Research Article

Emergence of a Pathogenic Strain of COVID-19

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Abstract

SARS-CoV-2 pandemic starting from Wuhan, China has now been spreading worldwide making the infection count more than 41 million. Within a short time span, many mutations are continuously occurring in the viral genome, be it point mutation or frameshift mutation. Scientists have been suggesting that, one of those numerous point mutations is becoming prevalent by replacing all the initial Wuhan strains of SARS-CoV-2. In this work, we have conducted a rigorous bio-informatic analyses and compared the properties of wild and mutant strains to find out the changes. Eventually, it is considered to be a more pathogenic and infective strain by our theoretical reports with a change in amino acid position number 614, which coincidentally converges with one or few publications mentioning emergence of new pathogenic D614G strain. Here we describe our approach to arrive at the conclusion.

Keywords: Mutation; SARS-CoV-2; D614G; Pathogenicity; q_R; Polar plot; Protein

1. Introduction

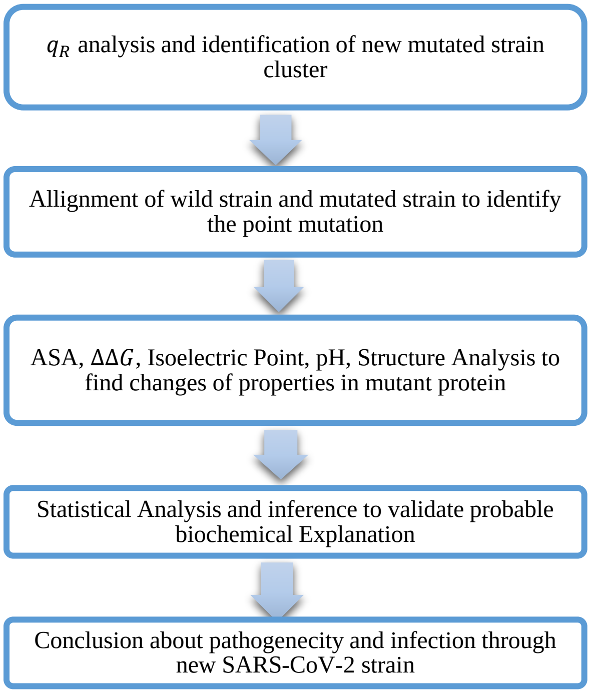
SARS-CoV-2 or COVID-19 being a pandemic (declared by W.H.O. on 11th March, 2020) has already spread to 211 countries worldwide, making the total count of infection cases to 7,273,958 with 413,372 deaths as on 11th June, 2020 [1]. SARS-CoV-2, commonly called as COVID-19, is an enveloped, single-stranded positive sense RNA virus, which falls under the family of *Coronaviridae* and has most likely jumped the species boundary [2] as other coronaviruses likely did. The Reproduction Number (R_0) of COVID-19 is approximately 3.28 [3], so it is highly contagious. It spreads via respiratory droplets and contact routes, infecting the upper and lower respiratory tracts which increases its potential to spread in a community [4]. People infected by this virus show a variety of symptoms with a median incubation period of 5.1 days [5].

It has been observed that unlike other RNA viruses, SARS-CoV-2 mutates at a slower rate [6]. Recently a prominent mutation has been observed in the spike glycoprotein of many reported viral sequences [7]. This spike glycoprotein helps the virus to attach with the host cell receptor, ACE2 (Angiotensin Converting Enzyme 2) and TMPRSS2 [8]. Point mutations in COVID-19 spike glycoprotein can lead to change in its infectivity and may also require extensive studies for sustainable vaccine designing [7, 9].

In this article we have analyzed the above-mentioned mutations in order to find out if any particular mutation is predominant. We have also discussed through various bioinformatics tools probable effects on the biochemical nature of the protein due to the predominant mutation and how it can affect the host receptor cells. Our analysis consists of mutation analysis, checking surface exposure and thermodynamic stability. These give us an interpretation about how the mutated protein is going to interact with the receptor with respect to wild protein.

