Advanced Studies in Biology, Vol. 14, 2022, no. 1, 111 – 127
HIKARI Ltd, www.m-hikari.com
https://doi.org/10.12988/asb.2022.91457

Study of Surface Potential and Hydrophobicity of Amino Acids in Mutated Spike Proteins of Variants of SARS-CoV-2 and Its Impact on Attachment and Internalization of Virus with Human Host Cells

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Abstract

The Spike protein as a whole and Receptor Binding Region in particular are the main molecules which participate in the process of attachment and internalization of SARS-CoV-2 into human cells. Detailed study of physical and chemical changes in spike protein of SARS-CoV-2 from Wuhan to Omicron strains is needed for the public health and therapeutic utility. Amino acid sequences of all strains of novel coronavirus (Wuhan, alpha, beta, delta, gamma and omicron) were studied and analysed using software, Clustal Omega. Present paper reports analysis of all mutations in the Spike protein of different strains focusing on the chemical and physical interaction of the amino acids of RBD of Spike Protein with amino acids of ACE-2 receptor, to explain basis of transmissibility and clinical severity of viral strains. We report how potential based affinity of Spike protein and host ACE-2 receptor has affected the virus-host attachment and that how hydrophobicity of amino acids in the RBD region will affect the internalization of virus molecule into host cells.

Keywords: SARS-CoV-2, mutations, Spike proteins, hydrophobicity

1 Introduction

Spike protein as a whole and Receptor Binding Domain (RBD) Region in particular are the main molecules which participate in the process of attachment and internalization of SARS-CoV-2 (Severe Acute Respiratory Syndrome-Coronavirus 2) into human cells. The process of attachment of any two protein molecules is accomplished by non-covalent bindings such as hydrogen bonding and Vander wall forces etc. which involves the role of positive or negative potential carried by the interacting molecules [1]. This has also been reported that

the attachment and internalization of enveloped viruses into the host cells, in general, are determined by complementarity of surface potential of spike protein and ACE-2 (Angiotensin Converting Enzyme-2) receptor and the hydrophobicity of the amino acids constituting binding zones [2]. Although mutations in spike proteins [3] have been reported but the altered physical and chemical properties due to mutations have not been studied as possible basis of transmissibility and severity of specific viral variants. We have undertaken a detailed analysis of all mutations in the Spike protein of different strains including those in Receptor binding domain (RBD) focusing on the chemical and physical-charges in amino acids to explain the transmissibility and clinical severity caused by different variants of SARS-CoV-2. Present paper reports how potential based interactions of Spike protein and host ACE-2 receptor has affected the virus-host attachment and that how hydrophobicity of amino acids in the RBD region will affect the internalization of virus molecule into host cells.

2 Materials and Methods

In the present study amino acid sequences of all strains of novel coronavirus (Wuhan, alpha, beta, delta, gamma and omicron) were analysed at Centre of Excellence in Virology & Immunology, Department of Life Sciences, School of Basic Research, Sharda University, Greater Noida. A systematic search of published papers on structural protein of SARS-CoV-2 from different countries/population groups globally was attempted. Amino acid sequences of all strains of coronavirus were obtained from a software, Clustal Omega. The obtained amino acid sequences were compared with reference to the replacement patterns between two strains. The changing patterns of amino acids were observed to understand their corresponding role in the ultimate pathogenicity and severity of the particular viral strain. This focused on exploring the mutations reported as well as the molecular aspects of vaccine production using electronic online databases. To study the altered structure of spike protein and their complementarity with available vaccines, bioinformatics study was conducted using NCBI databank and Clustal Omega.

3 Results

Amino acid constitution of spike proteins of different strains

Table 1 shows the number of amino acids constituting spike proteins in different strains of the SARS-CoV-2. Number of amino acids in the initial Wuhan strain were 1273. Subsequently, number of amino acids building spike proteins

have increased from 1273 initial number in Wuhan strain to 1285 in Alpha strain, 1285 in Beta strain; 1283 in Gamma strain; 1281 in delta strain and 1285 amino acids in Omicron strain, of SARS-CoV-2. The details of individual amino acid of each strain replacing amino acids of Wuhan strain is given in Tables 2-6.

Replacement pattern (Mutations trend) of Amino acids in spike proteins in different variants of SARS-CoV-2

Keeping Wuhan as reference strain, it was observed that in Alpha strain 6 changes or mutations were observed. Asparagine in the 501th position was replaced by Tyrosine, Alanine was replaced by Aspartic Acid in 570th position and so on. The others to be replaced were Aspartic acid to Glycine, Proline replaced by Histidine; Threonine replaced by Isoleucine; Serine replaced by Alanine and Aspartic Acid replaced by Histidine in Alpha strain (Table 2).

