REVIEW ARTICLE

Tsunami of India's Second COVID-19 Wave: B.1.617 and Black Fungus

Nayanika Chakraborty[1,2], Rajesh Pandey[3], Hemant K. Gautam[2]

[1]Department of Chemistry, University of Delhi, New Delhi, India, [2]Immunology and Infectious Disease Lab, CSIR-Institute of Genomics and Integrative Biology, New Delhi, India, [3]INtegrative GENomics of HOst-PathogEn (INGEN-HOPE) Laboratory, CSIR-Institute of Genomics and Integrative Biology, New Delhi, India

Abstract

The catastrophic second wave of COVID-19 caused over 26 million cases in India making it the epicenter of the global pandemic. The sudden surge in COVID-19 infections is intertwined with various variants including B.1.617. Globally, health authorities have expressed major concerns that key mutations L452R and E484Q located at the receptor-binding domain of the spike protein would have additive effects on SARS-CoV-2 evasion from the vaccine-elicited antibodies. As India struggles with COVID-19 cases spiraling out of control, it is simultaneously caught by escalating cases of "Black Fungus." Researchers are hurrying to determine the many circulating variants and to know the unknowns about biology and pathology of the mutating SARS-CoV-2 to analyze the threat possessed by them.

Key words: B.1.617, breakthrough infections, COVID-19, mucormycosis, SARS-CoV-2 and spike mutation

INTRODUCTION

The global cataclysmic condition due to the outbreak of the 2019 novel coronavirus (2019-nCoV) is not distinctive to the historical narrative of viruses but one that represents the disastrous effect of infectious diseases of the new century. In the recent past, other infections such as H1N1 influenza (swine flu) and Middle East respiratory syndrome coronavirus (MERS-CoV) and now the existing pandemic, coronavirus infectious disease-19 (COVID-19) have globally brought the scientific community together to rapidly explore antiviral drugs and vaccines. Universally, companies such as Moderna, the University of Oxford, BioNTech, Pfizer, Novavax, are among many others who have been able to develop vaccines in record time and at an unmatched pace when compared to older coronavirus outbreaks such as SARS-CoV and MERS-CoV. Masking, social distancing, testing, and contact tracing became armamentarium in the struggle to contain the spread of SARS-CoV-2. Israel, the United Kingdom, and the United States drove a rapid mass vaccination drive to fight back the COVID-19 infection spread. These countries used mRNA vaccines with a 95% efficacy rate against severe COVID which reduces the occurrence of these infections as mild cases of resurgence do not discharge enough viruses into circulation infecting others. The moment researchers slowed down the pace to investigate the unknowns about biology and pathology of the virus with the world striving for survival with vaccination as the hope, the SARS-CoV-2 virus stages Houdini-like ability to break the pipeline of evidence to fight back.

WHEN THE WAVE HIT INDIA HARD….

India witnessed an initial couple of cases of SARS-CoV-2 infection in Kerala in February 2020. Subsequently, it spread thick and fast, although the speed and severity were managed to a large extent by the national lockdown in 2020. After a lull during the past few months of 2020, India again witnessed a meteoric rise of COVID-19 cases in April 2021, wherein it was discovered that multiple variants were operating the chain reaction of COVID surges. The calamitous effect of the second wave of the pandemic has

Address for correspondence:
Dr. Hemant K. Gautam,
Immunology and Infectious Disease Lab, CSIR-Institute of Genomics and Integrative Biology, Sukhdev Vihar, Delhi - 110 025, New Delhi, India.
E-mail: hemant@igib.res.in

Received: 25-11-2021
Revised: 09-04-2022
Accepted: 30-04-2022

challenged the nation's health-care system and challenged the scientists to comprehend many variants of coronavirus, doing rounds. This led to a national initiative toward genome surveillance of SARS-CoV-2 through Indian SARS-CoV-2 Genome Sequencing Consortia (INSACOG). The change in the dynamics of transmission induced by the variants of the virus is a cause of major concern. Animal studies and other pieces of evidence indicate that one variant first observed in India is relatively more potent in evading the human immune system and has augmented transmissibility than other exciting variants. In a limited way, vaccine breakthrough has also been observed, although with milder disease severity. Instances of mucormycosis or black fungus are also showing significant cases, with the Government of Rajasthan declaring it as an epidemic as of May 19, 2021.