2. Methodology

The flowchart of the work procedure along with their objectives stand as the following:


q_R analysis and identification of new mutated strain cluster
Allignment of wild strain and mutated strain to identify the point mutation
ASA, $\Delta\Delta G$, Isoelectric Point, pH, Structure Analysis to find changes of properties in mutant protein
Statistical Analysis and inference to validate probable biochemical Explanation
Conclusion about pathogenecity and infection through new SARS-CoV-2 strain

We used 2D Polar Co-ordinate Representation of Amino Acid Sequences and q_R value characterization [10] to study the COVID-19 gene sequences. In this method, we assign angles to each amino acid depending upon their relative hydrophobicity indices and move one unit in the respective assigned direction. We assume the graph to contain unit masses at each co-ordinate and we determine the distance of center of mass of the graph from the origin, which is defined as the Quotient Radius or q_R value of the graph. This value is found to be a characteristic value of a sequence [10].

We investigated the spike glycoprotein sequences of COVID-19 through graph plotting, q_R value characterization and distribution plots. We have used *MEGA-X* to align the sequences and find out the mutational changes in it [11]. We used *iMutant 3.0* to find out the stability changes in the mutated sequence from the first reported Wuhan sequence due to single point mutations [12]. We have used *SABLE software* [13] and *RaptorX* [14] to identify the solvent accessibility and secondary structure of the individual amino acids present in the protein sequences. We used *Phyre2* [15] to generate pdb format for the spike protein sequences and also used *UCSF Chimera* [16] to visualize them.

Using the q_R plot we found out the dominant mutations in the recently reported strains of COVID-19. Sequences having identical q_R values indicate that they have identical amino acid sequences while sequences with close q_R values have one or more changes in sequences [10]. The mutations can be observed from the difference in the q_R values of Wuhan sequence and other sequences. We can obtain the value from

$$\Delta q_R = q_{R_i} - q_{R_{Wuhan}}$$

where q_{R_i} represents the q_R value of sequences other than original Wuhan sequence (YP009724390).

The solvent accessibility of individual amino acids indicates whether the particular amino acid is exposed to the solvent or buried inside the core of the protein or whether it is located in an intermediate zone. Amino acids that are more exposed at the surface have more probability in either directly taking part or catalyzing interactions between viral protein and host receptors. This Average Solvent Accessibility (ASA) profile is determined by using SABLE Software and RaptorX [14].

The change in Gibb's free energy (ΔG) for a protein is an important factor as it is directly related to protein folding and protein stability. We calculated the ΔΔG value for a single point mutated protein using iMutant 3.0 [12]. Where,

$$\Delta\Delta G = \Delta G_{mutated} - \Delta G_{wild\ type} \text{ in Kcal/mole}$$

If $\Delta\Delta G < -0.5$, then the protein is less stable while if $\Delta\Delta G > -0.5$ then the protein is relatively more stable.

In case of SARS-COV-2, the spike glycoprotein uses ACE2 (Angiotensin Converting Enzyme 2) as a receptor for attachment to the host cell. Then the host serine-protease, TMPRSS2 (Transmembrane Serine Protease 2) does S (Spike) priming allowing fusion between viral and host cell membrane and helps in the viral entry inside the cell.

So, by analyzing how the spike glycoprotein attaches with ACE2 we can understand how mutation has changed the infectivity of the new strain. ACE 2 is mainly attached to the cell membrane of lung type II alveolar cells. The pH of a healthy lung ranges between 7.38 to 7.42 [17]. All our calculations are done taking the average pH 7.40.

3. Results

3.1 q_R Characterization Analysis

We have analyzed the q_R values of the 46 sequences of Europe, 2052 sequence of North America and 14 sequences of India of Spike Glycoprotein of SARS-CoV-2 present in NCBI Database as on 9th May, 2020 [18].

