Symptomatic reinfection of SARS-CoV-2 with spike protein variant N440K associated with immune escape

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Keywords: SARS-CoV-2, reinfection, N440K immune escape variant

Dear Editor,

A number of cases of reinfections in individuals with SARS-CoV-2 have emerged across the globe [1,2]. With the emergence of genetic variants associated with immune escape in many regions of the world, it is being widely believed that immune escape variants could contribute to an increase in reinfections as well as potentially adversely affect the efficacy of vaccines [3,4]. A recent report also describes reinfection in a patient with neutralizing antibodies and identified an immune escape variant in the spike protein [5].

Here we describe a case of re-infection in an individual from South India characterized by whole genome sequencing of the virus isolated from both episodes. Analysis shows the presence of an immune escape variant N440K in the Spike protein in both episodes of infection. Incidentally, this variant was also found in a case of reinfection previously reported by us in a healthcare worker from North India [1].

In the first episode of infection, the 47-year-old male public service employee from Andhra Pradesh, India, was identified to be positive for SARS-CoV-2 on July 25, 2020, in nasopharyngeal specimens analysed as a part of routine surveillance and was asymptomatic. The cycle threshold values (ct) was 22.3 and 19.1 for ORF1ab and N genes respectively (LabSystems Diagnostic Inc.). The individual tested negative on August 2, 2020, and tested positive again on September 10, 2020, during routine surveillance, but during this episode, he was symptomatic with fever, cough and malaise. The ct values for the probes targeting ORF1ab and N genes were 21.9 and 19.2 respectively (LabSystems Diagnostic Inc.) during this episode and he tested negative after 14 days.

The RNA samples were sequenced using COVIDSeq protocol [6] on Illumina MiSeq (Illumina Inc) generating paired-end sequencing (75 × 2 bp) reads, which were analysed as per standard protocols [7]. Variants were called using VarScan [8]. Only variants having a frequency greater than 50% and a minimum depth of 50 reads were considered for further analysis. Lineages were assigned using PANGOLIN [9].

The analysis revealed a total of 15 and 17 genetic variants in the genomes from the two episodes E1 and E2 respectively of which 14 variants were common between the two episodes (Figure 1). A close comparison of the genetic variants with a compendium of immune escape variants revealed a 22882T>G (Spike: N440K) variant in the genomes isolated from both episodes of infection. The variant has previously been shown to emerge *in vitro* under selective pressure against the human monoclonal antibody C135 and show resistance to it [3]. The variant had a high prevalence of over 33% in the state of Andhra Pradesh [10] and has been reported previously in another case of SARS-CoV-2 reinfection from North India [1].

(A)

(B)

Figure 1 (A) Genetic variants in the genome isolates of the 2 episodes (denoted as Episode 1 and Episode 2) of SARS-CoV-2 infections. The 22882T>G (Spike: N440K) variant is marked with an arrowhead. **(B)** Phylogenetic context of the virus isolates of the 2 episodes with other global samples having the N440K variant

Phylogenetic analysis for the 2 isolates was done using all global genomes having the N440K variant, which includes 92 genomes from India sequenced in-house (BioProject ID: PRJNA655577). Analysis shows that genomes isolated from the 2 episodes fell under 2 distinct clusters of genomes (Figure 1B). The genomes clustered closely with other

genomes from Andhra Pradesh. Genome isolates of both episodes were assigned the PANGO lineage B.1.36. The close resemblance of the genome isolates from the 2 episodes of infection yet the presence of distinct variants in the 2 genomes suggests that the patient acquired both infections from the same location in Andhra Pradesh at two different time points.

Recent studies exploring the role of SARS-CoV-2 genetic variations in escaping immune response has shed light on the possible mechanisms of the pathogen to evade antibody response and immune reactions. The N440K variant has been reported to be resistant to class 3 monoclonal antibodies (mAbs) C135 and REGN10987 that are candidates for clinical development [3,11,12]. Both C135 and REGN10987 mAbs have been shown to have interactions focused on the N440 residue of the Spike protein and the close proximity of the N440 residue to the structural epitope of the mAbs potentially confers loss of binding and resistance to the neutralising effect of the mAbs [3,11]. The variant is also reported to have an enhanced binding affinity to the ACE2 receptor in humans [3,11]. Several studies have recently documented cases of reinfection with the presence of the variant E484K in the Spike protein in the second episode of infection, despite the presence of anti-SARS-CoV-2 antibodies in the patient [5,13,14]. The E484K is a defining variant for the two circulating SARS-CoV-2 variants of concern, B.1.351 lineage (20H/501Y.V2) and P.1 lineage (20J/501Y.V3), first identified in South Africa and Brazil respectively [15]. The variant has also been individually reported to be associated with escape from several mAbs [3,4,16] and a loss of neutralisation activity of antibodies elicited by vaccines [17,18] and its increasing prevalence across different regions raises concern about its potential impact. The high prevalence of N440K in India along with the findings presented here and in other related studies highlight the importance of analysing the potential impact of the variant and additional host factors on reinfections and immune evasion.

Acknowledgements

BJ, AJ, and MD acknowledge a research fellowship from CSIR India. The authors acknowledge grants from the Council of Scientific and Industrial Research (CSIR, India) through grants CODEST and MLP2005 which supported the genomic studies. The funders had no role in the design of the experiment, analysis, or decision to publish.

Conflicts of Interest

None Declared.

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