As the SARS-CoV-2 made its way around the world, mutation became inevitable in accordance with the concepts of Darwinian's natural selection. Countries with maximum infections propagate a maximal number of mutations. However, not all mutations contribute to the pathogenicity of the virus. Under evolutionary pressure, the virus strikes an equilibrium between infectivity and virulence which is dependent on the availability of the host population. Compared with HIV, SARS-CoV-2 changes at a rate of one-quarter,[1] which is very sluggish for an RNA virus, attributed to the talent of proof-reading the newly made RNA copies. Once inside the host cell, an opportunity to mutate is dependent on the time it spends with the host cells. Onset of some earlier mutations of SARS-CoV-2 might have occurred in immunocompromised patients, elderly patients, and patients on ventilators for prolonged periods. Viral sample sequences of SARS-CoV-2 variant B.1.617 were observed in India in December 2020, with the potency to dodge the immune system. Kerala witnessed the initial outbreak in January 2021, but no variant was identified as a matter of concern.

AS THE WAVE PROPAGATED SO DID THE COVID-19 CASES

On May 13, 2021, India reported about 4.14 lakh coronavirus infections spiking its total number to more than 23 million. B.1.1.7, first identified in Britain, was dominating the COVID-19 infection efflux in Delhi and Punjab as observed by genomic sequencing data. West Bengal was dominated by B.1.618 variant and B.1.617 was the causative variant in the state of Maharashtra. The first alert of the variant came from Rajesh Karyakarte group at BJ Medical College, Pune, in February 2021. The sequencing of the new SARS-CoV-2 variant B.1.618 is carried out extensively with analyses demonstrating E484K mutation same as Brazilian and South African variant. This mutation is robustly associated with the virus developing resistance to antibodies mediated through vaccines or past infection. Scientists are making concerted efforts to understand whether these variants make the SARS-CoV-2 virus more transmissible, deadly, and resistant all of which clues to whether these variants are plausible drivers of the current wave of COVID-19 infections witnessed by India. Global repository, GISAID, examined that B.1.618 is the third most common variant sequenced in the past 2 months at 12% whereas B.1.617 is the most common among sequencing at 28% followed by B.1.1.7. Soon after, B.1.617 outcompeted B.1.618 in West Bengal and also became the prevailing variant in other states. Delhi also saw the incidence of B.1.617 variant with L452R and E484Q mutations, as confirmed by National Centre for Disease Control, Delhi. Encompassing generously proportioned datasets and time span, the fitness of B.1.617 was observed to be better than circulating variants. This variant has a relatively better tenacity to transmit, causes an increase in severity of disease and higher escape potency for the acquired immunity than the variants already circulating in the population. Thus, in March 2021, National Institute of Virology, Pune, and INSACOG, established on December 30, 2020, approved B.1.617 as a "variant of concern" (VoC) in India. Tracing the earliest sequences of B.1.617 lineage goes back to December 2020 and determination of sub-lineages is underway. As of now, three sub-lineages have been reported; B.1.617.1 from 34 countries, B.1.617.2 from 31 countries, and only four nations have accounted B.1.617.3. E484Q mutation is not possessed by B.1.617.2 but it does contain T478K mutation not found in the other two subtypes. All the three sub-lineages contain two other mutations P681R and D614G, whose clinical significance needs to be investigated. The necessity of targeted sequencing and epidemiological knowledge is essential to fully comprehend the significance of these sub-lineages.

B.1.617 has not only become the dominant strain in India but has also super-speeded across 44 nations as of May 11, 2021, including the United Kingdom (February 22, 2021), the United States (February 23, 2021), Singapore (February 26, 2021), and Fiji (April 19, 2021). The government of the United Kingdom, on May 7, 2021, accounted for B.1.617.2 subtype as a VoC for the UK with cases spiking from 202 to 520 in a span of 7 days. On May 11, 2021, the World Health Organization also classified B.1.617 as the "variant of concern" stating that the variant has a rapid rate of transmission and reduced neutralization.

Variants of concern that impacted the human race globally and found in the community transmission stage in India include (i) B.1.351 originated in South Africa in late 2020, this variant vaccine breakthrough against the administration of University of Oxford –AstraZeneca jab, proving to be ineffective against this variant and is reported to be most immune evasive ascribed to E484K mutation, (ii) the tumultuous effects of P.1 variant witnessed in Brazil's second wave of pandemic earlier this year, are also able to escape immunity. This variant is majorly noticed in travelers and their contacts, and (iii) the variant B.1.1.7 debuted in the United Kingdom in late 2020 and continues to be globally most prevalent, in Delhi too but not observed in West Bengal.

WAVE CAUSED BY MOSAIC PATTERN?

