J Nepal Health Res Counc 2023 Jan-Mar;21(58):57-62

Genomic analysis of SARS-CoV-2 Circulating during Second and Third Wave of COVID-19 in Nepal

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ABSTRACT

Background: In Nepal, since the first detection of COVID-19 case in January 2020, the total cases have rose to almost a million with more than 12,000 deaths. Till now, WHO has classified 5 variants of SARS-Cov2 as variant of concerns at different time points causing many waves in different countries and regions at different time points. Nepal had also faced three distinct waves of COVID-19 caused by different variant of COVID 19. The objective of this study was to perform whole-genome sequencing of SARS-CoV-2 circulating in different waves of COVID-19 in Nepal and investigate its variant or lineage.

Methods: In this study, samples from 49 SARS-CoV-2 infected subjects from May 2021 to January 2022, were investigated. The methodology followed RNA extraction, real-time PCR for confirmation and whole-genome sequencing. The consensus genomes were interpreted with appropriate bioinformatics tools and databases.

Results: Sequence analysis of 49 genomes revealed to be of Delta (n=27) and Omicron Variant (n=22). The mutations in the consensus genomes contained the defining mutations of the respective lineages/variants. There were 20 genomes of Omicron sub-lineage BA.2, 1 of BA.1.1 and 1 of B.1.1.529.

Conclusions: This study provides concise genomic evidence of presence of Delta and Omicron variant of COVID-19 in Nepal. Delta and Omicron variants were driving the second wave and the third wave of COVID-19 respectively in Nepal. Therefore, the genomic surveillance must be increased to clearly map out the pandemic and strategize vaccination approaches in the country.

Keywords: COVID-19; delta, omicron; Nepal;SARS-CoV-2; whole-genome sequencing

INTRODUCTION

During early COVID-19 epidemic, wild type, Alpha-variant and other lineages were circulating in Nepal, resulting first surge of cases.[1,2] During April-June 2021, Nepal had second surge causing more than 350,000 infections. Similarly, third surge, which started from January-to-February-2022, had around 150,000 infections.[3]

Till now, WHO has classified 5 variants of SARS-CoV2 as variants-of-concerns: Alpha, Beta, Gamma, Delta and Omicron which have higher transmissibility or higher virulence.[4,5] The characteristic mutations for Delta-Variant make them more transmissible and virulent than previously circulating Alpha-variant. Similarly, the Omicron-variant carries more than 50 mutations with 26-32 mutations in spike-protein.[6-8] Additionally, it also has range of close mutations to the furin-cleavage site found in Delta-Variant.[9,10] Due to these mutations, Omicron can replicate 70-fold-faster, in the human bronchi, than Delta-Variant.

In this study, we performed whole genome sequencing of SARS-CoV-2 samples circulating in Nepal along with their mutation profiles, during second and third wave.

METHODS

For this study, a total of 49 SARS-CoV-2 positive samples (S1 to S49) were selected during the second and third wave, and processed at different time periods: May 2021 (4 samples: S1 to S4), August 2021 (4 samples: S5 to S8),

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December 2021 (12 samples: S9 to S20) and January 2022 (29 samples: S21 to S49). The SARS-CoV-2 samples were provided by Nepal Health Research Council, Star Hospital and Dhulikhel Hospital, Kathmandu University Hospital. The samples in this study were of variable clinical conditions, mortality and vaccination status.