In Beta strain 9 changes or mutations took place with reference to Wuhan strain (Table 3). Leucine was changed to Phenylalanine on 18th position; Aspartic Acid to Alanine on 80th position; Aspartic acid to Glycine, Arginine to Isoleucine; Lysine to Asparagine; Glutamic acid to Lysine; Asparagine to Tyrosine, Aspartic acid to Glycine and Alanine to Valine. In Gamma strain 12 mutations took place. Leucine of Wuhan was replaced by Phenylalanine in Gamma strain on 18th position; Threonine to Asparagine on 20th position, Proline to Serine; Aspartic acid to Tyrosine, Arginine to Serine, Lysine to Threonine; Glutamic acid to Lysine; Asparagine to Tyrosine, Aspartic acid to Glycine, Histidine to Tyrosine etc as shown in Table 4. In the spike protein of Delta strain, a total of 15 mutations took place with reference to Wuhan strain. Threonine was replaced by Arginine; Valine to Phenylalanine, Threonine to Isoleucine, Glycine to Aspartic acid, Arginine to Glycine, Alanine to Valine, Tryptophan to Leucine, Lysine to Asparagine etc as shown in Table 5. The Omicron strain had 41 changes as compared to Wuhan strain. Detailed changes are shown in Table 6. Salient features of mutations in spike protein of Omicron variant was that 6 different amino acids of Wuhan have been replaced by Lysine in Omicron strain, 4 other amino acids have been replaced by Serine and 6 other amino acids of Wuhan getting replaced by Proline in Omicron strain.

Common and Uncommon mutations in strains of SARS-CoV-2

Comparing the common mutations that arised in strains, N501Y was seen in the three i.e. Alpha, Beta, Gamma and Omicron strains. D614G was found in all the four strains including Delta. P681H was found in Alpha and Omicron strain while in case of Delta, the mutation at the 681st position was P to R. L18F mutation was found in Beta and Gamma strain. K417N was found in Beta, Delta

and Omicron while in the same position in case of Gamma strain it was K417T. E484K mutation was seen in gamma and while in the same position it was Q in case of Delta and A in case of Omicron strain. H665Y was seen in Gamma and Omicron strain. T95I, G142D and T478K was seen common in Delta and Omicron strain. Rest all the mutations reported were uncommon among strains (Table 2 to 6).

Molecular, Physical and Structural analysis of amino acids of Spike protein in different variants of SARS-CoV-2

Of the seven changes that occurred in Alpha strain, all the seven amino acids were replaced in terms of molecular, physical and structure was seen, viz; three Hydrophilic neutral amino acids (Asparagine, Threonine and Aspartic acid) was replaced by hydrophobic molecules (Tyrosine, Isoleucine and Alanine respectively) while at two places, a Hydrophilic negatively charged amino acid (Aspartic acid) was replaced by Hydrophobic molecule (Glycine) at 614^{th} position and by Hydrophilic positively charged molecule (Histidine) at 1118^{th} position. Two hydrophobic molecules (Alanine & Proline) were replaced with Hydrophilic negatively charged amino acid (Aspartic acid) ay 570^{th} position while with Hydrophilic positively charged amino acid at the 681^{st} position. The ratio of Hydrophobic: Hydrophilic nature observed was 4:3 in case of Alpha strain (Table 2; Figure 1).

In Beta strain, out of 9 mutations, 7 amino acids showed changes in terms of physicochemical nature. Four of these reflected changing of Hydrophilic negatively charged amino acids (3 Aspartic acid & 1 Glutamic acid) to Hydrophobic molecule (1 Alanine and 2 Glycine) and one Hydrophilic positively charged amino acid (Lysine). Two Hydrophilic positively charged amino acids (Arginine & Lysine) were replaced with Hydrophobic and Hydrophilic neutral (Isoleucine & Asparagine respectively). One Hydrophilic neutral (Asparagine) was replaced with Hydrophobic amino acid (Tyrosine) (Table 3, Figure 1). The ratio of Hydrophobic: Hydrophilic nature observed was 7:2 in case of Beta strain.

