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MUTATIONAL INSIGHT OF ORF8 PROTEIN OF SARS-CoV-2

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ABSTRACT: SARS-CoV-2 (Severe Acute Respiratory Syndrome), the causative agent of COVID-19, creates a devastating situation all around the globe. Nine accessory proteins are encoded by the genome of SARS-CoV-2. These accessory proteins are not required for viral replication but are essential for its interaction with the host. ORF8 among one of the accessory proteins of SARS-CoV-2 helps in immune evasion of the host. ORF8 disturbs the MHC pathway of the host. Present study includes, a total of 1175 ORF8 sequences from India for mutational study. Altogether 265 point mutations were identified in ORF8 protein sequences of India and among them D34G, V62L, S69L, L84S and F120L were the most frequent ones. Further, the mutations were identified as deleterious or neutral. The physicochemical properties and hydrophobicity was estimated. To characterize the immunogenicity of ORF8 protein B-cell epitopes and their antigenicity was calculated.

keywords: SARS-CoV-2, COVID-19, ORF8, Mutation**,** B-cell epitopes.

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1.INTRODUCTION

An unusual pneumonia has been reported in the December 2019 in Wuhan city of China. The causal microorganism named as SARS-CoV-2 or 2019-nCoV [1, 2]. This virus soon spread across the world and has caused enormous health risks and economic disruption [3]. The World Health

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Organization has declared COVID-19 as a pandemic disease. Due to these health calamities, morbidity and mortality rate is increasing at an alarming rate and still its high time to develop safe and more effective vaccines that can also act easily against several important variants of SARS-CoV-2.The ORF8 protein of SARS-CoV-2 is a unique accessory protein, which plays a crucial role in host pathogen interaction [4]. It has been evident from the several previous studies that the ORF8 possess multiple functions which interfere with the host immune responses such as the down regulation of class I MHC molecules as well as a strategies of host immune evasion [5]. The present study was undertaken to study the effect of mutation on the structure of ORF8 protein sequences from India and compared it with the Wuhan type isolates. Significant alterations have been found in the physico-chemical, immunological and structural aspects of SARS-CoV-2 ORF8 protein. The present work was, therefore, undertaken to explore an important accessory protein like ORF8, essential for host pathogen interaction and evasion of host immune response.

2. MATERIALS AND METHODS

2.1. Detection of ORF8 protein mutants

The amino acid sequence of ORF8 protein was retrieved from NCBI virus sequence collection database. Only those sequences submitted from India since the disease first originated till 18th January 2022 were selected and downloaded. A total of 1175 sequences of ORF8 protein were sequenced from India and were used in this study along with a reference sequence of Wuhan type virus. To detect the ORF8 mutants, a protein sequence alignment was done with a wild type by using Clustal Omega online server and the aligned files were visualized.

To check the mutations were synonymous or non-synonymous the ORF8 protein variants were submitted in PROTEAN v1.1.3 server and a cut off value of -2.5 was finalized to check these mutants. [6]

2.2. Determination of physicochemical properties of ORF8 protein and its hydrophobicity

The physicochemical properties which include the basic components of a protein like amino acid composition, its total molecular weight, its hydrophobicity was calculated using Protparam tool of Expasy online program. Protscale tool of expasy was used for preparing hydropathy plot of ORF8 protein [7].

2.3. Prediction of 3D structures of ORF8 protein

The three dimensional structure of ORF8 protein were predicted using Chimera along with the wild type protein [8]. This prediction helps in identification of change in protein structure of ORF8 protein upon mutation.

2.4. B-cell epitope prediction and immunogenicity

The epitopes of B-cell were identified using IEDB [9] server and the antigenicity of each of the ORF8 protein was predicted using Vaxijen v2.0 server which estimates antigenicity according to the auto cross-covariance (ACC) transformation of the protein sequences [10].

