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ORIGINAL ARTICLE

Correlation of COVID-19 Genome with Hypoxemia and Co-morbidities: Experimental Study

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ABSTRACT

Background: The Covid-19 pandemic has wreaked havoc throughout the world, with 150 million cases to date and over 3 million lives claimed worldwide.
Aim: To explore the hypoxemia in COVID-19 patients in relation with genomic mutation and co-morbidities.
Study Design: Experimental Study.
Methodology: A total of 16 COVID-19 positive patients admitted to Aziz Bhatti hospital were included in this study. COVID-19 was confirm through nasopharyngeal swab specimen diluted in normal saline subsequent RT-PCR was performed as per the standard operating procedure. Genome sequencing and interpretation of analysis was done through Illumina MiSeq.
Results: There was statistically significant difference ($P < 0.05$) in SaO2 in patients with N (Nucleocapisd) protein mutation compared with NSP13(Helicase) mutation.
Conclusion: It was concluded that mutation of N (Nucleocapisd) protein causes more pronounced hypoxia compared with Helicase mutation of COVID-19 genome.
Keywords: COVID-19, Hypoxia, Mutation and Genome.

INTRODUCTION

Corona virus or COVID-19 is a single stranded positive RNA virus with high infectivity and transmission rate. Currently COVID-19 spread has been declared as pandemic by WHO and is leading causes of apprehensions about the public health system[1]. As pandemic gets worsen it's becoming increasingly important to try and look for novel therapeutic agents and dig in deeper to pathophysiological causes of its sign and symptoms especially the hypoxia[2]. Some studies suggested that hypoxia due to COVID-19 caused by micro-thrombi in pulmonary vasculature. However later few studies pointed to the fact that extensive inflammation and cytokines storm leads to more pronounced hypoxia compared with micro-thrombi[1,2]. Respiratory damage due to COVID-19 can lead to variety of health issues including silent hypoxia. Silent hypoxia can be defined as extremely low levels of oxygen saturation in blood as anticipated, and patient doesn't show any signs of hypoxia or difficult breathing[3]. The exact pathophysiological cause of silent hypoxia is still unknown, but recent studies had indicated that it is possibly due to increased levels of carbon dioxide in blood of COVID-19 induced silent hypoxia patients. Whereas in ordinary hypoxic conditions a small change in levels of carbon dioxide couples with low levels of oxygen can lead to dyspnea an important symptom of hypoxia[3,1].

Hypoxia caused by COVID-19 can be different in intensity based on different mutants of viruses and might depend on various factors: virus having effect on brain or CNS therefore causing alteration in respiratory regulation centers, or virus effecting normal hemodynamics of blood vessels. Our study had tried to correlate these factors with hypoxia in COVID-19 patients[2,3]. There are seven species of corona virus known till date, out of which four are known to cause lower respiratory tract infection while other three only cause upper respiratory tract infection. SARS-CoV-2 just like the species of same family is a single stranded enveloped RNA virus with a genome size of 30kb[4]. The genomic study of SARS-CoV-2 reveled four sequences encoding for structural proteins and 16 sequence for non-structural proteins. Viral replication and pathogenesis require non-structural proteins, while structural proteins are for vaccine response and viral typing. The genomic study of SARS-CoV-2 showed many phylogenetic similarities to SARS-CoV specially receptor binding domain and S protein[5,6]. These phylogenetic genomic similarities proved the direct human to human transmission. Even though genomic studies showed it is only 75% identical to SARS-CoV but interestingly studies showed more resemblance to bat coronavirus particularly to SARSr-CoV RaTG13[7]. Recent studies coupled with phylogenetic mapping showed bats are reservoirs of coronaviruses. The comparative genomic studies showed sequences similarity of about (96.2 %) between SARS-CoV-2 and BatCoV[8]. The sequence analysis of worldwide SARS-CoV-2 showed mutations in receptors binding domain (RBD) which affects virus capability to use different hosts for replication[9]. The mutations in RBD were also found in pangolin SARSr-CoVs similar to SARS-CoV-2 that may have been used for species jump into humans. Many studies from worldwide reported genomic sequences of SARS-CoV-2 and their mutations which explains the spread of virus globally[8,9]. Initially studies found two variant of SARS-CoV-2 (L and S subtype) distinguished based on polymorphisms in two different nucleotides. These polymorphisms can be attributed to presence of RNA polymerases that can cause mutations and recombination more frequently as it was the case in Wuhan China where the predominant form as L subtype, the more aggressive and highly contagious compared with original S subtype.

As COVID-19 pandemic goes on the SARS-CoV-2 keeps on mutating in different strains. Therefore genomic sequencing is essential to explain COVID-19 related mortality and morbidity and to further explore the treatment options[8]. Genomic analysis of SARS-CoV-2 is essential to understand disease global dispersion, and epidemiology. It can also help to calculate the spread of virus load in a community where no proper testing facilities are present[8,9]. This is an ongoing process to further map the various genomic variants of SARS-CoV-2 to broaden our knowledge of its epidemiology and regional spread.