In Gamma variant, 12 mutations have taken place with reference to Wuhan strain of which 9 amino acids showed changes in terms of physicochemical nature. Three hydrophilic negatively charged amino acids (2 Aspartic acid & 1 Glutamic acid) were replaced with 2 hydrophobic amino acids (Tyrosine & glycine respectively) and 1 Hydrophilic positively charged amino acid (Lysine). Three Hydrophilic positively charged amino acids (Serine, Threonine and Tyrosine were replaced with Hydrophilic neutral and one hydrophobic amino acid. Two hydrophilic neutral were replaced with hydrophobic molecule while one

Hydrophobic amino acid (Proline) was replaced with Hydrophilic neutral amino acid (Serine). The ratio of Hydrophobic: Hydrophilic nature observed was 7:5 in case of Gamma strain (Table 4, Figure 1).

In Delta strain, 15 mutations have taken place with reference to Wuhan strain of which 12 amino acids showed changes in terms of physicochemical nature. There were 3 Hydrophilic negatively charged amino (2 Aspartic acid & 1 Glutamic acid) which was replaced by 1 Hydrophobic and 2 Hydrophilic neutral amino acids. Two Hydrophilic positively charged amino acids was replaced with Hydrophobic and Hydrophilic neutral amino acid respectively. Three Hydrophilic neutral amino acids were replaced by 2 Hydrophilic positive and one Hydrophobic amino acid. 4 Hydrophobic amino acids were replaced with 1 Hydrophilic negatively charged amino acid and 3 Hydrophilic positively charged amino acids. The ratio of Hydrophobic: Hydrophilic nature observed was 6:9 in case of Delta strain (Table 5, Figure 1).

Omicron variant has undergone maximum number of mutations (41 changes in amino acids) of which nearly 32 had changes in terms of their Surface potential, physical nature and structure. 3 hydrophilic negatively charged amino acids (1 Glutamic acid and 2 Aspartic acid) were replaced with hydrophobic molecules (Alanine, Glycine & Tyrosine). 7 Hydrophilic positively charged amino acids were replaced with Hydrophilic negatively charged molecules, 3 hydrophilic neutral and 3 hydrophobic molecule. 16 Hydrophilic neutral amino acids were replaced with 6 hydrophobic and 10 hydrophilic positively charged molecules. 6 Hydrophobic molecules were replaced with 2 Hydrophilic negatively charged molecules, 2 Hydrophilic neutral and 2 Hydrophilic positively charged molecule. The ratio of Hydrophobic: Hydrophilic nature observed was 20:21 in case of Omicron strain (Table 6, Figure 1).

Table 1. Number of amino acids constituting spike protein (SP) in different variants of SARS-CoV-2

S. No.	Strain	Country of Origin	No. of amino acids
1.	Wuhan	China	1273
2.	Alpha	United Kingdom	1285
3.	Beta	South Africa	1285
4.	Gamma	Brazil	1283
5.	Delta	India	1281
6.	Omicron	South Africa	1285

Table 2: Replacement of amino acids in Alpha strain when compared to Wuhan strain

S. No.	Mutation	Replaced amino acid of Wuhan strain	Phyisco-chemical profile of amino acid	New amino acid in alpha strain	Physio-Chemical profile of new amino acids
1.	N501Y	Asparagine	Hydrophilic neutral	Tyrosine	Hydrophobic
2.	A570D	Alanine	Hydrophobic	Aspartic acid	Hydrophilic negatively charged
3.	D614G	Aspartic acid	Hydrophilic negatively charged	Glycine	Hydrophobic
4.	P681H	Proline	Hydrophobic	Histidine	Hydrophilic positively charged
5.	T716I	Threonine	Hydrophilic Neutral	Isoleucine	Hydrophobic
6.	S982A	Serine	Hydrophilic neutral	Alanine	Hydrophobic
7.	D1118H	Aspartic acid	Hydrophilic negatively charged	Histidine	Hydrophilic positively charged

Table 3: Replacement of amino acids in Beta strain when compared to Wuhan strain

S. No.	Mutation	Replaced amino acid of Wuhan strain	Phyisco-chemical profile of amino acid	New amino acid in alpha strain	Physio-Chemical profile of new amino acids
1.	L18F	Leucine	Hydrophobic	Phenylalanine	Hydrophobic
2.	D80A	Aspartic acid	Hydrophilic negatively charged	Alanine	Hydrophobic

Table 3 (continued): Replacement of amino acids in Beta strain when compared to Wuhan strain