The quality of the obtained samples was assessed by RNA extraction and qPCR. The RNA extraction was performed using QIAamp Viral RNA Mini Kit (Cat. No. 52904) and real-time PCR was performed on BioRad CFX96 Detection System (1855195) using commercially available RT-PCR Kit. The library preparation was performed following the protocol provided by Illumina Inc. for Ampliseq Amplicon Sequencing with some modifications, which was validated in another study.[2] This protocol was successfully implemented for whole genome sequencing of SARS-CoV-2, in one of our previously published studies.[2] Briefly, the RNA samples were subjected to DNaseI (ZymoResearch, Cat. No. E1010) aimed for digestion of DNA (if any) in the sample. This was followed by clean-up using 2X SPRI (Solid Phase Reversible Immobilization) beads. The eluted RNAs were then quantified using Qubit™ RNA HS Assay kit (Invitrogen Cat. No. Q32852). After that, cDNA was synthesized for all the samples using AmpliSeq™ cDNA synthesis for Illumina® (Cat. No. 20022654). The core library preparation was performed using AmpliSeq™ Library PLUS for Illumina® (Cat. No. 20019101), partially digested with FuPa reagent, ligated with AmpliSeq™ UD Indexes for Illumina® (Cat. No. 20019104) and the prepared individual libraries were cleaned up with 1X SPRI beads. The library was amplified with Library Amplification Mix, followed by two step clean-ups (0.5X and 0.8X SPRI beads). The quality control analysis of the prepared library was done through PCR using Kapa Library Quantification Primer Premix for Illumina (KAPA Biosystems Cat. No. KP0005), followed by agarose gel electrophoresis of the amplicons and analysis in Agilent Tapestation 4150. Upon visualization of adapter-dimers in the library, it was cleaned up according to the requirement. Libraries without any adapter dimers are not cleaned up beads, whereas, the libraries that have adapter-dimers are cleaned accordingly. The libraries with low concentration were amplified using Kapa Library Amplification Kit for Illumina (KAPA Biosystems Cat. No. KK2612) Subsequently, all the individual libraries were quantified using Qubit™ 1X dsDNA HS Assay kit (Invitrogen Cat. No. Q33231). Finally, 100pM of the pooled library, along with 5% PhiX control, was loaded on the Illumina iSeq100.

The genomic sequences from the Illumina iSeq100 were base-called, and subsequently demultiplexed using Illumina BaseSpace. The generated fastq files were imported to CZID that uses Consensus Genome Piepline v3.4.7 and aligned to the reference genome (MN908947.3). CZID creates consensus fasta sequence from the aligned reads. The generation of consensus genome was validated by independent command line pipeline. Thus obtained consensus genome were further analysed in Nextclade, GISAID Covsurver and Phylogenetic Assignment of Named Global Outbreak LINeages (PANGOLIN). The data were, additionally, studied through various bioinformatics tools such as Clustal Omega. The sequences were, subsequently, submitted to GISAID with available subject information.

This study was ethically approved from Nepal Health Research Council (NHRC).

RESULTS

The subjects, whose samples were taken, were of mean age 37.7 years. The female to male ratio of the subjects was 1.04.

Table 1. Subject Characteristics in Age, Gender, Travel and Vaccination History.

SN	Characteristics	Frequency n (%)
Age		
1	0-10	0
2	11-20	4 (8.16%)
3	21-30	19 (38.77%)
4	31-40	11 (22.44%)
5	41-50	4 (8.16%)
6	51-60	2 (4.08%)
7	61-70	3 (6.12%)
8	>70	5 (10.20%)
9	Unknown	1 (2.04%)
Gender		
1	Male	25 (51.02%)
2	Female	24 (48.97%)
Travel History		
1	International Travel	3 (6.12%)
2	No Travel	29 (59.18%)
3	Unknown	17 (34.69%)
Vaccination		
1	Sinopharm Verocell	11 (22.44%)
2	AstraZeneca COVISHIELD	15 (30.61%)
3	Not Taken	6 (12.24%)
4	Unknown	17 (34.69%)

Three of the subjects were deceased. Most of the subjects had COVID-19 like illness of some type. Three of the all subjects had international travel history. Six of the subjects were not vaccinated, among which two were deceased. 7 were vaccinated with double dose of ChAdOx1 CoviShield, 10 with double dose of SinoPharma VeroCell. Additionally, 8 subjects had already taken booster dose prior to infection while 6 were unvaccinated until the SARS-CoV-2 infection (Table 1).

The previously confirmed SARS-CoV-2 positive samples were re extracted and RT-PCR was performed to verify the presence of SARS-CoV-2 in the samples.

The consensus genomes generated from our study had average genome coverage of 97.9% (77.3% to 99.8%) and depth of 2809.4x. Out of 49 genomes, 27 belonged to different sub-lineages of Delta Variant while 22 belonged to Omicron variant as shown in Supplementary Table 2.

