Surge of Severe Acute Respiratory Syndrome Coronavirus 2 Infections Linked to Single Introduction of a Virus Strain in Myanmar, 2020

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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is a major health concern globally. Genomic epidemiology is an important tool to assess the pandemic of the coronavirus disease 2019 (COVID-19). Several mutations have been reported by genome analysis of the SARS-CoV-2. In the present study, we investigate mutational and phylogenetic analysis of 30 whole genome sequences for genomic characteristics of the virus in the specimens collected early phase of pandemic, (March-June, 2020) and sudden surge of infection (August-September, 2020). Phylogenetic analysis revealed that 4 samples of L strain and 1 GR strain in early stage of local transmission, while 6 returnees by rescue flights showed GH (India) and GR strains (China and Philippines) with no evidence of local spread. However, all 19 whole genome sequences in sudden surge of local transmission showed genetically distinct clade GH (Lineage B.1.36). Surge of second wave on SARS-CoV-2 infection was linked to the single-introduction of the GH strain that may be a result of strict restriction of international travel and containment efforts. These genomic data provides the useful information to disease control and prevention strategy.

Introduction

The novel respiratory coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been a source of major concern globally. In Myanmar, the first cases of COVID-19 were reported in March 2020 and only a few local cases were reported until the end of July 2020 as the result of effective and enormous efforts to contain the virus. Moreover, since March 2020, restriction on international flight have been started to prevent the introduction of the virus. Only returnees Myanmar citizens by rescue flight were selectively allowed and they were checked for two times swab examination by molecular method within 21 days quarantine period. Consequently, only 379 confirmed cases were reported by August 18, 2020 in Myanmar¹. However, after August 19, a sudden increase in local cases without any history of contact with known cases, were reported in Rakhine State, western Myanmar. Within a week, local transmission was reported in the metropolitan city, Yangon. Up to September 30, 2020, a total of 13,373 confirmed cases were recorded with 310 deceased².

The molecular epidemiology of the SARS-CoV-2 strain provides useful information to formulate the strategy for prevention and control of the disease^{3,4}. Mutational analysis and phylogenetic studies revealed that the virus originated from Wuhan was replaced by mutant strains in many countries⁵. However, up to now, no known study on genomic analysis of SARS-CoV-2 has been documented in Myanmar.

Preliminary data with history of travelling abroad, the imported confirmed cases from returnees indicated that different strains from various geographical territories had been introduced into Myanmar. We report the whole genome sequence analysis of SARS-CoV-2 strains detected in Myanmar to provide the genetic evidence on spread and dynamics of virus dissemination in this country.

Methods

Ethics statement

The study was reviewed and approved by the institutional review board of the Department of Medical Research (Approval ID: Ethics/DMR/2020/073). The study was registered at Myanmar Health Research Registry with ID: PLRID-00476_V5). All procedures were conducted according to the institutional guideline on responsible conduct of research.

Study population

This study used the confirmed positive remaining nasopharyngeal swab samples after molecular diagnosis. According to the ARTIC protocol, samples with low Ct value were involved in this study for whole genome analysis. Based on the daily positivity rate of confirmed COVID-19 cases, early phase of the pandemic wave was defined before the August-15, 2020 and afterward, sudden surge of infection was observed. We selected 4 samples in early phase of pandemic wave and 6 samples from returnees from China and India. A total 9 samples from sudden surge of infection and one returnees from Philippines in August, 2020 and another 10 samples, in which 9 of them were deceased cases, were collected in September, 2020 (Table 1).

RNA extraction and confirmation of the infection

We follows the procedures for whole genome sequencing as described previously⁶. Briefly, nucleic acid was extracted from nasopharyngeal swab using QIAGEN QIAamp® Viral RNA Mini Kit according to the manufacture's instruction. The SARS-CoV-2 infection in the samples were confirmed by novel coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (Sansure Biotech, Republic of China) using BioRad CFX96 touch real-time PCR detection system according to the manufacturer's instruction.