3.	D215G	Aspartic acid	Hydrophilic negatively charged	Glycine	Hydrophobic
4.	R246I	Arginine	Hydrophilic Positively charged	Isoleucine	Hydrophobic
5.	K417N	Lysine	Hydrophilic Positively charged	Asparagine	Hydrophilic neutral
6.	E484K	Glutamic acid	Hydrophilic Negatively charged	Lysine	Hydrophilic Positively charged
7.	N501Y	Asparagine	Hydrophilic neutral	Tyrosine	Hydrophobic
8.	D614G	Aspartic acid	Hydrophilic negatively charged	Glycine	Hydrophobic
9.	A701V	Alanine	Hydrophobic	Valine	Hydrophobic

*Green color indicates no change in amino acid nature

Table 4: Replacement of amino acids in Gamma strain when compared to Wuhan strain

S. No.	**Mutation**	**Replaced amino acid of Wuhan strain**	**Phyisco-chemical profile of amino acid**	**New amino acid in alpha strain**	**Physio-Chemical profile of new amino acids**
1.	L18F	Leucine	Hydrophobic	Phenylalanine	Hydrophobic
2.	T20N	Threonine	Hydrophilic Neutral	Asparagine	Hydrophilic neutral
2.	P26S	Proline	Hydrophobic	Serine	Hydrophilic neutral
4.	D138Y	Aspartic Acid	Hydrophilic negatively charged	Tyrosine	Hydrophobic

Table 4 (continued): Replacement of amino acids in Gamma strain when compared to Wuhan strain

5.	R190S	Arginine	Hydrophilic Positively charged	Serine	Hydrophilic neutral
6.	K417T	Lysine	Hydrophilic Positively charged	Threonine	Hydrophilic Neutral
7.	E484K	Glutamic Acid	Hydrophilic Negatively charged	Lysine	Hydrophilic Positively charged
8.	N501Y	Asparagine	Hydrophilic neutral	Tyrosine	Hydrophobic
9.	D614G	Aspartic Acid	Hydrophilic negatively charged	Glycine	Hydrophobic
10.	H655Y	Histidine	Hydrophilic positively charged	Tyrosine	Hydrophobic
11.	T1027I	Threonine	Hydrophilic Neutral	Isoleucine	Hydrophobic
12.	V1176F	Valine	Hydrophobic	Phenylalanine	Hydrophobic

*Green color indicates no change in amino acid nature

Table 5: Replacement of amino acids in Delta strain when compared to Wuhan strain

S. No.	Mutation	Replaced amino acid of Wuhan strain	Phyisco-chemical profile of amino acid	New amino acid in alpha strain	Physio-Chemical profile of new amino acids
1.	T19R	Threonine	Hydrophilic Neutral	Arginine	Hydrophilic Positively charged
2.	V70F	Valine	Hydrophobic	Phenylalanine	Hydrophobic
3.	T95I	Threonine	Hydrophilic Neutral	Isoleucine	Hydrophobic

Table 5 (continued): Replacement of amino acids in Delta strain when compared to Wuhan strain

4.	G142D	Glycine	Hydrophobic	Aspartic Acid	Hydrophilic negatively charged
5.	R158G	Arginine	Hydrophilic Positively charged	Glycine	Hydrophobic
6.	A222V	Alanine	Hydrophobic	Valine	Hydrophobic
7.	W258L	Tryptophan	Hydrophobic	Leucine	Hydrophobic
8.	K417N	Lysine	Hydrophilic Positively charged	Asparagine	Hydrophilic neutral
9.	L452R	Leucine	Hydrophobic	Arginine	Hydrophilic Positively charged
10.	T478K	Threonine	Hydrophilic Neutral	Lysine	Hydrophilic Positively charged
11.	D614G	Aspartic Acid	Hydrophilic negatively charged	Glycine	Hydrophobic
12.	P681R	Proline	Hydrophobic	Arginine	Hydrophilic Positively charged
13.	D950N	Aspartic Acid	Hydrophilic negatively charged	Asparagine	Hydrophilic neutral
14.	E484Q	Glutamic acid	Hydrophilic Negatively charged	Glutamine	Hydrophilic neutral
15.	L452R	Leucine	Hydrophobic	Arginine	Hydrophilic Positively charged

*Green color indicates no change in amino acid nature

Table 6: Replacement of amino acids in Omicron strain when compared to Wuhan strain

