Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 9[12] November 2020 :160-164

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Mutational Analysis of outbreak of COVID-19 and its implication on drug development

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ABSTRACT
SARS-CoV-2 (Severe Acute Respiratory Syndrome) has emerged as a global heath pandemic. The causative agent of COVID-19 is a novel betacoronavirus. On 11 March 2020, the World Health Organization has declared worldwide emergency. This pandemic originated from an animal market in Wuhan city of Hubei province, China. Thousands of patients were admitted with the symptoms of unusual pneumonia, fever, cough, sore throat, breathlessness, fatigue in various hospitals, and they were found not responding to usual treatment. Currently, no effective drugs are available against this contagious disease. Since, SARS-Cov-2 is a member of plus strand RNA viruses; therefore, they have an inherent ability to produce high frequency of mutations. Due to occurrence of multiple mutations in its genome, viruses acquire antigenic shift to escape host immunity and develop drug resistance. Therefore, the aim of the present study is to identify the mutational sites that would help in the development of antiviral therapeutic agents.
Keywords *: SARS-CoV-2, Pandemic, COVID-19, Wuhan, pneumonia*

Received 11.08.2020 Revised 04.10.2020 Accepted 26.10.2020

INTRODUCTION

During the last two decades, humans have been suffering from several viral infectious diseasessuch as AIDS, Influenza, herpes, etc. Outbreak of SARS-CoV-2 pandemic originated from Wuhan City of Hubei Province, China, is an emerging viral infections that is rapidly spreading across the globe [1]. The SARS-Cov-2 spreads primarily through droplets of an infected person. This virus is infecting millions of people daily but unfortunately, no antiviral drug or vaccine has yet been developed for the treatment. As of today 14 August 2020, WHO has reported 20,439,814 confirmed positive cases of COVID-19, including 744,385 causalities from 216 countries.

Being an unique organism, a virus can alter their protein structure by changing amino acid sequences in such a way that they have still retained the infectious properties, evading the immune system of host. Viruses most often acquire drug resistance because of high rate of genetic variability shown by them [2]. SARS-CoV-2 is a single stranded positive sense viruse having 30 kb of genome size and accommodates as many as 14 ORFs that encode 29 proteins including four structural proteins such as S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins that are required to make complete virion particle. Additionally, viral genome encodes 16 non structural proteins (nsp) and 9 accessory proteins [3,4] including viral replication/transcription mediating protein; the RNA dependent RNA polymerase (RdRp) (also called as nsp12). In the present study, we have identified multiple mutations in the virus protein sequences isolated from India and comparing it with the Wuhan isolates (Wu et al. 2020). Our observations suggest that mutational analysis of SARS-CoV-2 might be considered as new approach to develop antiviral therapeutics.

METHODS

Sequence retrieval of SARS-CoV-2 from India

SARS-CoV-2 protein sequences collected from India in the month of June 2020 were downloaded from NCBI virus database. Only those sequences with full length protein of 7096 amino acid length were retrieved for further analysis. A total of 60 sequences were downloaded along with the Wuhan sequence as reference (Accession number YP_009724389) [4].

Multiple sequence Alignment and detection of mutation

The full length protein sequences downloaded from India and that of Wuhan were aligned using Clustal Omega software to detect mutation in the sequences, if any. Clustal Omega is a programme which performs multiple sequence alignment using HMM profile techniques and seeded guide trees. The sequence alignment file was used to record mutations in the Indian sequence with that of Wuhan virus sequence.

RESULTS

There were 60 sequences of SARS-CoV-2 full length protein from India, samples of which were collected in June 2020. Wuhan virus sequence was used as a reference as it was the first sequence submitted for SARS-CoV-2 virus when this COVID-19 disease first occurred in Wuhan province, China. The accession number with all details of the sequences retrieved is shown in table 1.

These sequences were aligned using Clustal Omega software to detect for similarities or difference in the sequences of India with that Wuhan. Clustal Omega performs pairwise alignments and alignment is viewed using Jalview. The aligned files were observed carefully to record the mutation in different sequences at different sites. A table was prepared of the mutated accessions with site of mutation and the difference in the wild type and mutated sequence. A total of 45 mutations were detected in 32 isolates of India. Out of these, 2 isolates showed triple mutation at the sites G112C, E658K, S1534I (QLH64939) and G327D, H2620R, C6742Y (QLI49779). Nine isolates showed double mutations at sites G519S and G3073C (QLF98210), G519S and G3073C (QLF98222), R4510C and W6152R (QLI52067), P1573A and T6297I (QLH64927), A1812D and R3993C (QLH93199), T6297I and D6900Y (QLF98246), K2732D and H5569R (QLH64777), W6152R and R4510C (QLI52055) and D6249Y and K6464N (QLI49731). Out of 60 isolates from India 31 isolates showed single point mutation other than those found with triple and double mutations.