Sequencing procedures

We performed the amplification of the SARS-CoV2 whole genome according to the ARTIC nCoV-2019 protocol. Briefly, cDNA was synthesis using GoScript™ Reverse Transcriptase Kit (Promega). Amplification was done using Q5 hot-start high-fidelity DNA polymerase (NEB). Amplicon was checked by capillary electrophoresis (LabChip GXTouch 24 nucleic acid analyzer). Overlapping amplicons of 400 bp were purified by Illumina sample purification bead (Illumina, USA). The cleanup amplicons were normalized before library preparation by Nextera DNA Flex Library preparation Kit (Illumina). The library was checked the quality by capillary electrophoresis and quantify using Qubit dsDNA HS (High Sensitivity) Assay Kit. Normalization was carried out before pooling on 10 samples in each run. These pooled samples were denatured by NaOH and 11 pmol library was loaded with 10% PhiX spike control to run 151 paired end in MiSeq next generation sequencer.

Sequence-analysis

The output sequences were proceeded for reads quality control by BBDuk (BBTools 38.57) and BBMap (BBTools 38.57). Read alignment against SARS-CoV-2 reference genome sequence (NC_045512.2). The sequences were checked by genome detective virus tool (<https://www.genomedetective.com/>)⁷. The variant calling was done by CoV-GLUE (<http://cov-glue.cvr.gla.ac.uk/>)⁸ and NextClade v0.5.0 (<https://clades.nextstrain.org/>)⁹ to assign the clade. The SARS-CoV-2 lineages was assessed by Pangolin COVID-19 Lineage Assigner (<https://pangolin.cog-uk.io>)¹⁰ The maximum likelihood phylogeny was done after aligned with reference sequence Wuhan-Hu-1 (NC_045512.2) and visualized by iTOL v5.6.3 (<https://itol.embl.de/>).

We submitted our sequences to NCBI SRA with accession numbers PRJNA659630, PRJNA655384 and PRJNA667200.

Result

We analyzed 30 nasopharyngeal swab specimens from patients with COVID-19 to conduct whole genome analysis. Reads were aligned to the reference genome (Wuhan Hu-1; GenBank MN908947.3) and consensus sequences generated. After the quality control, median number of the reads was 2,228,704 with mean sequencing depth of 8,376-fold covering more than 99% of the genome in all samples. We have identified 12 mean mutations in which contains 30 synonymous, 34 nonsynonymous substitutions and 5 mutation at 5'UTR site along the whole genome (Table 2).

According to the GISAID clade system (<https://www.gisaid.org>), four local cases that were collected in early stage of pandemic spread is clade 19A and one local case is clade 20B. Among the returnees, 4 were from India (clade 20A), one from china (clade 20B) and one from Philippine (clade 20B). All 4 local cases in early stage of pandemic spread was L clade (GISAID clade analysis) and B.6 lineage (PANGOLIN analysis: <https://pangolin.cog-uk.io/>). All returnees from India and local cases detected in the sudden surge of pandemic spread were GH strain and B.1.36.1 lineage (Table 1).

Lineage B.6 was mainly detected in Southeast Asia in March and April 2020¹¹. In our study, the local transmission cases had the history of primary and secondary contact with confirmed cases returned back from Singapore. Among the samples taken from the returnees from India in April and May, we identified GH strain with Lineage B.1.36.1. Meanwhile, the samples from the returnees from China and Philippines showed GR strain with Lineage B.1.80 (China) and B.1.1 (Philippine). According to the PANGOLIN analysis, B.1.36.1 is an India lineage circulating in India. The SARS-CoV-2 detected from the persons coming back from India showed B.1.36.1 lineage (Figure 1).

Among five local transmission cases before August 2020, we detected only one sample with D614G mutation in spike protein altogether with Trinucleotide-Bloc Mutation, 28881-28883 GGG>AAC (R203K and G204R) at nucleoprotein (N) gene that was denoted as GR strain. When we conducted mutational analysis on the samples of returnees from India (4 cases), China (1 case) and the Philippines (1 case), all were G

strains (GH strain in all samples from India returnees and GR strain from returnees from China and the Philippines).

After August 15, sudden increase in local transmission without any known contact history with confirmed cases was firstly reported in Rakhine State, western Myanmar and later in Yangon megacity and other regions in Myanmar. In our analysis, 9 samples from Rakhine State and 10 samples from Yangon City were included. Whole genome analysis on all these 19 samples revealed that those were GH strain with B1.36 lineage. There were same 12 mutations in all 19 samples (Table 2). Distinct synonymous mutation G18756T in ORF1b was observed in all these samples. Moreover, we observed C241T in 5'UTR, C3037T synonymous mutation at ORF1a, C14408T (P314L) at ORF1b, C18877T synonymous mutation at ORF1b, C22444T synonymous mutation at Spike, G25494T synonymous mutation at ORF3a, G25563T (Q57H) at ORF3a, C26735T in membrane protein, C28854T (S194L) at nucleoprotein in all samples taken from sudden surge of local transmission (Table 2).

