Correspondence

Quasispecies analysis of the SARS-CoV-2 from representative clinical samples: A preliminary analysis

Sir,

The current pandemic situation on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is expanding since its first report on December 2019. To date, 14,667,659 people are infected from SARS-CoV-2 all over the world, and India has reported on July 20, 2020, a total of 1,119,412 SARS-CoV-2 cases. SARS-CoV-2 is a single-stranded, positive-sense, poly-A-tailed RNA belonging to the family Coronaviridae. Its genome size ranges from 27 to 34 kb, which encodes for non-structural proteins, structural proteins and accessory proteins. To limit the spread of the virus, guidelines related to preventive measures are published by the World Health Organization as well as the Indian government as no vaccines or drugs are currently available. Processes for the identification of suitable antiviral drugs and vaccines have been initiated. Vaccine peptides based on the B-cell and T-cell epitope-inactivated and attenuated virus vaccines, subunit vaccine, DNA vaccines and mRNA vaccine are some of the approaches that are used for the design of the vaccine candidates for COVID-19. These approaches are implied based on the dominant population of the viral sequences present within the host cells. However, the viral RNA in the host cells is a heterogeneous population depending on the rate of mutations and their adaptation. The dominant population of the virus can be affected by the random mutations, a bottleneck event or changes that destabilize the present equilibrium.

The RNA-dependent RNA polymerase (RdRp) gene of the coronaviruses is known to be error prone, thereby leading to frequent mutation and recombination. The presence of quasispecies has been reported earlier from both SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV). Quasispecies analysis by Xu et al. from nine individual patient samples infected with SARS-CoV-1 revealed nine recurrent variant sites in a total of 107 variations. Park et al. studied 35 samples of 24 patients and identified a total of 16 nucleotide variant positions in MERS-CoV. Recently, Capobianchi et al. analyzed quasispecies in two SARS-CoV-2 patients in Italy. They could identify two nucleotide variations in ORF1ab gene at positions 2269 and 7388 having A\(\rightarrow\)T and G\(\rightarrow\)A substitutions, respectively. The present study was aimed to look upon the quasispecies in the different SARS-CoV-2 clinical samples sequenced and the consensus sequences for which were discussed in our previous studies.

The study was conducted in the ICMR-National Institute of Virology, Pune, India, during March to April 2020 after obtaining prior approval from the Instructional Ethics Committee. The next-generation sequencing data from Italian tourists, contacts of the Italian tourists and the Indian citizens sampled at Iran were used to analyze the quasispecies present within the clinical samples (nasal/throat swab). The samples were analyzed by using the variant detection tool as implemented in the QIAGEN CLC genomics workbench 20.0 (QIAGEN, Aarhus, Denmark). The reference sequence used for deriving variants from the sequenced reads was the SARS-CoV-2 Wuhan HU-1 strain (accession number: NC_045512).

Two different types of SARS-CoV-2 strains are classified depending on the nucleotide present at genomic position (GP) 8782 (gene: ORF1ab) and 28144 (gene: ORF8). The nucleotide change in the GP 8782 is a synonymous nucleotide substitution, whereas the GP 28144 has a non-synonymous nucleotide substitution, where Leu is observed when the nucleotide is T and Ser when it is C. The strain is classified as S-type when the nucleotides are T and C at GPs 8782 and 28144, respectively and is considered to
be non-virulent. However, when the nucleotides are C and T at those positions, respectively, it is classified as L-type, considered to be virulent in nature. However, there is no experimental evidence to support the method of classification. The reads of the clinical samples from the Italian tourists demonstrated that the GP 8782, on an average, had 54.6 per cent of T nucleotides in 50 per cent of the dataset analyzed (n=8) and the rest as C (Figure A). In contrast, GP 28144 had T for all the sample sets analyzed. The presence of TT nucleotides at the GP 8782 and 28144, respectively, in 50 per cent of the Italian samples was observed at the quasispecies level. Quasispecies was also observed in 50 per cent of the dataset analyzed (n=8), at GP 9561 and 22875, which led to non-synonymous change at the amino acid level (Ser→Leu and Ser→Phe, respectively) that had C→T nucleotide change for both positions.

The Indian contacts of the Italian tourists, however, had a different profile wherein the quasispecies observed in the Italian tourists were not observed for all the sample sets analyzed (n=7) (Figure B). The dominant nucleotide changes were reflected in the consensus sequences for the retrieved genome. For example, GP 241 (5’UTR) had T nucleotide (99.1%) compared to the Italian tourists (93%). The nucleotide deletion at the GP 28254 (ORF8 gene) was reduced by an average of 24 per cent when compared to the Italian tourists. The third sample set was from the Indian citizens sampled in Iran (Figure C), which showed quasispecies in more than 50 per cent of this sample set. This set had a single-nucleotide polymorphism (SNP) deletion in the ORF1ab gene (GP: 11083), with an average of 87.3 per cent, leading to early termination of the ORF1ab polypeptide (Figure C). The ORF1ab proteins in these cases was encoded by the remaining 12.7 per cent of the intact gene, which is present in minority. The remaining SNPs deletions observed in this set had an average above 98.7 per cent, indicating a lesser variation of the quasispecies population of the analyzed set (n=11). Variant analysis of a few isolates of the clinical specimens was also made, and it was observed that the nucleotide variation was similar to that of the respective clinical specimens (data not shown).

The quasispecies identified by Capobianchi et al. were different from those observed in our study. The quasispecies present in the three sets of the clinical samples analyzed revealed the presence of variation in the nucleotide positions and virus evolution to adapt towards the new host. This study also indicates that a quasispecies may vary depending on the location and indicates the further exploration of more massive dataset to identify variability among different clinical samples. Hence, it is vital to consider the heterogeneous virus population present within the host, when designing antiviral strategies. RNA viruses are known to have a higher mutation rate compared to their hosts, which leads to quasispecies formation in them. A higher mutation rate provides the virus an opportunity to expand its quasispecies population to adapt either to the same host or to the others under the influence of selection/environmental pressure and generate into different clades. However, the other mechanisms, such as random mutations, changes due

![Figure](image-url)
to RNA editing or error in proofreading mechanisms that can lead to changes in the nucleotides cannot be neglected. Such changes increase the likelihood of generating an escape mutant\textsuperscript{21,22}, which may be predominately influenced by selection/environmental pressure.

In brief, this study points towards the variations observed in the SARS-CoV-2 quasispecies sampled from different locations and indicates the need of analyzing a larger set of population from varied locations, which is also the limitation of this study.

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