In Europe out of those 46 sequences, 11 sequences had q_R value as around 30.16105055, which is equal to Original Wuhan sequence q_R value (YP009724390). So, we expect no mutations to take place in these sequences. Whereas in all other sequences, the q_R was different. However, one significant cluster can be made amongst them, where 16 sequences of the rest had same q_R value as 29.76636445. This q_R value (29.76636445) is first observed in a sequence from Spain, collected on 9th March, 2020 (QIU78707).

In case of North America, there was much abundance of sequences, the number of sequences being 2052. We computed all of them through our q_R characterization analysis. 601 of them had q_R value as 30.16105055. So, it implies that they were same as initial wild strains collected from Wuhan. Amongst the rest of North American sequences, we again found a large cluster, containing 1067 sequences and having the same q_R value as of European mutated cluster, being 29.76636445. The said American cluster first appeared in database on 11th March 2020, collected from San Diego County, California, USA (QIK50427).

From India, there were 14 sequences uploaded in database, which we computed through q_R characterization. 5 of them had original Wuhan Strain's q_R value, while 4 out of the 14 had this mutated q_R value. The mutated sequence first appeared on 11th March, 2020 (QJF77858). The mutated strain can be identified from $\Delta q_R = -0.3946861$.

A very similar observation of this particular cluster of q_R values in USA was mentioned in one of our earlier papers [7]. From the obtained dataset we see that this mutated strain (of q_R value 29.76636445) is abundant in whole of the world, irrespective of demography and also the count of such sequences is more than original wild strain in most of the cases. This observation tells us about the importance of this mutation. We plotted the 2D polar graphs of q_R values for spike glycoprotein of Wuhan strain and the mutated strains in Figure 1.

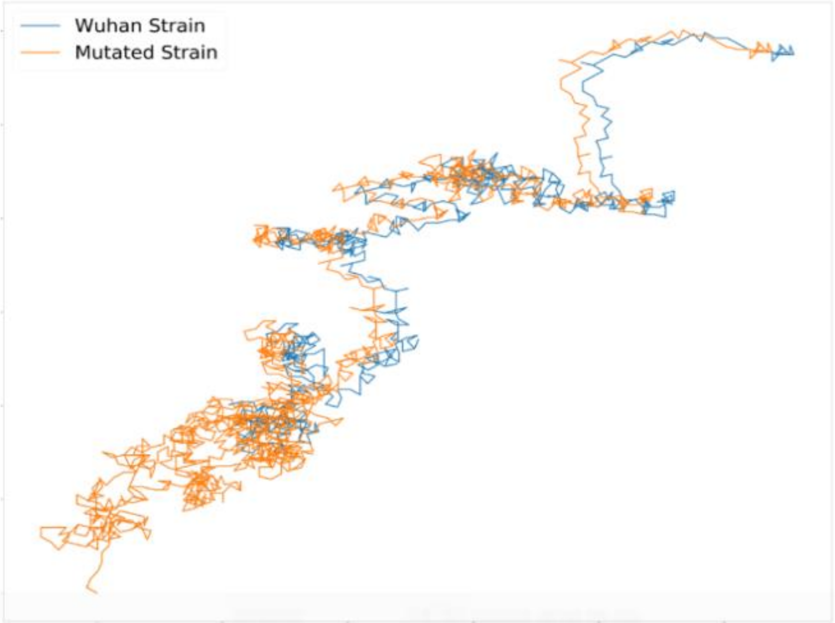


Figure 1: 2D Polar Plot of Wild strain (in blue) and Mutant strain (in red) of SARS-CoV-2 Spike Glycoprotein.

It can be observed from Figure 1 that both of them shows similar graphs except that the mutated sequence has formed a parallel shift leftwards after an initial part of the sequence. From our principle of q_R plotting, this indicates an occurrence of point mutation in its peptide sequence.

3.2 Alignment

We used *MEGA-X* to align the sequences. The mutation was found at the position 614 of the peptide sequence where an Aspartic Acid (D) was replaced by Glycine (G). This mutation was previously reported as D614G [19, 20].

3.3 Amino Acid Solvent Accessibility

The amino acid solvent accessibility was calculated simultaneously for the initial Wuhan sequence and the D614G mutated sequence. The results are shown in Table 1.