S.No.	Mutation	Replaced amino acid of Wuhan strain	Phyisco-chemical profile of amino acid	New amino acid in alpha strain	Physio-Chemical profile of new amino acids
1.	A67V	Alanine	Hydrophobic	Valine	Hydrophobic
2.	T95I	Threonine	Hydrophilic Neutral	Isoleucine	Hydrophobic
3.	G142D	Glycine	Hydrophobic	Aspartic Acid	Hydrophilic negatively charged
4.	N211I	Asparagine	Hydrophilic neutral	Isoleucine	Hydrophobic
5.	L212V	Leucine	Hydrophobic	Valine	Hydrophobic
6.	R216E	Arginine	Hydrophilic Positively charged	Glutamic acid	Hydrophilic Negatively charged
7.	G339D	Glycine	Hydrophobic	Aspartic Acid	Hydrophilic negatively charged
8.	S371L	Serine	Hydrophilic neutral	Leucine	Hydrophobic
9.	S373P	Serine	Hydrophilic neutral	Proline	Hydrophobic
10.	S375F	Serine	Hydrophilic neutral	Phenylalanine	Hydrophobic
11.	K417N	Lysine	Hydrophilic Positively charged	Asparagine	Hydrophilic neutral
12.	N440K	Asparagine	Hydrophilic neutral	Lysine	Hydrophilic Positively charged
13.	G446S	Glycine	Hydrophobic	Serine	Hydrophilic neutral
14.	S447N	Serine	Hydrophilic neutral	Asparagine	Hydrophilic neutral

Table 6 (continued): Replacement of amino acids in Omicron strain when compared to Wuhan strain

15.	T478K	Threonine	Hydrophilic Neutral	Lysine	Hydrophilic Positively charged
16.	E484A	Glutamic acid	Hydrophilic Negatively charged	Alanine	Hydrophobic
17.	Q493R	Glutamine	Hydrophilic neutral	Arginine	Hydrophilic Positively charged
18.	G496S	Glycine	Hydrophobic	Serine	Hydrophilic neutral
19.	Q498R	Glutamine	Hydrophilic neutral	Arginine	Hydrophilic Positively charged
20.	N501Y	Asparagine	Hydrophilic neutral	Tyrosine	Hydrophobic
21.	Y505H	Tyrosine	Hydrophobic	Histidine	Hydrophilic positively charged
22.	T547H	Threonine	Hydrophilic Neutral	Histidine	Hydrophilic positively charged
23.	D614G	Aspartic Acid	Hydrophilic negatively charged	Glycine	Hydrophobic
24.	H655Y	Histidine	Hydrophilic positively charged	Tyrosine	Hydrophobic
25.	N679K	Asparagine	Hydrophilic neutral	Lysine	Hydrophilic positively charged
26.	P681H	Proline	Hydrophobic	Histidine	Hydrophilic positively charged
27.	R682G	Arginine	Hydrophilic Positively charged	Glycine	Hydrophobic

28.	R683S	Arginine	Hydrophilic Positively charged	Serine	Hydrophilic neutral
29.	R685S	Arginine	Hydrophilic Positively charged	Serine	Hydrophilic neutral
30.	N764K	Asparagine	Hydrophilic neutral	Lysine	Hydrophilic Positively charged
31.	D796Y	Aspartic Acid	Hydrophilic negatively charged	Tyrosine	Hydrophobic
32.	F817P	Phenylalanine	Hydrophobic	Proline	Hydrophobic
33.	N856K	Asparagine	Hydrophilic neutral	Lysine	Hydrophilic Positively charged
34.	A892P	Alanine	Hydrophobic	Proline	Hydrophobic
35.	A899P	Alanine	Hydrophobic	Proline	Hydrophobic
36.	A942P	Alanine	Hydrophobic	Proline	Hydrophobic
37.	Q954H	Glutamine	Hydrophilic neutral	Histidine	Hydrophilic positively charged
38.	N969K	Asparagine	Hydrophilic neutral	Lysine	Hydrophilic Positively charged
39.	L981F	Leucine	Hydrophobic	Phenylalanine	Hydrophobic
40.	K986P	Lysine	Hydrophilic positively charged	Proline	Hydrophobic
41.	V987P	Valine	Hydrophobic	Proline	Hydrophobic