3. RESULTS AND DISCUSSION

3.1. Detection of ORF8 non-synonymous mutants

From India, 1175 sequences of ORF8 protein were submitted among which a total of 265 point mutations were identified. Out of these point mutants, the most frequent were identified as D34G, V62L, S69L, L84S and F120L (Figure 1). Out of these 5 frequent mutants, only three were neutral (V62L, S69L and L84S) while two were deleterious at 2.5 cut off values of PROVEAN score (Table 1).

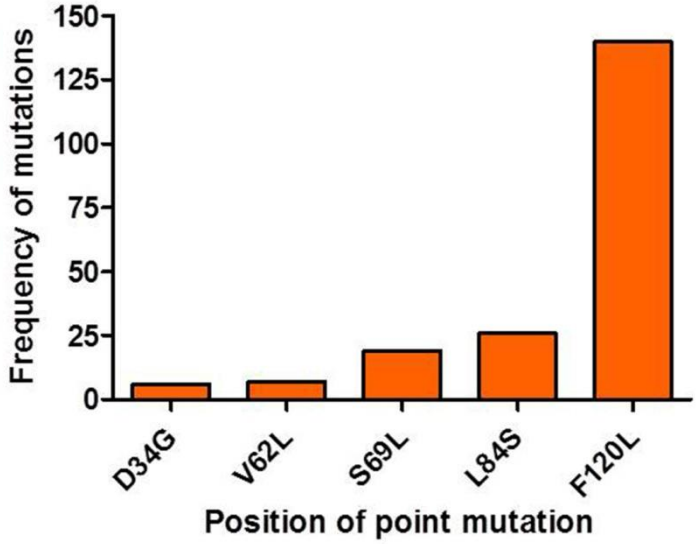

Figure 1. Frequent mutations of ORF8 protein from India

Table 1. List of nonsynonymous amino acid substitutions in ORF8 protein (cut off = -2.5)

Variant	PROVEAN score	Prediction (cutoff= -2.5)
D34G	-3.778	Deleterious
V62L	-0.722	Neutral
S69L	3.833	Neutral
L84S	2.333	Neutral
F120L	-2.667	Deleterious

3.2. Determination of physicochemical properties and hydrophobicity of ORF8 protein

Analysis of the amino acid sequence of ORF8 protein showed that it is 121 amino acid long with a molecular weight of 13831 Da. The theoretical pI of this protein is 5.42 which show it is slightly acidic in nature. The instability index of this protein is 45.79. The protein consists of more negatively charged proteins than positively charged (Table 2). The hydrophobicity estimation shows that the N-terminal of ORF8 protein is more hydrophobic than the C-terminal end (Figure 2).

Table 2. Physicochemical properties of ORF8 protein

Physicochemical properties	ORF8	Amino acid composition	No.	Percent composition (%)
Molecular weight	13831	Ala (A)	5	4.1
No. of amino acids	121	Arg (R)	4	3.3
Theoretical pI	5.42	Asn (N)	2	1.7
Instability index	45.79	Asp (D)	7	5.8
No. of negatively charged (Asp+ Glu)	13	Cys (C)	7	5.8
No. of positively charged (Arg+ Lys)	9	Gln (Q)	6	5.0
Aliphatic index	97.36	Glu (E)	6	5.0
Grand average of hydropathicity	0.219	Gly (G)	5	4.1
Estimated half-life (mammalian reticulocytes, in vitro)	30 hours	His (H)	4	3.3
Atomic composition		Ile (I)	10	8.3
C	633	Leu (L)	10	8.3
H	961	Lys (K)	5	4.1
N	155	Met (M)	1	0.8
O	177	Phe (F)	8	6.6
S	8	Pro (P)	7	5.8
Formula	$C_{633}H_{961}N_{155}O_{177}S_8$	Ser (S)	9	7.4
Total number of atoms	1934	Thr (T)	5	4.1
		Trp (W)	1	0.8
		Tyr (Y)	7	5.8
		Val (V)	12	9.9
		Phy (O)	0	0.0
		Sec (U)	0	0.0