SEQUENCE	POSITION: 614		
	Amino Acid	**Accessibility Nature**	**Value**
Wuhan (Wild)	D (Aspartic Acid)	Intermediate	0.305
D614G (Mutant)	G (Glycine)	Exposed	0.337

Table 1: Solvent Accessibility Table for Wild and Mutant Strain.

Glycine is more exposed towards the solvent than Aspartic acid, though between them, Aspartic acid is more water soluble (polar nature) and should have a higher solvent accessibility. This anomalous behavior of Aspartic acid and Glycine may be related to its function in the host cells.

3.4 Calculation of ΔΔG Value

The ΔΔG values were bioinformatically calculated for the Wuhan sequence and the Mutated sequence. The result obtained is shown in Table 2.

pH	**Temperature**	**ΔΔG = $\Delta G_{mutated}$ - ΔG_{Wuhan}**
7.40	298.0 K	-0.94 Kcal/mol

Table 2: ΔΔG value of wild and mutant strain.

The decrease in the ΔΔG value due to the single point mutation at site 614 from Aspartic Acid to Glycine indicates a large decrease in stability of the mutated protein. Since, the stability of a protein is somewhat balanced with its activity [21], there is a large probability of alteration in the Spike-protein interactions and activities due to the mentioned ΔΔG change.

4. Discussions

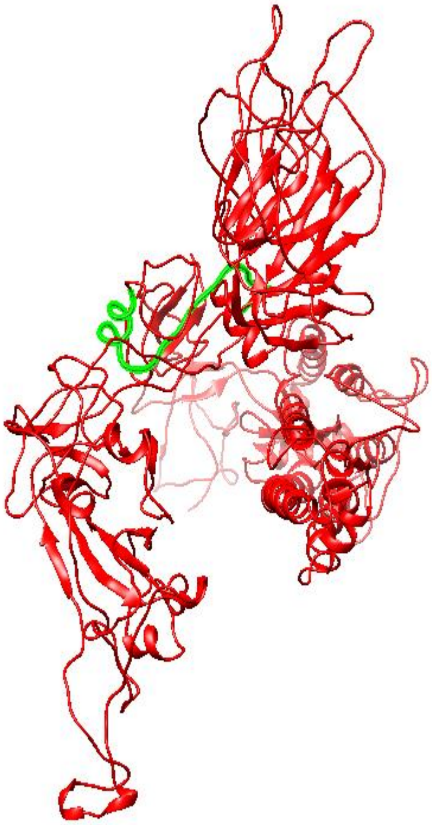


Figure 2: Protein Structure of Spike Glycoprotein of Wuhan (Wild) type strain of SARS-CoV-2.

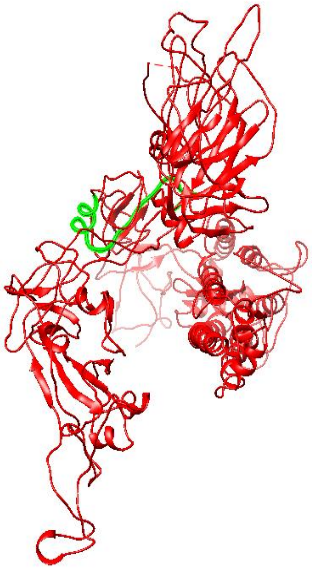


Figure 3: Protein structure of Spike Glycoprotein of Mutant D614G strain of SARS-CoV-2.

Figure 2 and 3 respectively shows parts of the spike glycoprotein of initial Wuhan sequence (Y009724390) and D614G strain obtained from UCSF Chimera from the same perspective. The highlighted blue region is the aspartic acid in the sequence of Wuhan strain (*GTNTSNQVAVLYQ**D**VNCTEVPVAI)* and in the case of D614G strain it is the Glycine amino acid (*GTNTSNQVAVLYQ**G**VNCTEVPVAI*). A difference in the structure of the two proteins can be observed within the circled region in the figures. This difference is probably caused due to the D to G mutation in the sequence.

