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Evolving biothreat of variant SARS-CoV-2 – molecular properties, virulence and epidemiology

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Abstract. – SARS-CoV-2 are enveloped RNA viruses that belong to the family Coronaviridae of genus Beta coronavirus, responsible for the COVID-19 pandemic. The mutation rate is high among RNA viruses and in particular, coronavirus replication is error prone with an estimated mutation rate of 4x10-4 nucleotide substitutions per site per year. Variants of SARS-CoV-2 have been reported from various countries like United Kingdom, South Africa, Denmark, Brazil and India. These variants evolved due to mutations in spike gene of SARS-CoV-2. The most concerning variants are Variant of Concern (VOC) 202012/01 from United Kingdom and B.1.617 variant of India. Other variants include B.1.351 lineages, cluster 5/SARS-CoV-2 variant of Denmark, 501.V2 variant/SARS-CoV-2 variant of South Africa, lineage B.1.1.248/lineage P.1 of Brazil. Mutations in S protein may result in changes in the transmissibility and virulence of SARS-CoV-2. To date, alterations in virulence or pathogenicity have been reported among the variants from many parts of the globe. In our opinion, since the S protein is significantly altered, the suitability of existing vaccine specifically targeting the S protein of SARS-CoV-2 variants is a major concern. The mutations in SARS-CoV-2 are a continuous and evolving process that may result in the transformation of naïve SARS-CoV-2 into totally new subsets of antigenically different SARS-CoV-2 viruses over a period of time.

Key Words:
SARS-CoV-2, Variants, Lineages, Molecular properties, Spike protein, Naïve SARS-CoV-2.

Introduction

COVID-19 pandemic, due to SARS-CoV-2 is still looming at large causing severe morbidity, mortality and global economic crisis[1]. SARS-CoV-2 are enveloped viruses that belongs to the family Coronaviridae of genus Beta coronavirus[2]. It has a single stranded positive sense RNA (ss RNA) genome of 26-32 kb size with six major open reading frames (ORFs). The ORF includes replication enzyme coding region (ORF 1a and 1b), E gene (envelope protein), M gene (membrane protein), S gene (spike protein), and N gene (nucleocapsid protein) that are common to coronaviruses[3].

The first new strain of variant SARS-CoV-2 was reported from England and Northern Ireland on 14th December 2020. It is called SARS-CoV-2 - VUI 202012/01 (variant under investigation, year 2020, month 12, variant 01) or VOC (variant of concern) 202012/0[4]. Variants from UK, VUI-202012/01 are designated as B.1.1.7 lineage. As on 13th December 2020, over 1108 cases positive for SARS-CoV-2 VUI 202012/01 have been identified. Preliminary studies[4,5] revealed that the variant has the ability to spread rapidly in the community. Currently, virologists and health authorities of UK are investigating the virulence properties, clinical features, mechanism of pathogenesis and efficacy of existing COVID-19 vaccine on the variant strain[6]. This variant SARS-CoV-2 was detected during the epidemiological surveillance in Southeast England following a sudden surge in COVID-19 cases. This variant SARS-CoV-2 strain exhibited more than three-fold increase in positive cases compared to naïve SARS-CoV-2[5]. Majority of this variant SARS-CoV-2 infections were noticed in patients under 60 years of age. This variant exhibited fourteen mutations and three deletions. The rapid transmissibility of variant strain may be attributed to these mutations. An important mutation detected was N501Y which resulted in change in amino acid asparagine of the receptor binding domain (RBD) to tyrosine at position 501[7]. The Global Initiative on Sharing

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Avian Influenza Data (GISAID) authorities also reported that the same RBD mutation on N501Y has been noticed in South African and Australian variants[8]. Molecular investigations showed that N501Y mutation of SARS-CoV-2 variant identified in UK and South Africa evolved separately[6,9]. On RBD, another important mutation, P681H (proline to histidine at residue position 681) was also observed. Mutation by deletion at 69/70 position of S gene may interfere in RT-PCR test results that use S gene target[8]. However, most RT-PCR tests throughout the world employ many molecular targets, hence identification of variant using routine naïve SARS-CoV-2 - RT-PCR is not problematic[8,10]. Later on, Italy, Denmark, Netherlands and Iceland also reported with the cases of new variant, VUI-202012/01. Laboratory investigations are conducted to assess the difference in biological features between VUI-202012/01 variant and naïve SARS-CoV-2. Currently very little information is available on the biological properties of the variant strain. The UK virologists have already uploaded the whole genome sequencing of VUI-202012/01 variant at GISAID. Since the spread of VUI-202012/01 variant is faster than the naïve SARS-CoV-2, tier 4 restrictions were imposed in the affected areas of UK and other countries in order to control the spread. Most of the viruses change their genomic or other molecular sequences over a period of time by mutation. Usually, these viral mutations are not favorable to their normal replication. More extensive virological and molecular studies should be carried out on the variant strains to know the efficacy of therapy, diagnosis and prophylaxis used for all SARS-CoV-2 strains. Many researchers noticed that multiple strains of SARS-CoV-2 variants are circulating globally[11,12].