Table 1: Details of SARS-CoV-2 sequences retrieved from India in June 2020

Accession	Authors	Geo_Location	Collection_Date
YP_009724389	Baranov,P.V., Henderson,C.M., et al.	China	2019-12
QLI49695	Trivedi,N., Khatri,H., et al.	India: Himatnagar	6/14/2020
QLI49707	Chauhan,N., Mullan,S., et al.	India: Choryasi	6/13/2020
QLI49719	Mullan,S., Gamit,A., et al.	India: Choryasi	6/13/2020
QLI49731	Gamit,A., Puvar,A., et al.	India: Surat	6/13/2020
QLI49743	Puvar,A., Raval,J., et al.	India: Surat	6/13/2020
QLI49755	Gandhi,M., Trivedi,P., et al.	India: Surat	6/13/2020
QLI49767	Trivedi,P., Pandya,M., et al.	India: Surat	6/13/2020
QLI49779	Patel,K., Pandya,L., et al.	India: Choryasi	6/13/2020
QLI49791	Pandya,L., Ansari,A., et al.	India: Choryasi	6/13/2020
QLI49803	Ansari,A., Trivedi,N., et al.	India: Surat	6/13/2020
QLI49815	Mullan,S., Gamit,A., et al.	India: Surat	6/13/2020
QLI52055	Trivedi,N., Chauhan,N.,et al.	India: Surat	6/13/2020
QLI52067	Chauhan,N., Mullan,S., et al.	India: Surat	6/13/2020
QLH64777	Pandya,L., Ansari,A., et al.	India: Vadodara	6/5/2020
QLH64789	Gandhi,M., Trivedi,P., et al.	India: Rajkot	6/12/2020
QLH64801	Pandya,M., Patel,N., et al.	India: Rajkot	6/12/2020
QLH64813	Puvar,A., Raval,J., et al.	India: Modasa	6/14/2020
QLH64825	Pandya,L., Ansari,A., et al.	India: Rajkot	6/12/2020
QLH64837	Ansari,A., Trivedi,N., et al.	India: Rajkot	6/12/2020
QLH64849	Raval,J., Patel,Z., et al.	India: Jetpur	6/12/2020
QLH64861	Patel,Z., Gandhi,M., et al.	India: Jetpur	6/12/2020
QLH64875	Gandhi,M., Trivedi,P.,et al.	India: Rajkot	6/12/2020
QLH64887	Trivedi,P., Pandya,M., et al.	India: Rajkot	6/12/2020
QLH64899	Kumar,D., Saiyed,Z., et al.	India: Surat	6/11/2020
QLH64915	Saiyed,Z., Patel,K., et al.	India: Surat	6/11/2020
QLH64927	Patel,K., Pandya,L., et al.	India: Surat	6/11/2020
QLH64939	Pandya,L., Ansari,A., et al.	India: Surat	6/11/2020

QLH90040	Ansari,A., Trivedi,N., et al.	India: Surat	6/12/2020
QLH90076	Trivedi,N., Chauhan,N., et al.	India: Surat	6/12/2020
QLH90088	Pandya,M., Patel,N., et al.	India: Choryasi	6/13/2020
QLH90101	Savaliya,N., Kumar,R., et al.	India: Surat	6/13/2020
QLH93117	Kumar,R., Kumar,D., et al.	India: Surat	6/13/2020
QLH93129	Kumar,D., Saiyed,Z., et al.	India: Surat	6/13/2020
QLH93185	Saiyed,Z., Patel,K., et al.	India: Surat	6/13/2020
QLH93199	Patel,N., Savaliya,N., et al.	India: Surat	6/13/2020
QLH93212	Raval,J., Patel,Z., et al.	India: Surat	6/13/2020
QLH93283	Patel,Z., Gandhi,M., et al.	India: Surat	6/13/2020
QLF97937	Patel,K., Pandya,L., et al.	India: Gandhinagar	6/11/2020
QLF97949	Ansari,A., Trivedi,N., et al.	India: Vadodara	6/8/2020
QLF97985	Khatri,H., Gandhi,M., et al.	India: Himatnagar	6/14/2020
QLF97997	Gandhi,M., Puvar,A.,et al.	India: Ahmedabad	6/14/2020
QLF98069	Raval,J., Patel,Z., et al.	India: Prantij	6/19/2020
QLF98081	Patel,Z., Gandhi,M.,et al.	India: Talod	6/19/2020
QLF98093	Trivedi,P., Pandya,M., et al.	India: Rajkot	6/12/2020
QLF98105	Patel,N., Savaliya,N., et al.	India: Rajkot	6/12/2020
QLF98117	Savaliya,N., Kumar,R.,et al.	India: Chuda	6/12/2020
QLF98129	Kumar,R., Kumar,D., et al.	India: Chuda	6/12/2020
QLF98141	Kumar,D., Saiyed,Z., et al.	India: Junagadh	6/12/2020
QLF98153	Saiyed,Z., Patel,K., et al.	India: Junagadh	6/12/2020
QLF98165	Patel,K., Pandya,L., et al.	India: Rajkot	6/12/2020
QLF98178	Trivedi,N., Dhruv,G., et al.	India: Rajkot	6/12/2020
QLF98198	Dhruv,G., Trivedi,A., et al.	India: Rajkot	6/12/2020
QLF98210	Trivedi,A., Puvar,A., et al.	India: Rajkot	6/12/2020
QLF98222	Puvar,A., Raval,J., et al.	India: Rajkot	6/12/2020
QLF98234	Pandya,M., Patel,N., et al.	India: Gondal	6/12/2020
QLF98246	Patel,N., Savaliya,N., et al.	India: Gondal	6/12/2020
QLF98258	Savaliya,N., Kumar,R.,et al.	India: Surat	6/11/2020
QLF98275	Gamit,A., Puvar,A., et al.	India: Olpad	6/13/2020
QLF98287	Puvar,A., Raval,J., et al.	India: Olpad	6/13/2020