The analysis of B.1.617 is under process and the data regarding the mutations have just started pouring in. According to GISAID, B.1.617 variant's first detection dates back to October 5, 2020. INSACOG amplified the network of large-scale sequencing of viral genomes to understand the rising number of variants and to fully understand the fitness of the new mutants. Scientists found that B.1.617 variant was predominant in Maharashtra accounting for 60% of the cases by mid-February 2021. The selective pressure of augmenting the affinity toward the angiotensin-converting enzyme 2 (ACE2) receptor and enhancing the evading power from neutralizing antibodies favor mutations of SARS-CoV-2.

Genomic and structural analysis revealed that within B.1.617 clade, four sub-clusters are linked to alterations specific to the spike region. The rate of recurrence of mutations L452R and E484Q in the receptor-binding domain (RBD) of the spike protein and mutations G142D and P681R within the spike but outside the RBD has gained speed from January 2021.[2] H1101D and T95I are the other reported mutations. In late March, one cluster of mutations, though small, did not possess E484Q but had T19R and D950N mutation associated with spike protein. Another synonymous mutation, namely, D111D arose along with the RBD L452R and E484Q mutations but was not witnessed in clusters where E484Q mutation was absent. It was also interesting to note that since February 2021, the frequency of the occurrence of non-synonymous mutations is ever-increasing.

Compared to wild-type virus strain, L452R and E484Q mutations reduce inter- and intra-molecular interaction of spike protein with the ACE2 receptor on human cells, suggested by crystal structure analysis. L452R alteration decreases the hydrophobic interactions with the nearby residues (L492 of the RBD). The switch from hydrophobic L452 to hydrophilic 452R results in greater stability of the complex attributed to interaction with water molecules. At the interaction interface, E484Q mutation interrupts the electrostatic bond of the spike RBD residue E484 with K31 located in the ACE2 and is touted to be similar to E484K mutation (lineage B.1.1.7 and lineage B.1.351). The P681R mutation associated with the polybasic S1-S2 furin cleavage site of the spike protein; could help in increased membrane fusion and internalization leading to enhanced transmissibility. Furthermore, both L452R and E484Q mutations disturb the interaction of the REGN10933 and P2B-2F6 neutralizing antibody with the spike proteins, hampering the neutralization effect. Previous data reported the ability of L452R mutation hindering the neutralizing effects of various monoclonal antibodies (mAbs), thus contributing to potential escape from cellular immunity and augmenting viral infectivity and sponsoring replication. The combination of triple mutation L452R, E482R, and P681R indicates the convergent viral evolution to develop a potentially immune evasive variant, recurrently adapting and tricking the human hosts.

CAN VACCINES TAME THE WAVE TURNING INTO TIDE?

The genomic data were correlated with findings of a preprint[3] from a German team led by Markus Hoffman, infection biologist at Leibniz Institute for Primate Research, Göttingen, that demonstrated B.1.617 variant is better at entering the human lung cells and intestines as compared to the variants already in circulation. Another preprint study[4] echoed the same concern of pathogenicity of the variant with hamsters indicating more inflammation in their lungs than when infected with other variants. The potential of the severity of the infection caused by this SARS-CoV-2 variant needs a further detailed examination.

A preliminary insight came when researchers from the laboratory of Ravindra Gupta demonstrated that antibodies generated from vaccines may be less effective against B.1.617 than other disseminating variants.[5] The team collected sera from nine individuals who obtained one jab of BNT162b2 (Pfizer vaccine) and tested it against a pseudo-typed viral vector modified with SARS-CoV-2 spike protein with mutations of B.1.617 variant. Researchers found that B.1.617 variant's spike confers decreased susceptibility to the BNT162b2 mRNA vaccine manufactured neutralizing antibodies rendering them 80% less effective to some mutations of the variant. In addition, the study also revealed that Delhi health care workers who encountered extensive viral load and obtained ChdOx-1vaccine (Indian version of AstraZeneca vaccine) in early 2021 reported a number of cases of "breakthrough" infection in the second wave with the majority cases coupled with B.1.617 variant.

Scientists at Emory University reported in a preprint study[6] that there is a 6.8-fold reduction in the ability of antibodies to block the variant when compared to wild type, for which the vaccine was pre-meditated analyzing 24 convalescent sera samples and 24 samples from individuals who obtained mRNA vaccines (Moderna, Pfizer). Despite this antibody resistance, the protective immunity developed from mRNA vaccine tested in this study will perpetuate against B.1.617 variant.