Recently, South African variants of SARS-CoV-2 are labelled as B.1.351 lineage. Researchers have observed that South African variants are actually mutants of B.1.1.7 lineage[11]. Bosch et al[13] revealed that both B.1.1.7 and B.1.351 lineages have mainly acquired mutations in genes responsible for forming S protein of SARS-CoV-2. Due to the alteration in the nucleotide sequence of spike gene, it has resulted in the change of sequence of amino acid of S protein. Studies also exhibited that significant changes have occurred within S gene. Due to these genetic changes both the variants expressed high transmissibility compared to naïve SARS-CoV-2. Since the variants exhibited altered S protein, there may be changes in their immunological responses compared to naïve SARS-CoV-2[14]. So, the vaccines developed for naïve SARS-CoV-2 may or may not offer protection for both B.1.1.7 and B.1.351 lineages. Since some dissimilarities exist between naïve SARS-CoV-2 and its lineages, the transmission, virulence and pathogenesis appear to be different among them. Whereas some diagnostic RT-PCR kit developed in some parts of the world may face difficulty in detecting SARS-CoV-2 lineages using probes used for naïve SARS-CoV-2. Apart from these stated countries, there are also some other lineages that have been increasingly reported from countries like Denmark and Brazil. This review article puts forward the updates on variants of SARS-CoV-2.

Materials and Methods

Detailed literature review was conducted on SARS-CoV-2 and variants of SARS-CoV-2 published in various journals and scientific reports. Articles on original research, reviews, case studies, book chapters, WHO and CDC reports published from March 2020 to April 2021 were included in this study. The Medical Subject Headings (MeSH) related to SARS-CoV-2, SARS-CoV-2 variants/lineages, COVID-19 were duly considered in this study. References were obtained from MEDLINE/PubMed, the NLM Catalog and other NLM databases. Research articles from SCOPUS, Web of Science, Science Direct, and Google Scholar were also used. Latest trends and developments on molecular properties, virulence, epidemiology of variant SARS-CoV-2 and naïve SARS-CoV-2 were taken as inclusion criteria in this study. Three dimensional structures of S protein of naïve SARS-CoV-2 and variants were retrieved and used to make figures from RCSB-PDB (Research Collaboratory for Structural Bioinformatics – Protein Data Bank) resources. The PMID (PubMed Identifier) protein sequence analysis for spike D614G variant, minus RBD was obtained from 32991842, PMCID: PMC7492024.

Variants of SARS-CoV-2

Molecular Characteristics of Spike Protein (S) of Naïve SARS-CoV-2

The SARS-CoV-2, S protein is approximately 180 KDa and exists as a trimer. This S protein is composed of 1273 amino acids. It has N-termi-

nus, transmembrane domain and short C-terminal segment[13]. S protein trimer consists of signal peptide at the N-terminus and two subunits S1 and S2. Residues 1-13 forms the signal peptide, residues 14-685 and 686-1273 are the S1 and S2 subunit, respectively. RBD is comprised of 319-541 residues of S1 subunit. Residues 788-806 forms the fusion peptide in the S2 subunit[15]. The S1 and S2 subunits form the head and stalk of SARS-CoV-2 S protein[16]. The RBD region is an important target for neutralizing antibodies. SARS-CoV-2 has approximately 80% amino acid sequence identity with SARS-CoV. However, the RBD domain in S1 is less conserved (64% identity) compared to the fusion protein (FP) (90% identity) in the S2 subunit. Remarkably, antigenicity differences exist between the RBD of SARS-CoV and SARS-CoV-2 despite 64% sequence identity. In the S2 subunit residues 912-984 and 1163-1213 form the heptapeptide repeat sequence (HR) 1 and HR2, respectively[17]. The repetitive heptapeptide sequence is HPPHCPC. HR1 and HR2 is essential for the viral fusion and entry function of the S2 subunit[18].