Table 2: Mutational locations after multiple sequence alignment of SARS-CoV-2 full length protein sequences with position and sequence

S.No.	Accession No.	Position of mutation	Wild sequence	Mutated sequence
1.	QLH64939	112	G	C
		658	E	K
		1534	S	I
2.	QLI49779	327	G	D
		2620	H	R
		6742	C	Y
3.	QLF98210	519	G	S
		3073	G	C
4.	QLF98222	519	G	S
		3073	G	C
5.	QLI52067	4510	R	C
		6152	W	R
6.	QLH64927	1573	P	A
		6297	T	I
7.	QLH93199	1812	A	D
		3993	R	C
8.	QLF98246	6297	T	I
		6900	D	Y
9.	QLH64777	2732	K	D
		5569	H	R
10.	QLI52055	6152	W	R
		4510	R	C
11.	QLI49731	6249	D	Y
		6464	K	N
12.	QLF97997	309	P	A
13.	QLF97985	1940	S	Y

14.	QLF98081	2046	P	L
15.	QLI49791	2620	H	R
16.	QLI49707	2980	D	G
17.	QLF97949	3983	S	F
18.	QLI49719	5766	R	Q
19.	QLF97937	5928	N	H
20.	QLH93212	6199	A	S
21.	QLH93185	6297	T	I
22.	QLF98105	6297	T	I
23.	QLH90076	6297	T	I
24.	QLF98129	6297	T	I
25.	QLF98287	6297	T	I
26.	QLH64837	6297	T	I
27.	QLF49767	6297	T	I
28.	QLH64887	6297	T	I
29.	QLH64899	6297	T	I
30.	QLH83117	6297	T	I
31.	QLH93283	6297	T	I
32.	QLH64699	6297	T	I

DISCUSSION

Mutational Analysis of SARS-CoV-2

In the month of January 2020, the whole genome sequence (Wuhan-Hu-1) of SARS-CoV-2 deposited in the NCBI Gene bank. [4]. RNA viruses including SARS-CoV-2 exhibit high frequency of mutation which is prove to be advantageous for these viruses to adapt and survive in different climatic conditions (temperature, humidity and seasonal variations) which increases its transmissibility throughout the world [5,6]. Mutations also favours natural selection process and help in selection of those viral strain or subtypes which are more potent and fit [7] that may lead to drug resistance as well as immune evasions[8].

The results of the present study revealed that a total of 45 mutations have been observed in 32 India isolates. Out of these, 2 isolates showed triple mutation, nine showed double mutation and 31 isolates could able to show 31 mutations respectively. These mutational sites would be of great significance for predicting protein structures. Due to the effects of these multiple mutations, significant alterations in the protein structure of these viruses might have happened, consequently nullify the effect of antiviral therapeutic drugs. There is no specific antiviral drugs are available for the treatment of SARS-CoV-2 till date, however, various repurposing drugs like chloroquine, favipiravir, remdesivir and TMPRSS2 protease inhibitors have been found to be effective in combating COVID-19 infections. Personal protective strategies should be strictly followed to prevent this contagious viral infection till specific and safe antiviral agents have been made available.

CONCLUSION

Present findings suggest that evolbality of SARS-CoV-2 is associated with the onset of new mutations that spread at new locations of the viral genome and also provides great insight to develop effective control strategies against COVID-19 infections.

FINANCIAL SUPPORT

Nil

DECLARATION OF CONFLICTING INTERESTS

The authors declare that they have no conflict of interests among them.

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CITATION OF THIS ARTICLE

D K Jha, N Yashvardhini and V P Kesari .Mutational Analysis of outbreak of COVID-19 and its implication on drug development . Bull. Env. Pharmacol. Life Sci., Vol 9[12] November 2020 :160-164