Furthermore, researchers from NYU Grossman School of Medicine, New York, USA, led by Professor Nathaniel R. Landau examined the resistance to neutralization of convalescent sera, vaccine manufactured antibodies, and therapeutic monoclonal antibodies against B.1.618 and B.1.617[7] SARS-CoV-2 variant spike protein. The study was carried by modification of lentiviral virions pseudo typed with the B.1.618 and B.1.617 spike proteins. The authors found that there was average decrement of 3.9-fold and 2.7-fold in the neutralization of B.1.618 and B.1.617 spikes against IC50 for convalescent sera and antibodies generated by Pfizer BNT162b2 and Moderna mRNA1273 vaccines, respectively. The B.1.617 spike was neutralized by Regeneron monoclonal antibody concoction (constituting

REGN10933) with a reduction of 4.7-fold attributed to the L452R mutation. The authors' state that the current vaccines with modest neutralization resistance will be able to protect from these variants.

In another preprint[8] research group led by Pragya Yadav, a virologist at NIV, Pune, investigated the neutralization efficiency of convalescent sera and sera obtained from people vaccinated with BBV152 (Covaxin, Bharat Biotech). The neutralizing capability dropped by 2-fold for Covaxin elicited antibodies against B.1.617.

The B.1.617 variant of SARS-CoV-2 does have the fitness to survive than the other variants, chiefly in patients whose immunity is compromised due to earlier infection or vaccination, according to Hoffman. Although the vaccine remains effective, the trend is toward less effective.

WAVE SETS DOUBLE BLOW TO INDIA: BREAKTHROUGH INFECTIONS AND "BLACK FUNGUS"

"Breakthrough" infections, even after full vaccination, are being observed with new mutants. All the systemic vaccines designed to date, function to provide systemic defense against virus, high protection from severe disease, needless hospitalization, and escape death after being infected with SARS-CoV-2. Clinical trials also examine all these end points and not against the degree of infection. With the vaccines in India showing efficacy of 62–81%, it is possible to witness cases of severe COVID-19 and mild COVID even more so. Dr. Shashank Joshi who is an expert on the Maharashtra state COVID-19 task force points out that all the vaccines in use are early generation emergency use authorization vaccines. Better pharmacovigilance and more datasets are required to get close view into the efficacy of the vaccines against "breakthrough" infections and to refine them in future. Furthermore, aging immune system might not respond against antigens and vaccines when compared with younger immune system. For a given COVID-19 vaccine, there is a measurable variation in concentration of neutralizing antibodies in the aged individuals with some generating no neutralizing antibodies even after two doses of vaccination.

Coronavirus disease (COVID-19)-associated mucormycosis (CAM) is adding to the burden of health-care challenges in India; already being stretched due to COVID-19. As of May 21, 2021, CAM has infected about 7250 people in India and caused 219 fatalities and the numbers are increasing. Mucormycosis affects the nose, spreads to the eyes causing blindness, and the brain causing seizures or headaches. Mucormycosis fungi causing human disease proliferate well at body temperature and in acidic mediums such as when tissue is decomposing or associated with uncontrolled diabetes. Mucormycosis is not new to India with 14 in every 100,000 people getting infected even before COVID-19. A major factor for the widespread nature of reports of CAM in India could be diabetes.

Opportunistic fungal infection mucormycosis, often called "black fungus," is increasingly reported in India in patients with COVID-19 or who are recovering from coronavirus. A recent review[9] on CAM explained that 94% of patients are diabetic, and in 67%, it is poorly controlled. Obesity and diabetes make people immunocompromised and increase the risk of COVID-19 infection. After the world witnessed the potential of dexamethasone against cytokine storm induced by a coronavirus, it became the go-to treatment during the second hit from SARS-CoV-2 with severe COVID-19 cases started getting administered with systemic glucocorticoids. Tocilizumab, another corticosteroid, is an even stronger immunosuppressant used and being used as one of the medication options. The dangerous cocktail of corticosteroids along with diabetes probably enhanced the risk of CAM.

In addition, CAM is predominantly noticed in patients on prolonged ventilator support and administered steroids as a standard treatment, which was not the case in the first wave. The medical oxygen requires humidification before getting administered to the patients; so it is passed through sterile water. If this procedure is followed with non-sterile water, it can be a hotspot for black fungus infection. Without humidification, medical oxygen has drying effects on the mucosal membrane which leads to disruption of the airway.

WHAT LIES AT THE SHORE: "WHITE FUNGUS AND YOUNGSTERS IN THE EYE OF CYTOKINE STORM?"