After the S-protein binds the human angiotensin converting enzyme 2 receptor (hACE2) conformation changes of the S-protein leads to the fusion of virus with the host cell membrane. When the RBD binds to hACE2, the FP, HR1 and HR2 domains play crucial role to bring viral envelope and cell membrane into proximity for viral fusion and entry into target cell. The RBD present in the S1 domain is chiefly responsible for binding of the virus to the receptor. The S2 domain which contains the HR domain, including HR1 and HR2 is related to virus fusion[19]. The cells expressing the hACE2 are lung, intestine, heart, kidney, and alveolar epithelial type II cells[20]. SARS-CoV-2-S binds to hACE2 with 20 times higher affinity than SARS-CoV[21]. This shows that SARS-CoV-2 may have enhanced infectivity and the infectious dose might be less in comparison to SARS-CoV. Independent research studies show that the SARS-CoV-2-S contains several furin cleavage sites cleaved by furin like proteases[22]. Another protease that cleaves the S protein of SARS-CoV-2 is Trypsin[23]. The cleavage of SARS-Co-V2-S into S1 and S2 is the basis of fusion. After the S protein is cleaved the FP is exposed and undergoes conformational changes and gets inserted into host membrane. The formation of 6 helical bundle by HR1 and HR2 in the S2 is also essential for viral fusion[24].

Notable Variants and Mutations of SARS-CoV-2

At the first week of February 2020, a variant of SARS-CoV-2 with D614G substitution in the spike glycoprotein was reported[25]. After many months, the variant with D614G (aspartic acid to glycine at residue 614) substitution replaced the naïve SARS-CoV-2 strain, originated from China. By the end of June 2020, this variant became predominant form with global presence. Animal model studies and human respiratory cell studies revealed that the variant with D614G mutation has enhanced infectivity and resulted in rapid global spreading. Remarkably, despite having higher infectivity rate the variant D614G did not elicit severe disease. The variant also does not affect the efficacy of available laboratory testing, treatment, immune prophylaxis or preventive public health measures. The S protein structure of variant of SARS-CoV-2 with D614G substitution is shown in the Figure1.

Astonishingly, recent reports exhibited that other variants reported from many parts of the world demonstrated increased infectivity, virulence and may be a challenge to existing vaccines used to prevent naïve SARS-CoV-2[26]. Further studies are required to assess the pathogenicity, virulence, therapeutics aspects, and immune prophylaxis for variant strains of SAS-CoV-2.

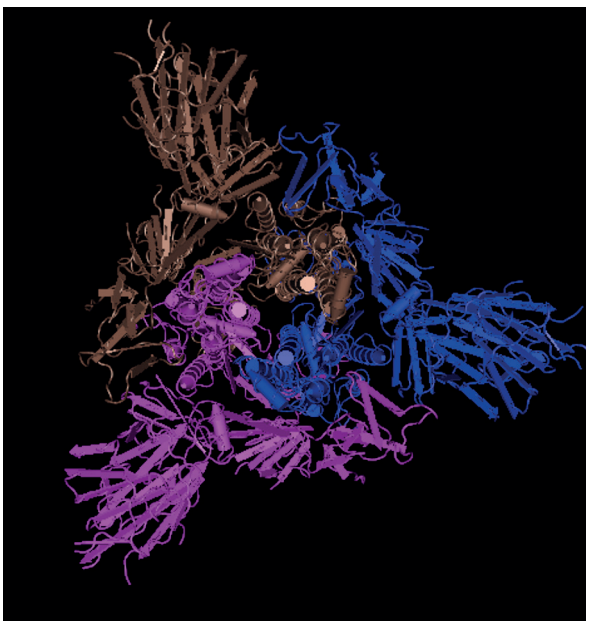

Figure 1. S protein structure of variant of SARS-CoV-2 with D614G substitution.

Cluster 5/SARS-CoV-2 Variant of Denmark

Cluster 5 also referred as ΔFVI-spike by the Danish State Serum Institute is a variant of SARS-CoV-2 discovered in Denmark in early November[27]. Minks were found to be the reservoir, five clusters of mink variants of SARS-CoV-2 were found designated as clusters 1-5 (Danish: cluster 1-5) by the Danish State Serum Institute[28]. Several mutations were observed in the S-protein of Cluster 5. The mutations include 69-70 delta HV (deletion of a histidine and valine at amino acid positions 69 and 70 in the N-terminal domain of the S1 subunit); I692V – a conservative substitution of isoleucine to valine at position 692 that is located seven amino acids downstream of the furin cleavage site. Additionally, Y453F (tyrosine to phenylalanine at residue position 453 in the RBD, M1229I (methionine to isoleucine at residue position 1229), and S1147L, a non-conservative substitution of serine to leucine at residue position 1147 in the S2 subunit, were also noted[29].