The phylogenetic analysis revealed that multiple introduction of the SARS-CoV-2 strains is possible in early phase of the pandemic wave in Myanmar. However, single strain introduction was observed in the cases in sudden surge of the pandemic wave (Figure 2). Moreover, SARS-CoV-2 strains in deceased cases were not different with that in recover cases.

Discussion

Genetic epidemiology provides the useful information for prevention and control of the diseases. Whole genome data can be applied for disease tracking in local transmission cases. Unlike the other diseases, COVID-19 become pandemic spreading to many countries within a short period and genomic studies revealed that SARS-CoV-2 mutation rate is same as other RNA virus affecting the spread of the diseases globally¹². Taken together, genomic surveillance is important to know the transmission of the disease by which further action for containment, control and prevention of the disease¹³.

Among the reported mutations, non-synonymous mutation D614G was firstly observed in early 2020 but spread widely within a few weeks in many countries. The Spike D614G mutation is reputed to SARS-CoV-2 infection in vitro suggesting it enhances viral infectivity that may result in increased of G strain globally^{14,15}.

In the present study, only one cases of GR strain was observed in local transmission cases in early pandemic wave of infection. Among the returnees, GH strains from India and GR strain from China and Philippines were observed. Because of the strict quarantine rules, infections confirmed in theses returnees might not be spread locally in Myanmar.

Unfortunately, local transmission cases without history of contact on known cases were reported in late August 2020 in western Myanmar, Rakhine State. Within a few days, many local cases were detected not only in the Rakhine State but also in Yangon and other areas in Myanmar. In our analysis on 19 samples in theses sudden surge of infection, showed GH strain with distinct non-synonomous mutations.

Mutation at the 5'UTR region may affect the viral transcription and replication leading to the speed of the viral infection cycle^{16,17}. In our study, all D614G variants were coexistent with C241T at the 5'UTR. Although the role of that mutation, G18756T is still not clear, it was reported in the sample of the second time infection in South America and India¹⁸.

Among all mutations detected in the present study, the leader sequence mutation C241T is co-evolved with three important mutations, C3037 T, C14408T, and A23403 G, which result in amino acid mutations in nsp3 (synonymous mutation), RNA primase (P323L), and spike glycoprotein (S protein, D614G), respectively. These co-mutations are in critical proteins for RNA replication and for binding to ACE2 receptor¹⁹. However, virulence of the infection might not be related to the strains detected in this present study as the genetic characteristics of the SARS-CoV-2 of deceased cases were similar with that of recover cases.

In the present study, whole genome analysis on these local cases in sudden surge of infection revealed that all were GH strain (Lineage B.1.36). Unlike the other countries, Myanmar limits the international travel and only returnees of Myanmar citizens were allowed with limitations by relief flights. Consequently, only few local cases were observed in early pandemic wave before August 2020 although multiple introduction of the SARS-CoV-2 were observed. In spite of the containment efforts, single introduction of GH strain (Lineage B.1.36) was observed and spread causing sudden surge of COVID-19 wave in late August 2020 in Myanmar.

One limitation of our study is that only a subset of the laboratory confirmed samples was included and further study focusing on the molecular surveillance for better understanding on the SARS-CoV-2 in Myanmar should be carried out.

Conclusion

Mutational and phylogenetic analysis on whole genome data in our study suggests multiple introductions of the SARS-CoV-2 in Myanmar. Since March 2020, local transmission of SARS-CoV-2 was reported, but genomic data indicated that early local transmission cases were L strain with B.1.6 lineage which differed from the samples taken from the sudden surge of local transmission cases after August, 2020 (GH strain with B.1.36 lineage). Although the real impact on these strains on different aspect of pathogenicity and virulence is undetermined, dynamics of the viral sequences related to the sudden surge of the infection should be taken into high concern in control of pandemic COVID-19.

Declarations

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M.S.H, W.Y.M, P.P.T performed the RNA extraction and laboratory experiments, M.H.N, H.M.T wrote the manuscript, all read and approve the manuscript.

