Original Article

Molecular Medical Diagnosis of COVID-19 and Omicron Variant

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Abstract

Genetic lineages of severe acute respiratory syndrome corona virus-2 (SARS-CoV-2) have continued to emerge and circulate around the world since the onset of the COVID-19 pandemic. There are numerous variants of SARS-CoV-2, the virus that causes corona virus disease 2019 (COVID-19). Like other viruses, SARS-CoV-2 evolves over time. Most mutations in the SARS-CoV-2 genome have no impact on viral function, but certain variants have gained worldwide attention because of their rapid emergence within populations, evidence of transmission, and clinical implications. During the pandemic, most parts of India were affected, including Odisha, leading to high rates of morbidity and mortality. For the present study, 368,303 samples were received by the COVID-19 lab i.e., medical (Virus Research Diagnostic Laboratory) VRDL from six districts of western Odisha, including approximately 25,000 COVID-19-positive samples. The diagnostic method of the quantitative RT-PCR cannot be used to distinguish among the variants created by mutation of the genes initially. Therefore, selected positive clinical samples were sent in cold chain for whole genome sequencing (WGS), and disease severity was sequenced using the Illumina Seq at ILS, BBSR for variant detection. The reported observation from the next generation sequencing (NGS) based sequenced samples of western Odisha updated in the INSACOG-WGS portal confirms the presence of Delta (B.1.617.2) and Delta sub lineages, Omicron (BA.2), and Omicron (B.1.1.529). Maximum infection was caused by Delta sub lineages

(83.5%) irrespective of age, sex, and geographic area followed by Delta and Omicron. Molecular diagnosis and WGS based study reveal the widespread transmission of the fatal virus, significantly affecting every corner of the globe.

Keywords: Western Odisha, SARS-CoV-2, Delta (B.1.617.2), Omicron (B.1.1.529), Omicron (BA.2)

Introduction

Severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) has a positive-sense single-stranded RNA genome approximately 30,000 nucleotides in length. The genome encodes 27 proteins, including an RNA-dependent RNA polymerase (RdRP) and four structural proteins. RdRP acts in conjunction with nonstructural proteins to maintain genome fidelity. A region of the RdRP gene in SARS-CoV-2 was shown to be highly similar to a region of the RdRP gene found in bat corona virus Rhinolophus affinis (RaTG13), with a 96% similarity in the overall genome sequences. The four structural proteins of SARS-CoV-2 include the spike surface glycoprotein (S), the small envelope protein (E), the matrix protein (M), and the nucleocapsid protein (N). In corona viruses, the S gene codes for the receptor-binding spike protein that enables the virus to infect cells; this spike protein mediates receptor binding and membrane fusion, which determines its host tropism and transmission capabilities.[1] According to a World Health Organization (WHO) report, the outbreak of COVID-19 has been confirmed in every country (WHO, 2022): the virus has infected 642 million people worldwide and claimed the lives of 6.6 million as of November 2022. The most severely affected countries include the United States, India, and Brazil. Currently, India is reporting 2,638 new cases, one new case per 100,000 people (–55%), and 43 new deaths, and one new death per 100,000 (+33%) per day. [2]

Seven corona viruses (CoVs) have been found to infect humans, including the four so-called common cold viruses (i.e., human corona virus [HCoV]-NL63, HCoV-229E, HCoV-OC43, and HKU1) and the three potentially lethal viruses of SARS-CoV, Middle East respiratory syndrome virus (MERS-CoV), and SARS-CoV-2. Out of these, SARS-CoV-2 created the ongoing COVID-19 pandemic that began in December 2019[3] and continues to evolve and adapt to the human population, as highlighted by the emergence of novel variants. Mutations within the spike protein of SARS-CoV-2 variants confer increased transmissibility and some degree of resistance to antibody-mediated neutralization. [4]

Like other viruses, SARS-CoV-2 evolves over time. Most mutations in the SARS-CoV-2 genome have no impact on viral function, but certain variants have gained worldwide attention because of their rapid emergence within populations, evidence for transmission, and clinical implications.[5,6] Viruses like SARS-CoV-2 continuously evolve as changes in the genetic code caused by genetic mutations or viral recombination occur during the replication of the genome. A lineage is a genetically closely related group of virus variants derived from a common ancestor, and a variant has one or more mutations that differentiate it from other variants. In some cases, a group of variants with similar genetic changes, such as a lineage or group of lineages, may be designated by health organizations as a variant being monitored, a variant of concern, or a variant of interest due to the attributes that may require public health action.[7]