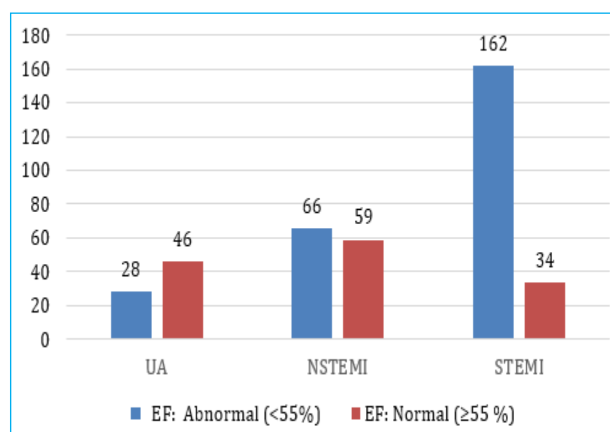

Figure1. Increasing number of cases, during second wave (starting from April'21 and peaking at May'21) and third wave (peaking in January'22) compared to sequencing results (GISAID) showing high percentage of Delta and Omicron Variants.

The mutation pattern analysis in the GISAID CoVsurver mutation platform showed mutations characteristic of Delta variant in spike protein of all the Delta Variant genomes (n=27): L452R, T478K, D614G and P681R. Additionally, substitution mutation K417N, which is characteristic to Delta+,was seen in genome S2, S7 and S8, however only S2 was dictated as Delta+ variant by Pango lineage. Additionally, genomes S27 and S29 to S46 had the numerous mutations specific to Omicron variant. S36 belonged to sub-lineage BA.1, S47 belonged to B.1.1.529 and the rest to sub-lineage BA.2. All the genomes (n=20) belonging to sub-lineage BA.2 did not have the mutation del69-70 in the spike protein while S36 (sub-lineage BA.1.1) had the mutation.

The whole genome sequencing results from Nepal, as a whole,[11] when compared with surge of COVID-19 cases during second and third wave, showed that Delta and Omicron variant led the second and third wave respectively.

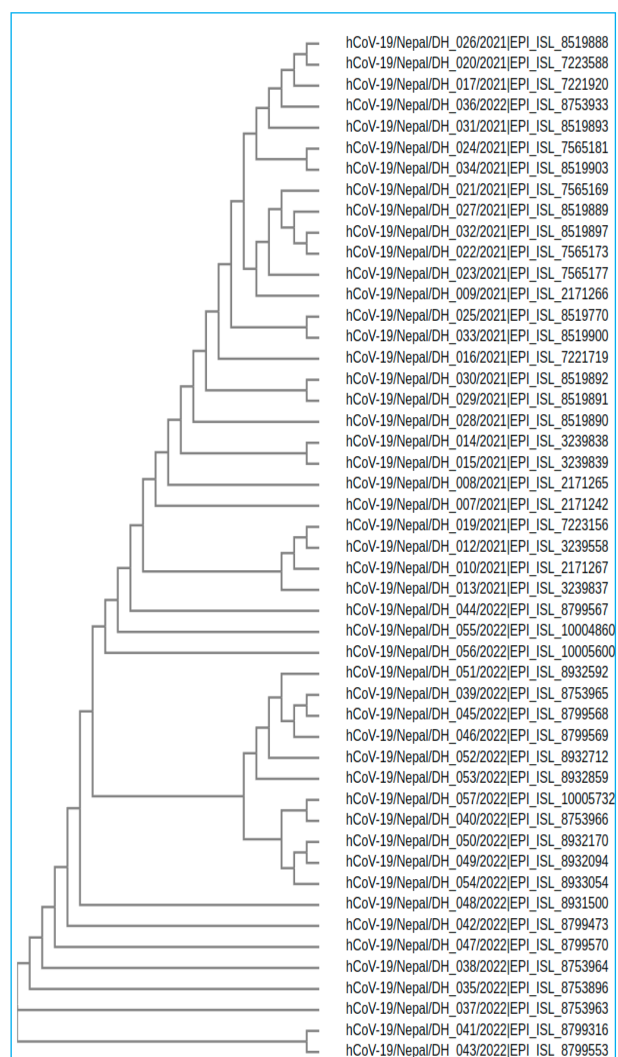

Figure2. Phylogenetic Tree of all 49 genome sequences used in this study. The tree was prepared using UShERtool from UCSC Genome Bioinformatics Group