Other than Denmark, the countries that reported the presence of SARS-CoV-2 in minks were Netherlands, Sweden and Spain. It was speculated that cluster 5 may have reduced efficacy to vaccine[11]. By 5th November 2020 around 200 cases of mink related COVID-19 were detected[30]. After 19 November 2020, no further cases were reported.

Variant 501.V2/SARS-CoV-2 Variant of South Africa

This variant is also known as 501Y.V2 variant or 20H/501Y.V2 or B.1.351 lineage[31]. This variant was first detected in South Africa on 18 December 2020. As of 26 January 2021, the variant has spread to 26 different countries with a total of 668 cases globally and 540 cases in South Africa alone. The last case was reported on 19 January 2021 with one case from Tanzania[25]. Three mutations are found in the RBD in the S-protein of the virus. They are N501Y (asparagine to tyrosine at residue position 501), K417N (lysine to asparagine at residue position 417), and E484K (glutamic acid to lysine at residue position 484)[32]. The 2D structure of S protein demonstrated by variant 501.V2 is shown in the Figure 2.

Among the three mutations in 501Y.V2 variant E484K mutation remarkably is associated with lack of sensitivity to neutralising antibodies that may affect the S protein based COVID vaccines. In Brazil, the E484K spike mutation was linked to a case of reinfection with the 501.V2 variant[33].

Lineage B.1.1.248/Lineage P.1

A new variant was detected in December 2020 in Manaus, Amazonas state, North Brazil[34]. The new lineage was named P.1, descendent of B.1.1.28. The following are the important mutations detected in this lineage such as E484K (Glutamic acid to lysine at residue position 484), K417T (lysine to threonine at residue position

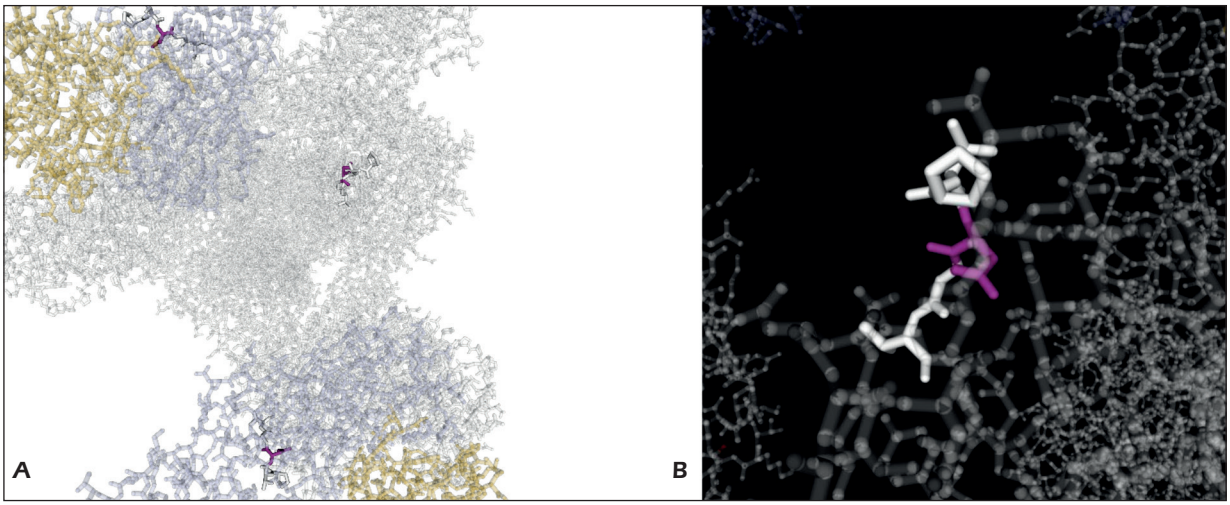

Figure 2. 2D structure of SARS CoV2 spike protein. **A,** Asparagine residue (*magenta color*) at position 501 in trimetric spike protein. **B,** Asparagine residue (*magenta*) at position 501 in close view flanked by threonine at position 500 and Glycine at position 502.

417), and N501Y (asparagine to tyrosine at residue position 501)[15]. Remarkably, the samples that were collected from March to November 2020 were negative for the variant but was positive for the samples collected after 15 December 2020. The lineage P.1 was detected in Tokyo, Japan on 6 January 2021, with a history of travel from Amazonas state of Brazil[35].