On March 11, 2020, WHO declared the spread of COVID-19 as a pandemic. [8] New variants emerged around the same time in multiple locations, independent of one another, beginning in September 2020. This began with the emergence of B.1.1.7 in the United Kingdom (UK), followed by B.1.351 in South Africa, followed by B.1.617 in India and P.1 in Brazil. These new variants have multiple mutations on their spike proteins and spread rapidly across the globe over a short period, suggesting that they are highly virulent. [9] However, COVID-19 spread throughout India at a slower pace in comparison to other countries. India's Ministry of Health and Family Welfare (MoHFW) has continued to closely monitor the COVID-19 outbreak, launching the Integrated Disease Surveillance Programme (IDSP) in the National Center of Disease Control to conduct surveillance within the country and on outside travelers. The data from each state surveillance control room are updated directly to MoHFW; as such, we extracted the data used in the present study for the analysis of COVID-19 cases nationwide from the official MoHFW website.[10]

Given the pandemic potential of the virus, most parts of India were affected including Odisha leading to high rates of morbidity and mortality. Respiratory samples from the major districts of western Odisha along with samples from the Veer Surendra Sai Institute of Medical Science & Research (VIMSAR) hospital were sent to its government-ICMR approved COVID-19 RT-PCR laboratory in Burla per ICMR guidelines for molecular diagnosis (i.e. quantitative RT-PCR testing). Due to ongoing mutations, multiple waves of infection were seen in many countries, including India, leading to complications and reinfection even among those who are partially or fully vaccinated. As such, the government aimed to study the full genome sequence of the virus to develop a comprehensive management plan, using a network of laboratories across the country for early intervention research and variant analysis. These laboratories are also linked under the Indian SARS-CoV-2 Genomics Consortium (INSACOG). The present study has been planned to further understand the sequencing profile of SARS-CoV-2 variants based on available laboratory records in western parts of Odisha.

Study Objective

The study objective is to evaluate the frequency and distribution of SARS-CoV-2 variants circulating in different part of western Odisha.

Methodology

Study Setting

We conducted this study at the COVID-19 RT-PCR testing laboratory (ICMR) at the Department of Microbiology, VIMSAR Burla in Sambalpur, Odisha, in collaboration with the Institute of Life Sciences (DBT, GOI) in Bhubaneswar, Odisha.

Study Design and Population

The current study is a retrospective observational study approved by the Institutional Ethical Committee (Ref. No-194-2022/-F-O/96/Dt. 05.08.2022) of VIMSAR. Respiratory samples (i.e., nasopharyngeal samples) from major districts of western Odisha and the VIMSAR hospital were sent to the government-approved COVID-19 RT-PCR laboratory in Burla per ICMR guidelines for molecular diagnosis. This laboratory is part of the INSACOG, jointly initiated by the Ministry of Health and Department of Biotechnology (DBT) with the Council for Scientific and Industrial Research (CSIR); the ICMR; and the Institute of Life Sciences in Bhubaneswar (ILS, BBSR), Odisha. The selected SARS-CoV-2 positive samples were sent to ILS, BBSR for WGS and further analysis.[25-67]

Laboratory Documentation and Evaluation

Samples Received and Documentation

Clinical samples in virus transport media of suspected cases fulfilling the case definition for SARS-CoV-2 were referred by the hospital authority and COVID-19 state collection centers. We received these samples in cold chain and registered them in our laboratory for further processing. As per ICMR COVID guidelines, we documented all received samples in the portal after testing.

Sample Processing and Molecular Diagnosis of COVID-19

We conducted sample processing and RNA extractions per kit manufacturer guidelines (supplied by OSMCL, Odisha) in the BSL-2 plus lab, followed by post viral lysis procedures in the designated RNA lab area. We conducted further detection of SARS-CoV-2 via a quantitative reverse transcriptase PCR (q RT-PCR); this is the best choice for SARS-CoV-2 detection because of its sensitivity and versatility in the detection and quantification of a wide range of specimens. We conducted analyses of SARS-CoV-2 mutations based on confirmatory gene statuses (Orf1/Rdrp/N) as kit literatures (supplied by ICMR, OSMCL) and lab controls. For this, we used approximately 30 different types of RNA extraction and RT-PCR kits cumulatively for SARS-CoV-2 diagnosis at our testing lab.[70-91]

Whole Genome Sequencing

The selected positive clinical samples (500 ul VTM, Ct < 26 for N/Orf1/Rdrp) were sent in cold chain for WGS, representing the geographical districts and disease severity (per guidelines from the Director of Public Health, Odisha, 332/24.01.2021) to ILS, BBSR. We then sequenced these clinical specimens using NGS with the Illumina Seq at ILS, BBSR for variant detection. All subject-related data were updated in the INSACOG-WGS portal.