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Conflict of interest statement

The authors declare no competing interests.

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Tables

Table 1. Demographic characteristics of the sequenced patients with severe acute respiratory syndrome coronavirus 2, Myanmar, 2020

ID	Age	Sex	Place	Contact history with known positive cases	Foreign country travel history	Diagnosis date (2020)	Outcome	Nextstrain clade	GISAID clade	PANGOLIN Lineage
MM1	35	M	Yangon	Yes	No	24-Apr	Recover	19A	L	B.6
MM2	31	F	Yangon	Yes	No	24-Apr	Recover	19A	L	B.6
MM3	53	M	Yangon	Yes	No	24-Apr	Recover	19A	L	B.6
MM4	48	M	Yangon	No	No	3-May	Recover	19A	L	B.6
MM5	28	F	Yangon	Yes	No	7-May	Recover	20B	GR	B.1.1
MM6	41	F	Yangon	No	Yes (India)	25-May	Recover	20A	GH	B.1.36.1
MM7	29	F	Yangon	No	Yes (China)	2-Jun	Recover	20A	GH	B.1.80
MM8	63	F	Yangon	No	Yes (India)	26-May	Recover	20A	GH	B.1.36.1
MM9	66	M	Yangon	No	Yes (India)	26-May	Recover	20A	GH	B.1.36.1
MM10	40	F	Yangon	No	Yes (India)	30-May	Recover	20B	GR	B.1.1
MM11	22	M	Sittwe	Yes	No	21-Aug	Recover	20A	GH	B.1.36
MM12	58	M	Sittwe	Yes	No	21-Aug	Recover	20A	GH	B.1.36
MM13	28	M	Sittwe	Yes	No	21-Aug	Recover	20A	GH	B.1.36
MM14	38	F	Sittwe	No	No	20-Aug	Recover	20A	GH	B.1.36
MM15	29	F	Sittwe	No	No	20-Aug	Recover	20A	GH	B.1.36
MM16	83	M	Sittwe	No	No	20-Aug	Recover	20A	GH	B.1.36
MM17	28	F	Sittwe	Yes	No	20-Aug	Recover	20A	GH	B.1.36
MM18	43	M	Sittwe	Yes	No	21-Aug	Recover	20A	GH	B.1.36
MM19	50	F	Sittwe	Yes	No	19-Aug	Recover	20A	GH	B.1.36
MM20	21	F	Yangon	No	Yes(Philippine)	19-Aug	Recover	20B	GR	B.1.1
MM21	75	M	Yangon	No	No	12-Sep	Deceased	20A	GH	B.1.36
MM22	84	F	Yangon	No	No	13-Sep	Deceased	20A	GH	B.1.36
MM23	58	M	Yangon	Yes	No	16-Sep	Deceased	20A	GH	B.1.36
MM24	67	M	Yangon	Yes	No	16-Sep	Deceased	20A	GH	B.1.36
MM25	24	M	Yangon	No	No	14-Sep	Deceased	20A	GH	B.1.36
MM26	74	M	Yangon	No	No	17-Sep	Deceased	20A	GH	B.1.36
MM27	75	F	Yangon	Yes	No	17-Sep	Deceased	20A	GH	B.1.36
MM28	48	F	Yangon	No	No	17-Sep	Deceased	20A	GH	B.1.36
MM29	23	F	Yangon	No	No	17-Sep	Deceased	20A	GH	B.1.36
MM30	53	M	Yangon	No	No	28-Sep	Recover	20A	GH	B.1.36

Table 2. Mutations detected in 30 whole genome sequences (table attached)

Figures

Figure 1

Number of daily confirmed COVID-19 cases in Myanmar within March to September 2020 with GISAID clades in analyzed 30 genomes. GH clade (D614G and Q57H synonymous mutation) in local cases were detected in second surge of the pandemic COVID-19 in Myanmar.

Figure 2

Maximum likelihood analysis clades of the 30 whole genome on SARS-CoV-2 in Myanmar, 2020. Green clades were local transmission cases in early pandemic time. Red clades indicated the samples of the

sudden surge of pandemic wave after August, 2020. NC 045512.3 denoted the reference strain. The remaining were imported cases.

Supplementary Files

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- [Table2.pdf](#)