We can write the reaction between the spike glycoprotein receptor of COVID-19 and host ACE2 as the following

$$a[spike\ glycoprotein] + b[ACE2] \rightarrow b[spike\ glycoprotein - ACE2\ complex] \quad (1)$$

That is, *a* moles of spike glycoprotein reacting with *b* moles of ACE2 form *b* moles of the complex, since the number of ACE2 receptors is a constant in the cell, that is, the number of ACE2 is not a function of number of viral spike protein. The number of complexes formed between host receptor and viral spike protein is totally dependent on the concentration of ACE2 in the cell which remains constant for each healthy individual.

Aspartic acid (D), located at a C-coil in the Wuhan strain, on mutation to Glycine (G) resulted in a ΔΔG value of -0.94 indicating a greater instability. Most mutations leading to novel functions are destabilizing [21]. A destabilizing mutation to Glycine changes the biochemical nature of the protein at that site by making Glycine more solvent accessible and making the mutation site more prone to novel chemical reactions [19]; finally, increasing its attachment potential with ACE2 and ultimately increasing its stability. We have also graphically plotted the distribution of this mutation versus date of collection which is shown in Figure 4.

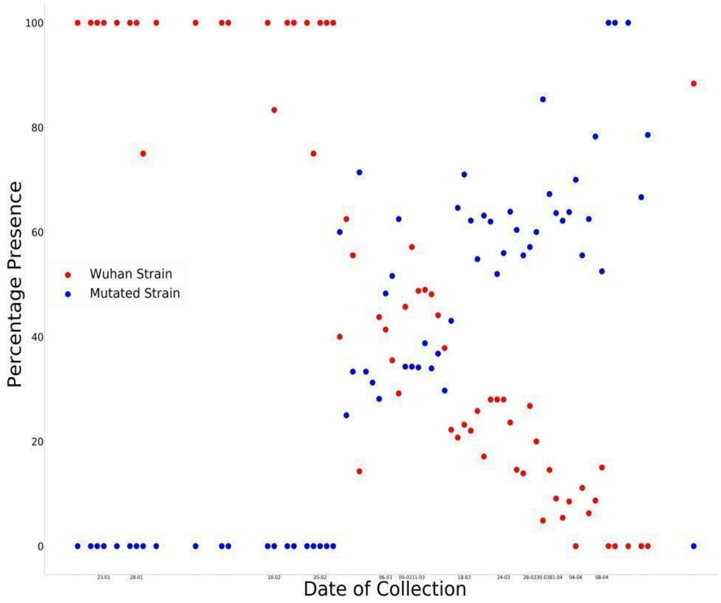


Figure 4: Graph of percentage of wild strains and mutant strains in collected sequences with respect to time.

In Figure 4, a point (x_i, y_i) represents that on the day x_i, if there were 100 collected sequences, then no. of sequences of that strain was y_i. The plot resembles the ideal graphical plot of concentration versus time of reactants and products. So, it can be mathematically written as,

$$rate\ of\ D614G\ strain\ abundance = -\frac{d(Wuhan\ strain\ abundance)}{dt}$$

The relative rate of formation of D614G strain is more prominent than any other mutation in the spike glycoprotein sequence. The more affinity towards the directed formation of the said mutation implies that this point mutation helps the virus to sustain itself. It also indicates that the D614G strain can attach more with ACE2 receptor and hence propagate, thus converging with our Bio-Chemical inference. Previously we saw that the mutation makes the new strain comparatively less stable; from these data we find out that the D614G strain is increasing in number, i.e. it has a higher infection rate; this is plausible if we consider that the region surrounding the mutation-site becomes a hotspot for chemical reaction favoring a stronger attachment with ACE2. This evidently explains the high surge in percentage of presence for this strain. The D614G strain is increasing in quantity in nature in due course of time, while the initial Wuhan strain is becoming obsolete.