Analysis of Variants

We used data available from laboratory records from the INSACOG-WGS portal to analyze frequency distributions and classify the mutation profile of SARS-CoV-2 variants (VOC/VOI). We used appropriate statistical analyses, including student t-tests and a one-way ANOVA, for the variant distributions and classifications.

Results

Timeline of Incidence Rate of SARS-CoV-2 for 2020, 2021, and 2022

Our retrospective analysis confirms the incidences and spread of different mutations of SARS-CoV-2 over various periods, demonstrating its variation and gradual evolution. Altogether, we received 368,303 samples from several districts in western Odisha to date, including 21,242 positives (excluding Rapid antigen test positives). We observed the highest peak of positive cases from April 26, 2021, to May 16, 2021 (second wave of Delta), and the second highest peak from January 6, 2022, to February 6, 2022 (third wave of Omicron). The first positive peak occurred from August 1, 2020, to September 25, 2020 (first wave of COVID-19).[11] There is a lack of uniformity in the time period ranges on our end due to limited file documentation (see Table 1.a and Figure 1). In addition, we analyzed post vaccination and reinfection rates among our sample population; however, the analysis and variant patterns are limited to the NGS analysis data set.

NGS Data Analysis and Demographic Studies

We compiled the demographic and epidemiological characteristics of confirmed COVID-19 cases through descriptive statistics, including the age distribution, sex (male-to-female ratio), and Ct values of individuals from six different western Odisha districts as well as foreign returnees. We used our data set of 224 samples for NGS analysis, per the mentioned guidelines. We analyzed the data for samples that exhibited complete SARS-CoV-2 sequences (n = 90). The mean age of patients in the study was 33.7 years, with a comprehensive range of 10–70 years. A total of 61 samples were from male patients with a mean age of 35.04 years, and the remaining samples were from female patients with a mean age of 44.44 years (see Tables 2 and 3).

Characterization of Circulating SARS-CoV-2 Variants and Vaccine Efficacy

In the present study, we investigated the role of different underlying mutations and their transmission during the pandemic. From the NGS data updated in the INSACOG-WGS, we observed four different types of mutations, including two different VOCs (see Table 2). Most of these mutations comprised Delta (B.1.617.2) and its sublineages, with 83.3% (75 of 90) affecting multiple districts of the study area. We observed Omicron (B.1.1.529) in two UK returnees from our lab, which demonstrates the migration and transmission of variants along with local evolutions (see Tables 2 and 3). In addition, documented vaccination history in our data set is irregular: approximately one third of the subjects (28 of 90) were vaccinated. Further, we observed several cases of reinfection: out of 28 subjects total (M/F = 24/4), 24 presented with Delta sub lineages, three presented with Delta (B.1.617.2), and one presented with (B.1.617.2).[12]

Pattern and Distributions of Delta Sublineages

The Phylogenetic Assignment of Named Global Outbreak Lineages (Pangoline) designation and assignment using SARS-CoV-2 spike gene nucleotide sequences is a tool used to study genetic diversity. We subdivided the Delta variants in the Pango lineage designation system into variants from AY.1 to AY 125. However, there is no information on whether such classification correlates with biological characteristic changes of the virus.. We observed that the Delta sublineage was AY.44 with an incidence of 25.8% (15 of 58), followed by nine cases of AY.4, eight cases of AY.12, and seven cases of AY.102. One case of death (male, 47 years old, uncertain vaccination history) from the set was diagnosed with AY.12 (see Figure 2).[13,14]

Discussion

Since its emergence in 2019, SARS-CoV-2 has evolved effectively, accumulating deleterious mutations in its genome (i.e., Alpha [B.1.1.7], Beta [B.1.351], Gamma [B.1.1. 28 P1], and Delta [B.1. 617.2]). Within the 5 months from January to May 2021, multiple highly transmissible SARS-CoV-2 variants were detected across the globe, possibly escaping both natural and vaccine-induced immunity, leading to increased SARS-CoV-2 infection. [15]