As of May 21, 2021, four cases of "white fungus" have been reported in Patna, which is said to be deadlier than black fungus given that it infects other organs in addition to lungs. Although the cause of the spread of white fungus stays the same as black fungus, control is imperative as it is the main reason for leukorrhea.

The youngsters showed a relatively higher pattern of infection during the second wave of SARS-CoV-2 compared to the first wave. As CMRI Hospital director of pulmonology Raja Dhar put forth that due to the stronger immune system of the young; the generation of cytokine storm affected majorly lungs, followed by heart, kidney, and the liver. Young individuals experienced respiratory distress with a requirement of oxygen support. Even though cytokine storm is partly responsible, other factors such as lack of vaccination and non-compliance to COVID appropriate behavior/s may also be contributory factors along with the new VoC, B.1.617.

CONCLUSION

A current preprint[10] reported by researchers at Gladstone Institute of Virology showed that Moderna and Pfizer vaccines induced forceful T-cell response against variants of SARS-CoV-2. They also demonstrated that in previously infected individuals', vaccination stage more robust harboring of T-cells specific to SARS-CoV-2 in the upper respiratory tract. T-cell immunity is less susceptible to evasion by variants. Therefore, a drop in the potency of neutralizing antibodies should not ward off people from getting vaccinated in a hope of an ideal vaccine.

Mass scale immunization, COVID appropriate behavior, and at least 2–5% successful genomic surveillance remain keys to managing the developing third wave.

Even though researchers and scientists globally continue their expedition to understand the viral sorcerer's tricks on the world; the tsunami of India's second COVID-19 wave put extensive stress on health infrastructure. With the explosive surge in COVID-19 infections, it is likely to become the hornet's nest culturing future SARS-CoV-2 variants to look out for.

ACKNOWLEDGMENTS

The author expresses special thanks to Director IGIB for the support. Ms. Nayanika Chakraborty would also like to acknowledge the CSIR for providing the fellowship.

REFERENCES

1. Callaway E. The coronavirus is mutating does it matter? Nature 2020;585:174-7.
2. Cherian S, Potdar V, Jadhav S, Yadav P, Gupta N, Das M, *et al.* Convergent evolution of SARS-CoV-2 spike mutations, L452R, E484Q and P681R, in the second wave of COVID-19 in Maharashtra, India. bioRxiv. 2021;2021:440932.
3. Hoffmann M, Hofmann-Winkler H, Krüger N, Kempf A, Nehlmeie I, Graichen L, *et al.* SARS-CoV-2 variant B.1.617 is resistant to bamlanivimab and evades 1 antibodies induced by infection and vaccination 2 3'. bioRxiv 2021;2021:109415.
4. Yadav PD, Sapkal GN, Abraham P, Ella R, Deshpande G, Patil DY, *et al.* Neutralization of variant under investigation B.1.617 with sera of BBV152 vaccinees. bioRxiv 2021;2021:441101.
5. Ferreira I, Datir R, Papa G, Kemp S, Meng B, Rakshit P, *et al.* SARS-CoV-2 B.1.617 emergence and sensitivity to vaccine-elicited antibodies. bioRxiv 2021;2021:443253.
6. Edara VV, Lai L, Sahoo MK, Floyd K, Sibai M, Solis D, *et al.* Infection and vaccine-induced neutralizing antibody responses to the SARS-CoV-2 B.1.617.1 variant. bioRxiv 2021;2021:443299.
7. Tada T, Zhou H, Dcosta BM, Samanovic MI, Mulligan MJ, Landau NR. The spike proteins of SARS-CoV-2 B.1.617 and B.1.618 variants identified in india 1 provide partial resistance to vaccine-elicited and therapeutic monoclonal antibodies. bioRxiv 2021;2021:444076.
8. Yadav PD, Mohandas S, Shete AM, Nyayanit DA, Gupta N, Patil DY, *et al.* SARS CoV-2 variant B.1.617.1 is highly pathogenic in hamsters than B.1 variant. bioRxiv 2021;2021:442760.
9. John TM, Jacob CN, Kontoyiannis DP. When uncontrolled diabetes mellitus and severe COVID-19 converge: The perfect storm for mucormycosis. J Fungi 2021;7:298.
10. Neidleman J, Luo X, McGregor M, Xie G, Murray V, Greene WC, *et al.* mRNA vaccine-induced SARS-CoV-2-specific T cells recognize B.1.1.7 and B.1.351 variants but differ in longevity and homing properties depending on prior infection status Equal contribution. bioRxiv 2021;2021:443888.

Source of Support: Nil. **Conflicts of Interest:** None declared.