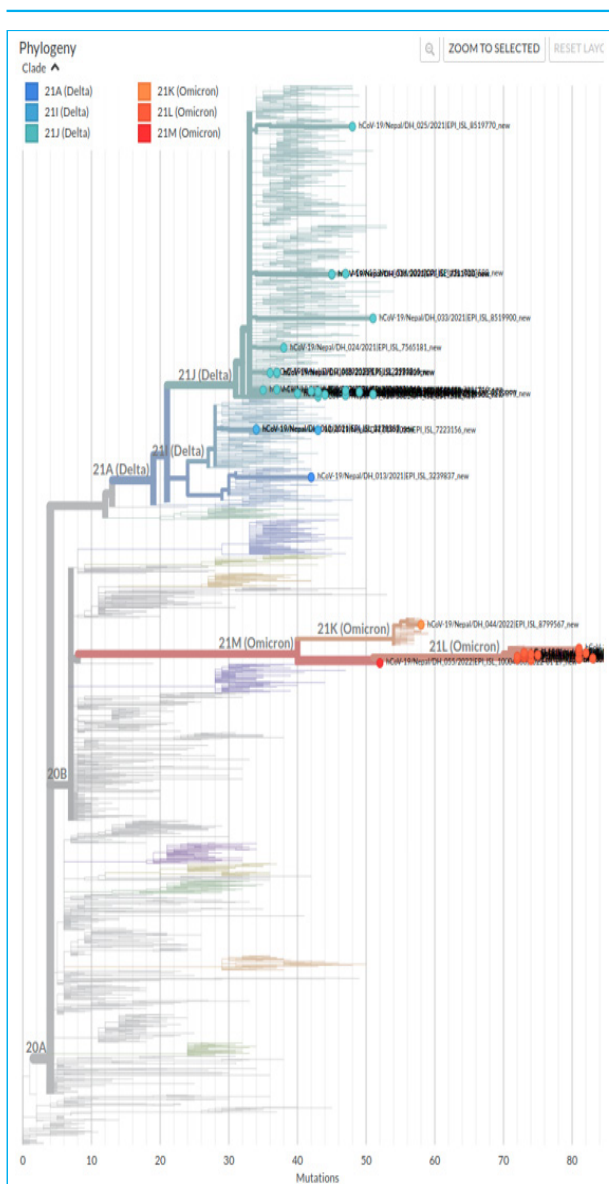

Figure 3. Global position of the 49 genome sequences, of this study, aligned to global phylogenetic tree of SARS-CoV-2 sequences. The tree was prepared using NextClade and Auspice which use submissions from GISAID.

DISCUSSION

In our study, when compared to the reference genome hCoV19/Wuhan/WIV04/2019 in GISAID, 27 genomes (S1 to S26 and S28) were of Delta Variant. This variant of concern of SARS-COV-2 was first identified in October 2020 in India, however, its exponential spread started in March 2021. The second wave of COVID-19 pandemic started in Nepal from April 2021, a month after. With one month of buffer time, the wave in Nepal was reported to be a spillover of India's outbreak across Nepal-India open border.[12] On May 2021, as reported from Nepal Government, 97% of 35 SARS-CoV-2 samples, sequenced for whole genome, were found to be variant B.1.617.2.[13] Similarly, out of 47 SARS-CoV-2 sample collected from May to mid-July, all of them were of Delta Variant, with three of them with K417N mutation.[14] Even though this wave subsided by September 2021,[3] according to our study, Delta predominated among the SARS-CoV-2 infection, in Nepal, throughout the sample collected in the time period of May-December 2021. Similarly, Omicron was first reported on 6 December 2021, in Nepal.[15] However, until 23 December, additional three cases of Omicron were reported, which were from the contact tracing of the first case. The third wave of COVID-19 lasted peaked throughout the month of January and waned by the third week of February.[5] Furthermore, according to GISAID database, after 24 December 2021 until February 2022 in Nepal, 611 out of 687 subject sequenced were of Omicron variant.[11] Similarly, as per our study, for samples collected in January 2022 all (n=22) except one (S28) was of Omicron Variant.