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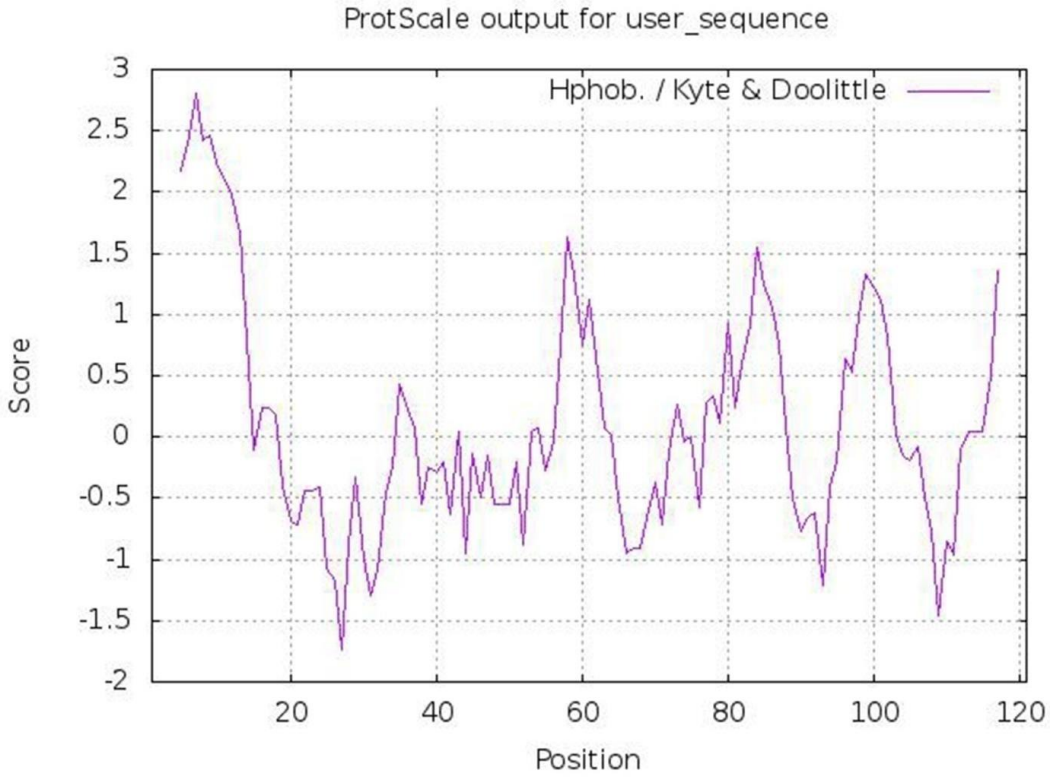

Figure 2. Hydropathy plot of ORF8 protein of SARS-CoV-2

3.3. Prediction of 3D models of ORF8 protein

The protein models of both wild type and mutated ORF8 protein were built using Chimera which showed the alterations in the protein structure upon mutation. The structure of both the proteins is sown in figure 3.