VUI/VOC 202012/01

In Kent, England there was an increase in incidence of COVID-19 cases in early December 2020[36]. Epidemiological investigations were conducted on 8 December 2020 to investigate the increase in incidence. Frequency of VOC detection and the reproduction number (Rt) indicates that the VOC is significantly associated with a higher Rt[26]. The new variant is defined by 23 mutations: 13-nonsynonymous mutations, 4 deletions and 6 synonymous mutations[4]. The non-synonymous mutations include a series of S protein mutations. The S protein mutations are HV (histidine and valine) 69-70 deletion, Y144 (tyrosine) deletion, N501Y (asparagine to tyrosine at residue position 501), A570D (alanine to aspartic acid at residue position 570), P681H (phenyl alanine to histidine at residue position 681). Others include T716I (threonine to isoleucine at residue position 161), S982A (serine to alanine at residue position 982), D1118H (aspartic acid to histidine at residue position 1118)[12].

Among these mutations, three mutations may have significant biological effects, such as N501Y, HV 69-70 deletion and P681H that is located adjacent to the furin cleavage site. Among the three mutations the most concerning mutation is N501Y[37]. N501Y is identified to have a higher binding affinity to hACE2 as it is one of the contact residues in RBD. It is suspected that N501Y mutation alone or in combination with HV 69-70 deletion in the N terminal domain (NTD) might enhance the transmissibility of the virus. As the asparagine to tyrosine substitution is in the RBD the neutralising antibodies may be less effective. Experiments with monoclonal antibodies showed decreased neutralizing effect on the SARS-CoV-2 variants at position 501. However, the data is not available for the polyclonal antibodies[38]. Experts believe that vaccines may be effective against the new VOC-202012/01 variant.

Devastating Variant B.1.617 of India

Currently in India (from April 2021) there is a severe second wave of COVID-19 due to a variant SARS-CoV-2. Molecular studies showed that this mutant belongs to B.1.617 variant of SARS-CoV-2. This variant acquired two mutations known as E484Q and L452R. E484Q mutation is similar to South African and British variants whereas L452R is similar to the variant of California. Interestingly, the two mutations evolved separately in other variants but found together in Indian variant. The second wave in India with enhanced Rt by the variant, exhibited rapid spreading and increased virulence affecting the younger adults. This variant is also commonly called 'double mutant variant' locally in Indian media.

Inadequate medical infrastructure, improper policy decision by policy makers and government mismanagement fuelled this second wave catastrophe in India. Without foreseeing and future planning against second wave, the Indian government supplied the vaccines manufactured in India to other countries. This is the main reason for the current shortage of vaccine in India. Furthermore, just before the second wave, the Indian government conducted elections in some states. During the election campaign COVID-19 containment measures were not followed. In addition, many religious festivals like 'Kumbh mela' involving thousands of people under crowed conditions were conducted. Furthermore, lack of sufficient medical oxygen availability in the country aggravated the situation leading to several deaths.

Conclusions

All clans of coronaviruses are notoriously known for its frequent high rate of mutation, due to errors during its replication cycle. It is observed that minor nucleotide sequence changes have been noticed between naïve SARS-CoV-2 and its associated variants etc. Alteration of nucleotide sequence were noticed in S and nucleocapsid genes of variants. Since the S protein is responsible for attachment and entry of SARS-CoV-2, the variation in its sequences may have a profound effect on the transmissibility of the variants. The S protein sequence changes in the SARS-CoV-2 lineages may be attributed to the evolution of the coronavirus to adapt to different hosts. The N501Y mutation is present in VOC, 501Y.V2 and P.1 variants. Although these three variants have evolved independently to each other, the N501Y mutation seems to be common,

enhancing the infectivity. We predict that in due course the evolved lineages of SARS-CoV-2 may further mutate and acquire enhanced virulence. A major concern is that these lineages may totally transform and acquire novel genes that are totally different from the naïve SARS-CoV-2 similar to the antigenic shift observed in Influenza A viruses. It may pose severe constraints in the suitability of existing COVID-19 vaccine against the evolved SARS-CoV-2 variants.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Ethics Approval and Consent to Participate
None required in this study.

Consent for Publication
All the authors give consent for publication.

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Authors' Contribution
Study idea: Dr. Kannan Subbaram Conceptualization: Dr. Shaik Syed Ali Pakeer Acquisition of data and analysis: Dr. Sheeza Ali Interpretation of the data: Dr. Sheeza Ali, Dr. Kannan Subbaram and Dr. Shaik Syed Ali Pakeer.

Availability of Data and Material
All datasets generated or analyzed during this study are included in the manuscript.

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