The objective of the study was to explore the difference in levels of SaO_2 of COVID-19 positive patients with and without COPD

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METHODOLOGY

Sample collection: Total of 16 patients with COVID-19 admitted to ICU ward of Aziz Bhatti hospital were included in this study. 4 were females and 12 were male. Out of which 8 were confirmed with COVID-19 with mutation in N (Nucleocapsid) proteins, and remaining 8 were Covid-19 positive with mutation in NSP13 (Helicase).

Sample processing: The sample was collected under strict SOP as advised by WHO and government of Pakistan. Samples were collected as nasopharyngeal swab specimen placed in 3 mL of normal saline. The diluted samples were then transferred to real time PCR unit and were analyzed and declared cases of COVID-19. Viral RNA extraction was done by manual extraction. And subsequent amplification was carried out. RNA quality was checked by using kit for quality control (Invitrogen USA). cDNA synthesis was carried out by iScript synthesis kit. Genome sequencing by random priming method was performed through illumine MiSeq[10].

Random sampling: Random sampling was performed on all subjects for measurement of SaO2 by using pulse oximeter (Masimo Radical -7 pulse oximeter). Any biased in oximeter reading was confirmed with arterial blood SaO2. All subjects were cases of COVID-19 as confirmed by real time PCR reports. Patients comorbidities, D dimers and LDH was also evaluated for any correlation with SaO2.

Statistical analysis: SPSS 25.0 statistical software was used to analyze the whole data. Student t test was used to evaluate the statistical significance of SaO2 and hypoxia among two groups of different mutations of COVID-19 genome.

RESULTS

Genome Analysis: A total of 16 COVID-19 positive patients samples were processed for genomic analysis. It was found that 8 samples were having mutation in N (Nucleocapsid) viral protein which increases virus stability and increase its virulence. Therefore patients with this particular mutations showed more signs of deterioration. Remaining 8 cases were detected having mutation in NSP13 (Helicase). All cases of this particular mutation were stable and recovered without deterioration.

Correlation of mutation with hypoxia:

There was statistically significant ($P < 0.05$) difference in SaO2 between patients with mutation in N (Nucleocapsid) protein and patients having mutation in NSP13 (Helicase) protein. SaO2 were found to be lowered in patients with N (Nucleocapsid) protein mutation and patients deteriorated further as time passes away as shown in table-1. Results were expressed as mean ± SD.

Correlation with co-morbidities: Total of 16 patients were included in this study out of which 14 were reported with comorbidities such as diabetes, Hypertension, Obesity, and cardiovascular diseases. We found out that correlation of mutation and hypoxia with comorbidities was in significant as shown in table-2. The exact cause of this in significance is not known. Results were expressed as mean ± SD.

Correlation with LDH and d-Dimers: Our study also explored any possible correlation of mutational hypoxia with LDH and d-Dimers. It was noted that all 16 patients were having mild to moderate increase in LDH and d-Dimers that could be due to other patho-physiological disturbances of COVID-19. Therefore it was concluded that LDH and d-Dimers were not having any significant impact on levels of SaO2 in patients with mutation of viral proteins as shown in table-2. Results were expressed as mean ± SD.

Table-1: Correlation of mutation with SaO_2

Subject	SaO2	*p*-value
N(Nucleocapsid mutation)	84.23±0.30	0.000*
NSP13(Helicase mutation)	92.4 ± 0.19	0.000*

*Statistically significant

Table-2: Correlation with LDH, D-Dimers and Co-morbidities

Subject	Age	LDH (U/L)	d-Dimers(ng/mL)	Comorbidities	SaO_2
N(Nucleocapsid mutation)	47.12±4.18	268.5±1.9	484.71 ±7.17	Hypertension, CVS diseases, obesity	84.23±0.30
NSP13(Helicase mutation)	46.2±4.31	269.1±1.6	485.01±7.21	Hypertension, CVS diseases, Obesity	92.4 ± 0.19

DISCUSSION

Coronavirus infection or COVID-19 was first identified in Wuhan, China had created havoc across the globe and became a nightmare for specialized healthcare system. As COVID-19 infections was increasing at alarming rate sequencing of viral genome become very important for vaccine and therapeutic purposes[10]. Since December 2019, many viral insolates had been sequenced for genomic studies and results were shared with international community through numerous studies. The first viral isolate to be studies at Wuhan was identified as L strain later on as diseases progressed into pandemic multiple strains were detected and named as S, V and G strains. Overall COVID-19 has 8 coding and 6 non-coding genes[11]. Earlier studies revealed that mutation is mostly confined to N (Nucelocapsid) proteins and Orf1ab regions. Later on it was observed Orf1ab and Orf3a genes caused evolutionary mutations and subsequent strains were then divided into S, V and G strains[12]. The first wave of COVID-19 infections were recorded in March to July 2021in Pakistan with a peak somewhere in mid-June where average 4000 to 6000 patients tested positive per day. Study of genomic analysis of COVID-19 helps in understanding the epidemiology and spread of virus. Globally genome sequencing of COVID-19 had helped us to understand variety of strains and their epidemiological variations and had contributed to creation of databases such as GISAID, and Nextstrain[13].