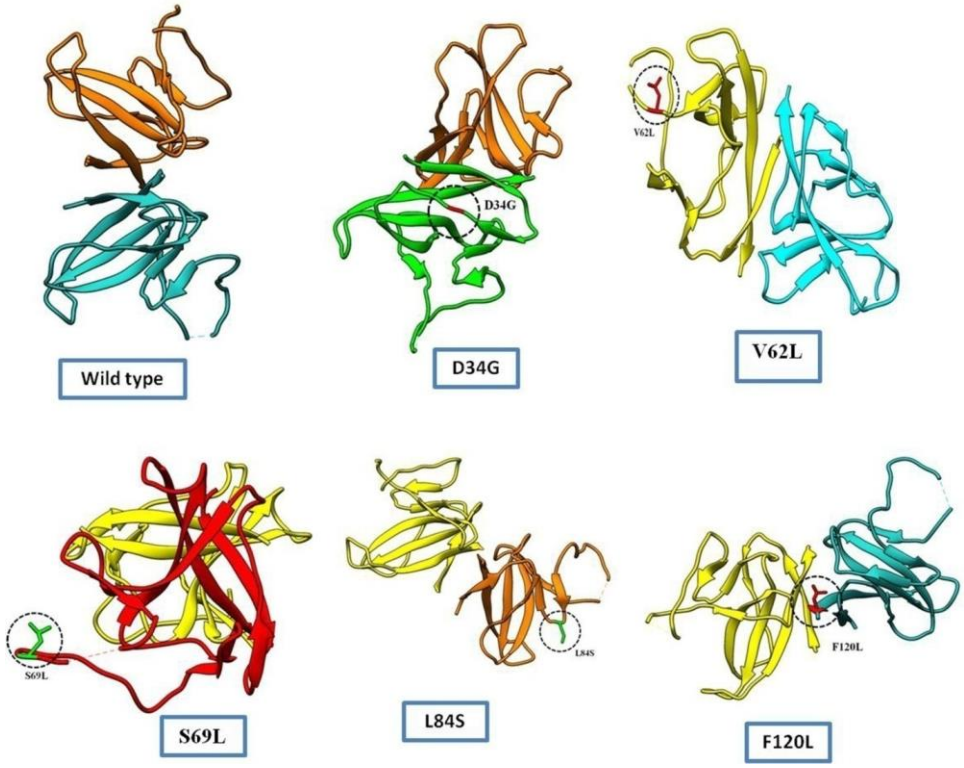

Figure 3. 3D models of wild type and mutated ORF8 protein

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3.4. Prediction of B-cell epitope and its antigenicity

Altogether 5 B-cell epitopes were found in the ORF8 protein (Table 3). These epitopes possess strong binding affinity with the B-cells and hence has high antigenic properties (Figure 4). The antigenicity of ORF8 protein was 0.64 which shows it is highly antigenic and can elucidate humoral immune response.

Table 3. B-cell epitopes of ORF8 protein of SARS-CoV-2

No.	Start	End	Peptide	Length
1	23	45	QSCTQHQPYVVDDPCPIHFYSKW	23
2	48	56	RVGARKSAP	9
3	63	78	DEAGSKSPIQYIDIGN	16
4	91	95	QEPKL	5
5	106	111	EDFLEY	6