CoVs can infect humans and animals to cause mild to severe disease or even death. They are divided into four genera: alpha- and beta-CoVs predominantly originate in bats and infect other mammals, whereas gamma- and delta-CoVs originate in and largely infect avian species.[1] All viruses, including SARS-CoV-2, change over time—and most of this change has no or little impact on the virus's properties. However, some changes may affect the virus's characteristics, such as how easily it spreads; the associated disease severity; or the performance of vaccines, therapeutic medicines, diagnostic tools, or other public health and social measures. The emergence of SARS-CoV-2 variants in places where the virus is uncontained poses a global threat from the perspective of public health and vaccine efficacy. Travel has been an important factor in the spread of SARS-CoV-2 variants worldwide; India has certainly observed the importation of SARS-CoV-2 variants via international travelers. [16]

Genomic sequencing is the process of deciphering the genetic material found in an organism to track its spread. It plays an important role in the identification of novel variants. Variants are challenging from the perspective of diagnosis, vaccine efficacy, and reinfection. The established nomenclature systems for naming and tracking SARS-CoV-2 genetic lineages via Global Initiative on Sharing Avian Influenza Data (GISAID), Nextstrain, and Pango are currently and will remain in use in scientific research. The Pango lineage nomenclature system is hierarchical; fine scaled, and designed to capture the cutting edge of pandemic transmission. There are more than 1,000 Pango lineages, compared to 12 and nine clades for the NextStrain and GISAID, respectively.[17]

India experienced a devastating second wave of the pandemic with the emergence of the Delta variant in April 2021. This variant has accounted for major breakthrough and reinfection cases in numerous countries, irrespective of the vaccine. Most of these cases were characterized by the Delta variant and its derivatives (i.e., AY).[14] Additionally, we noted varieties of mutations and their transmission in multiple districts simultaneously. Consequently, Omicron has become the dominant variant in many countries worldwide. The B.1.1.529 variant of Omicron, a new variant with a large number of mutations, was first reported as a VOC by WHO on November 26, 2021, and was identified from specimens collected from South Africa and Botswana via GISAID. According to preliminary evidence, this novel variant has increased the risk of reinfection compared with the other SARS-CoV-2 VOCs; it may be associated with enhanced transmissibility and reduced vaccine-induced immunity.[18] With the current study, we illustrated that Ct value-based distinctions among the variants were not precise, and thus the distribution of their severity and reinfection rates with respect to variants is least significant.

A recent ICMR epidemiological study documented a 4.5% reinfection rate of SARS-CoV-2 in India.[19] Cases of reinfection were also well documented in all countries with variable rates. Although age and gender vary, reinfection rates and vaccine effectiveness were significant issues at the country level. We noted similar observations in the present study: the frequency of reinfection was higher in male patients than in female patients, which may be the result of mobility. Reinfection rates, patient health updates, correct diagnoses, and vaccine efficacy were the most prominent issues for this disease.

Conclusion

The present research study concludes the analysis of COVID-19 transmission in western Odisha. Here, we report some of the important variants that resulted in millions of infections globally. The RT-PCR-based molecular diagnosis and WGS-based study reveal the widespread transmission of the fatal virus to every corner of the globe. Delta is a significant harmful variant of SARS-CoV-2 with devasting effects worldwide, and the Omicron variant was highly transmissible yet well documented. The COVID-19 pandemic has stressed the importance of health care and robust economic systems; rapid diagnostic testing, antiviral therapy, and active surveillance have ultimately reduced pandemic mortality.

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Conflict of Interest:

Authors report that they have no conflict of interest. The authors alone are responsible for the content and writing of this article.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institution with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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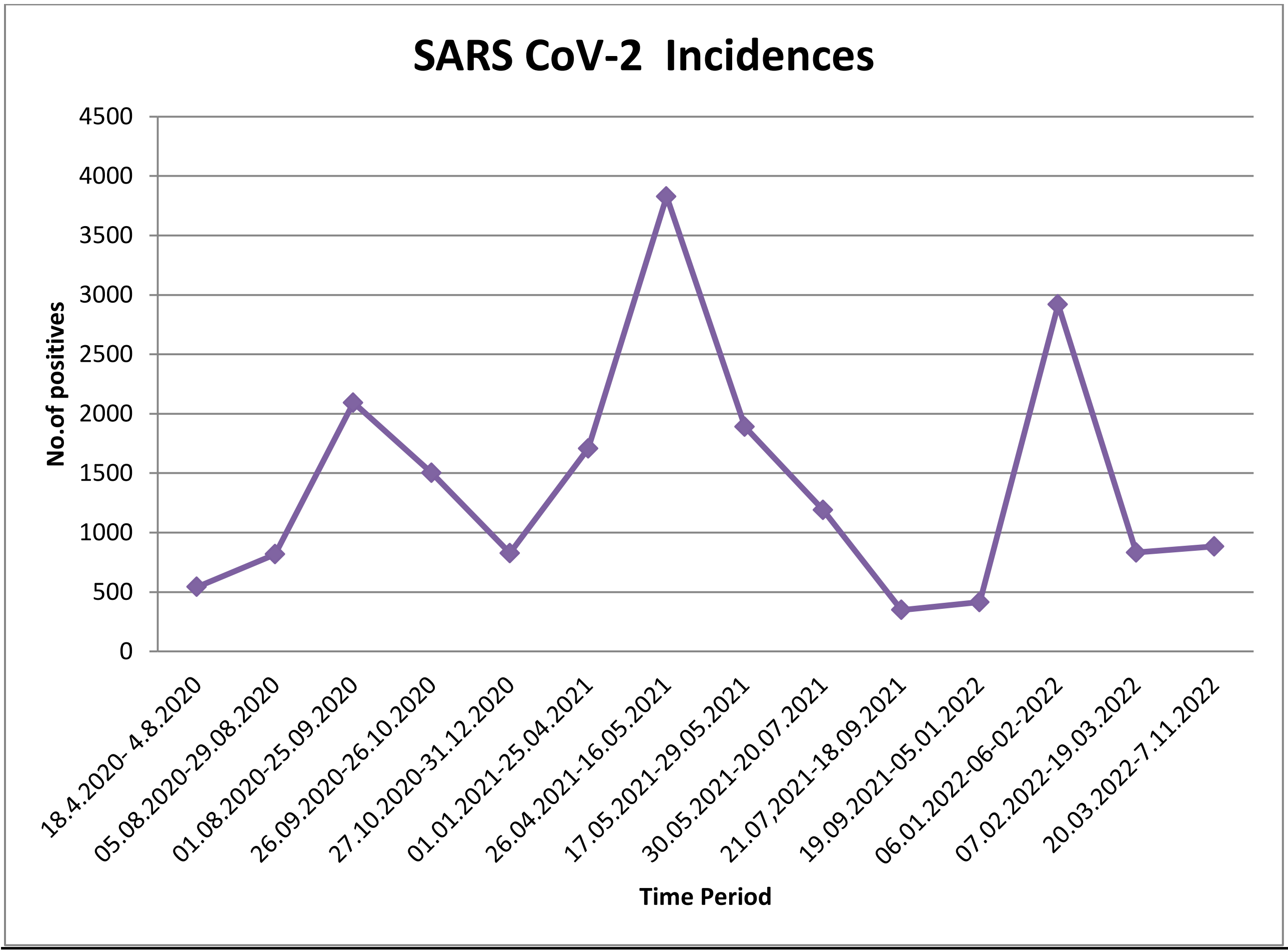
Fig: 1-Incidence rate of SARS-CoV-2 for 2020, 2021 and 2022 Time line



- Uniformity in time period range is lacking

Table:1-Types of Variant *vs*. Period of Incidences (ref: Sequencing updates INSACOG)

Types of Variant	**Period of Incidences**
Delta and its Sub Lineages (N= 75)	July 2021- Dec 2021

Omicron (B.1.1.529) (N=2)	Dec 2021
Omicron (BA.2) (N=13)	Jan 2022-March 2022

Table:2(a)-Age & Sex Distribution among Variants:

Type of Variant	No. of Subjects	Local Resident/ travel History	Gender Male/ Female	Mean Age (in yrs.)	Lowest Ct Value Mean± SD (Range)
Delta (B.1.617.2)	17	Multiple districts of State	12/5	33.5	19.29± 3.54 (14.0-26.0)
Delta Sub Lineages	58	Multiple districts of State	42/16	34.62	18.7±4.27 (13.0-27.0)
Omicron (BA.2)	13	Multiple districts of State	6/7	57.2	21.61±2.06 (19.0-25.0)
Omicron (B.1.1.529)	2	UK returnee	1/1	24	18.5

*Var. -15.91

#(Omicron -B.1.1.529 is not included in statistical analysis because of small sample size)

Table: 2(b)-Area wise Prevalence of Variants:

Type of Variant	Sambalpur	Bargarh	Jharsuguda	Deogarh	Sundergarh	Boudha	Foreign Returnee
Delta (B.1.617.2)	16	1	0	0	0	0	NA
Delta Sub Lineages	52	2	2	1	0	1	NA
Omicron (BA.2)	6	2	1	1	1	2	NA
Omicron (B.1.1.529)	2	-	-	-	-	-	Yes

Figure:2 - Incidences of Delta Sub Lineages (Pangolineage ref: WGS-INSACOG)