0
6	Suspected re-infection cases	6
7	Vaccine breakthrough cases	12

Table.2 Results of Sequencing among RTPCR Positive Samples

S.No	Variant of Sars-Cov-2	Total No. of Samples
1	B.1.617.2	36
2	DELTA LIKE VARIANT	14
3	AY.6	1
4	AY.4	9
5	AY.12	10

Table.3 Distribution of Variants among the Different Categories

S.No	Category Of Sample For Sequencing	Total Samples	B.1.617.2	Delta Like Variant	AY.6	AY.4	AY.12
1	Community cluster	15	5	6	0	1	3
2	Family cluster	11	6	1	0	3	1
3	Positive samples from children	7	4	1	0	1	1
4	Infection among young adults with severe lung infection,	19	10	4	1	2	2
5	Deceased patient without any co-morbidities	0	0	0	0	0	0
6	Suspected re-infection cases	6	4	0	0	1	1
7	Vaccine breakthrough cases	12	8	1	0	1	2

Table.4 Gender Distribution of Sars Cov-2 Variants

S.No	Variant	Males	Females
1	B.1.617.2	21	15
2	DELTA LIKE VARIANT	6	8
3	AY.6	0	1
4	AY.4	5	4
5	AY.12	5	5
	TOTAL	37	33

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