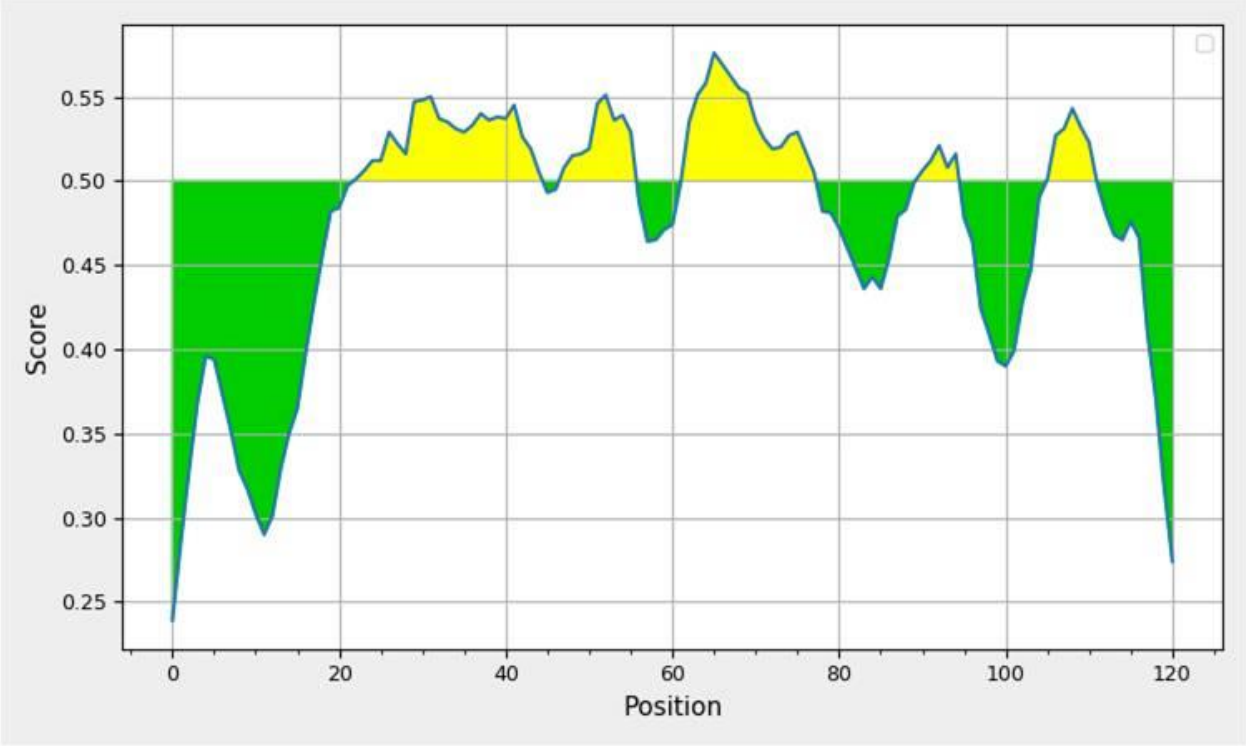

Figure 4. B-cell epitopes in ORF8 protein of SARS-CoV-2

SARS-CoV-2, is a member of coronaviridae family and nidovirales order having ssRNA as genetic material and have a high potential to exhibit mutations that are found to be beneficial for these group of viruses to evolve in ever changing climatic situations and also increases their infectivity worldwide [2,3]. Notably, mutations might also favour the process of natural selection and most often selecting those traits of viruses that are pre-requisite to survive in the highly dynamic environment of host [11, 12, 13, 14]. These properties of viruses (SARS-CoV-2) could complicate ongoing efforts to combat this contagious disease because the high frequency of mutation induces

drug resistance as well as immune evasions quickly [15,16,17]. It is evident from the previous studies on mutational aspects of SARS-CoV-2 that these mutations occurred in structural, non structural as well as accessory proteins of SARS-CoV-2. In the present study ORF8 protein sequence of SARS-CoV-2, has been studied which showed least homology with those of SARS- CoV [5, 18, 19]. In this study, we detected 265 point mutations, in ORF8 protein sequences of India. Out of them D34G, V62L, S69L, L84S and F120L were the most frequent ones. Further, the mutations were characterized as deleterious or neutral. The hydrophobicity and physicochemical properties was estimated. To characterize the immunogenicity of ORF8 protein B-cell epitopes and their antigenicity was calculated. Due to the impact of various mutations on ORF8 protein, its structure gets altered and hence it cannot bind with IRF3 and therefore, evasion of the host immune system as well as down regulation of MHCI protein can be easily accomplished [20,21]. Flower et al. (2021) have also reported that ORF8 protein in beta coronavirus is considered as fast evolving viral protein and therefore easily evade the immune system of the host organisms [5].

4. CONCLUSION

ORF8 protein of SARS-COV-2 plays an important role in host pathogen interaction. In this study, ORF8 protein mutants were identified from India and their protein structure was modeled. This study also predicted B-cell epitopes as well as antigenic properties of the virus which helps in identifying ORF8 protein as a vaccine candidate. Further *in vivo* studies needs to be done to utilize this protein as vaccine candidate.

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None

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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