So, all these data and values indicate a rightward shift in the chemical reaction (1). A more favorable formation of the complex simultaneously implies more infection and more spread of the D614G strain of COVID-19. The graph below shows the superimposed plot of presence of the studied mutated strains and reported infection in various states of USA. We can see that a surge in the number of reported sequences is proportional to the increase in infections.

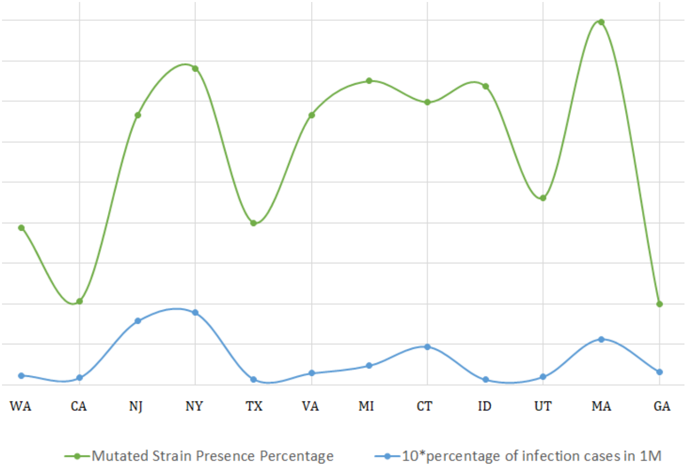


Figure 5: Superimposition of infection rate in states vs abundance ofD614G strain.

Also, if we plot the case fatality ratio vs the percentage presence of mutant D614G strain, again a very similar coherent pattern is found which again supports our theory. The graph is shown in Figure 6.

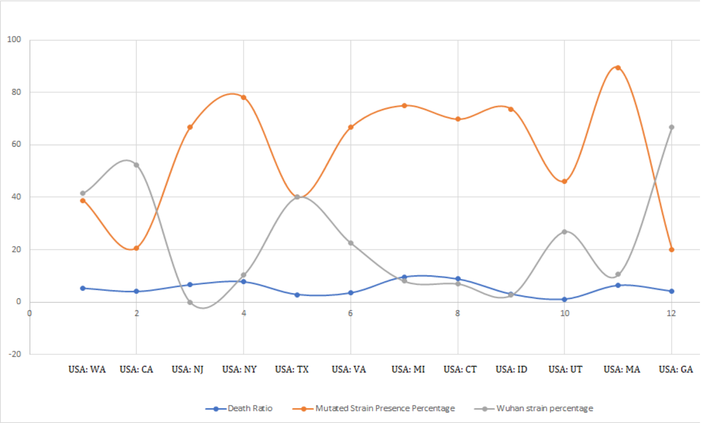


Figure 6: Superimposition of wild and mutant strain presence vs death ratio in various states.

In Figure 6, the orange curve represents the percentage presence of D614G strains and Gray curve shows percentage presence of Wuhan strain while the blue curve is the *Case Fatality Ratio*. *Case Fatality Ratio* is defined as the number of fatal cases of infection amongst total number of infected patients. We see the mutant strain abundance and case fatality ratio are exactly coherent and run with almost zero phase difference. That is, case fatality ratio amongst the infected people is high when the state has more number of D614G strains. The more is the number of D614G strains, the more is the chances of people being infected with this strain which eventually converges with higher death rate. This may be interpreted as its more pathogenic nature. Also, these mutated strains were first collected from various continents from March 9-11, 2020 onwards, which also satisfies the increasing (polynomial increase) counts from mid of March, 2020.

5. Conclusion

So, it is evident that a mutated strain D614G has been formed and is becoming quite dominant with respect to time replacing the initial Wuhan sequence. This new strain is probably more infectious due to its change in biochemical properties which increases its attachment potential with the ACE2 receptor. This mutation helps the virus to propagate, but is deadly for humans as it increases the rate of infection.

Author Contributions

Shreyans Chatterjee did the bioinformatic analysis of the sequences. Tathagata Dey and Smarajit Manna collected data and performed statistical analysis.

Declaration of Competing Interest

The authors declare no conflict of interest.

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