*Green color indicates no change in amino acid nature

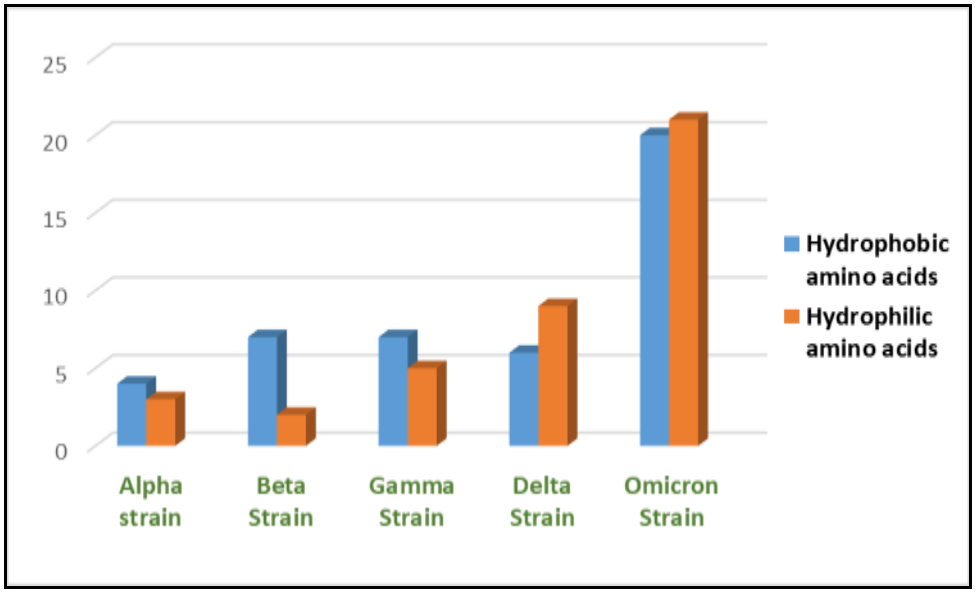

Figure 1. Physico-chemical profile of replaced Amino acid in various strains of SARS-CoV-2

4 Discussion

Present paper reports the physical and chemical analysis of amino acids of Spike proteins of different variants which have replaced the original amino acids constituting Spike protein of Wuhan strain. The detailed analysis of Wuhan strain and the subsequent mutations which took place in the mutated strains showed that number of amino acids constituting Spike protein of virus has increased gradually from Wuhan through Alpha, Beta, Gamma, Delta and Omicron variants. Earlier studies undertaken on SARS-CoV reported that surface potential of ACE-2 provides negatively charged ridges surrounding catalytic spot of receptor which interacts with RBD of Spike protein of SARS-CoV2 [4]. Keeping this model of ACE-2 receptor in reference, our analysis of different variants showed that presence of positively charged amino acids on the surface of Spike proteins will provide potential based attraction to a specific variant of virus towards ACE-2 receptor on the surface of human epithelial cells of naso-pharyngeal region and a variant having more positive potential on surface of spike protein will bind more strongly with the human receptor leading to more expulsion of human cells surrounded by virus particles during process of coughing and sneezing and hence facilitating faster pathogenic transmission. Previous studies have reported the genome length, segmented and unsegmented structure of viruses as the possible

basis of transmission of disease [5]. However, we report for the first time that increased positive potential on the surface of Spike protein of SARS- CoV-2 will increase the host-virus attachment ensuring the faster transmission, as has been observed in the case of fast spread of infection caused by Omicron variant.

Our study of analysis of physical and chemical changes of amino acids in the mutated Spike Proteins also revealed a chemical change in amino acids in mutated strains. We observed that hydrophobicity of amino acids of Spike Protein has increased during evolution of mutations from Wuhan to Omicron strain and that maximum number of hydrophobic amino acids are present in Omicron variant. Increased hydrophobic nature could impart the hydro inertness to the RBD of Spike Protein and could reduce chances of internalization of virus particle into human cell. This could be the possible reason as to why Omicron variant did not cause many clinical severity/mortality.

5 Conclusions

The detailed physical and chemical analysis of amino acids in mutated proteins have shown a trend in increasing positive surface potential of viral molecules and also its hydrophobicity as varying with travel of changes and that mutations though may make available vaccine and drug molecules less effective but they may also make a virus less virulent with time. Study reported here could be of prospective significance in predicting transmission and severity of future pandemics. In addition, the chemical and potential details of Spike Proteins could sharpen our future efforts of development of effective vaccine and anti-viral drugs.

Acknowledgements. This study was financially supported by the project "*A Case-Control study on large hospital data base to implicate role of co-morbidities causing mortality of COVID-19 patients and development of a new RT-PCR based algorithm as predictor of clinical outcome*". Project code: 2021-6369.

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Received: October 3, 2022; Published: November 2, 2022