The genomic sequences S1 to S26 and S28, of this study, had the characteristic mutations of Delta Variant. The mutations such as L452R and P681, in the Spike Protein, are known to impact the antibody binding while T478K affects the folding and attachment of the virus to ACE2 receptor.[16] Spike_P681R is the most significant mutation in the Delta lineage as it lies in the furin cleavage site and results in enhanced rate of membrane fusion, internalization and thus better transmissibility.[17] Genome S2, S7 and S8, belonging to Delta Variant, also had one of the mutations of Beta variant, i.e. K417N. Interestingly, this mutation is seen in all of the genome sequences of Omicron Variant. Until 29 January 2022, there are total of 396,415 such submissions in GISAID (including 21 from Nepal) which fall in the lineage AY.5 (Delta Variant that has K417N mutation). In a recent in-silico study, it was mentioned that K417N induced no major structural changes in spike protein and would seem neutral or even unfavorable for interaction with ACE2.[18,19] Though some comments from scientific community have speculated that Delta variant with K417N (nicknamed Delta Plus) could reduce effectiveness of vaccine with increased risk of reinfection, the sub lineage was reclassified as variant under investigation in October 2021.[20-22]

Omicron(21K), which is reported to have number of mutations in spike protein, receptor binding domain and N-terminal domain, could play vital role in antibody recognition and ACE2 binding.[23] It has been reported that the increased transmissibility of omicron could be the result of a cluster mutation at S1-S2 furin cleavage site (substitutions H655Y, N679K and P681H).[24] This

mutation of P681H causes the virus to spread more to wider range of tissues.[25] All of the genome sequences of Omicron variant sub-lineages BA.2 and B.1.1.529, in this study, have the aforementioned substitution in the furin cleavage site except genome S36, which falls under sub-lineage BA.1.1. The substitution mutation Q498R, in combination with N501Y, is reported to increase the ACE2 affinity by more than 1000 times.[26] Interestingly, none of the genome sequences, of Omicron variant, in this study contained the mutation Q498R. Additionally, the sequences S29, S32, S33, S34, S35, S40, S42 and S46 contained a mutation E484A, which has been associated with immune escape characteristic of the variant.[27] Some mutations in Orf1a such as deletions at 3674, 3675 and 3676 can compromise the host cell's ability to destroy the viral components.[28] In our study, all of the Omicron variant genome sequences contained the deletion at 3675 and 3676 except S48 which contained only the deletion at 3678. Interestingly, only S36 and SS47 had deletion at 3674 which were of sub lineage BA.1.1 and B.1.1.529 respectively.

The limitation of this study is that it did not include the samples from epidemiologically diverse regions of Nepal. As the number of COVID-19 cases were at its peak across the country in both waves, we have assumed the transmission potential to be high everywhere, and our sampling could represent the concise depiction of variants circulating in Nepal.

CONCLUSION

This study concludes the identification of SARS-CoV-2 Delta and Omicron by whole genome sequencing. Delta Variant dominated the second wave as well as post second wave infections, the initiation of third wave was caused by Omicron variant which dominated throughout the third wave.

Therefore, public health authorities should increase sequencing surveillance for early detection of circulating new variants so that early action could be initiated and reduce the impact of surge of cases.

ACKNOWLEDGMENTS

We want to acknowledge Bill & Melinda Gates Foundation for supporting iSeq100 platform which helped us to perform whole genome sequencing of SARS-CoV-2. We also would like to thank Nepal Health Research Council (NHRC) for facilitating us for the samples and Star Hospital and Dhulikhel Hospital for providing the samples for the study. Finally, we are appreciative of Chan Zuckerberg Biohub (CZ Biohub) for the support.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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