In Pakistan there is limited genomic studies on COVID-19 and data is limited to only few studies conducted recently. Initially the infection was associated with travelers coming from abroad, later on local transmission of COVID-19 was reported after 3 weeks. G and S strains were found to be main culprits behind the first wave of COVID-19 in Pakistan. Genomic studies of second wave of COVID-19 identified relatively new strains of COVID-19 such as B-1 and B-6[12,13]. Virus mutations depends on multiple factors including processes such as endonucleases activity and pressures such replicating or repairing. Our study and recent studies had identified the mutations at N (Nucleocapsid) proteins. This mutation was present at serine – arginine region close to N terminal domain. The nucleocapisd of COVID-19 contains an N protein of 419 amino acids and comprised of N terminal domain also called as RNA binding domain[13].

Compared with wild type of COVID-19 this mutations helps in better stabilization of virus and more molecular flexibility. This in turn causes virus to be more virulence and high infectivity. Viral propagation and RNA replication role was mainly played by Helicases[14]. Therefore many pharmacist believed helicases are ideal targets for antiviral therapy. Our study had found mutation in NSP13 compared with wild type. Therefore molecular flexibility decreases forming more interactions. This mutation also alters the immune interaction with virus and thus involved in changing the immunogenicity of COVID-19. 55% of this variant having low and high binding affinity for epitopes. However further investigations are needed to determine the binding affinity and changes in immune components that renders this particular strain to be less virulent and decrease infectivity[15].

Similarly there were many mutations that were reported in different countries in particular at Helicases but results showed a total different scenario of virulence and infectivity. Therefore authors suggest to further investigate this aspect and explore the genome sequence.

CONCLUSION

It was concluded that mutation of N (Nucleocapisd) protein causes more pronounced hypoxia compared with Helicase mutation of COVID-19 genome. However, co-morbidities correlation with hypoxemia was insignificant.

Limitations: Limitations included limited sample size, time frame, resources and financial constrains.

Conflict of interest: Nil

Author's contribution: SS&AAG: Conceptualized the study, analyzed the data, and formulated the initial draft, **SUA&MM:** Contributed to the proof reading, **MA&AF:** Collected data, **MZA,ZUS&TL:** Contributed to the proofreading the manuscript for intellectual content.

REFERENCES

1. Rahman, A., Tabassum, T., Araf, Y., Al Nahid, A., Ullah, M. and Hosen, M., 2021. Silent hypoxia in COVID-19: pathomechanism and possible management strategy. *Molecular Biology Reports*, 48(4), pp.3863-3869.
2. Kashani K. Hypoxia in COVID-19: Sign of Severity or Cause for Poor Outcomes. Mayo Clinic Proceedings. 2020;95(6):1094-1096.
3. Nitsure M, Sarangi B, Shankar G, Reddy V, Walimbe A, Sharma V. Mechanisms of Hypoxia in COVID-19 Patients: A Pathophysiologic Reflection. Indian Journal of Critical Care Medicine. 2020;24(10):967-970.
4. Lu G, Wang Q, Gao G. Bat-to-human: spike features determining 'host jump' of coronaviruses SARS-CoV, MERS-CoV, and beyond. Trends in Microbiology. 2015;23(8):468-478.
5. Kim D, Lee J, Yang J, Kim J, Kim V, Chang H. The Architecture of SARS-CoV-2 Transcriptome. Cell. 2020;181(4):914-921.e10.
6. Targeting Multiple SARS-Cov-2 And Human Proteins: In Silico Approach For COVID-19 Drug Repurposing. International Journal of Pharmaceutical Research. 2020;12(02).
7. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. New England Journal of Medicine. 2020;382(8):727-733.
8. Ceraolo C, Giorgi F. Genomic variance of the 2019-nCoV coronavirus. Journal of Medical Virology. 2020;92(5):522-528.
9. Belouzard S, Millet J, Licitra B, Whittaker G. Mechanisms of Coronavirus Cell Entry Mediated by the Viral Spike Protein. Viruses. 2012;4(6):1011-1033.
10. Khan M, Ali S, Khan A, Muhammad N, Khalil F, Ishfaq M et al. SARS-CoV-2 Genome from the Khyber Pakhtunkhwa Province of Pakistan. ACS Omega. 2021;6(10):6588-6599.
11. Lai C, Wang C, Wang Y, Hsueh S, Ko W, Hsueh P. Global epidemiology of coronavirus disease 2019 (COVID-19): disease incidence, daily cumulative index, mortality, and their association with country healthcare resources and economic status. International Journal of Antimicrobial Agents. 2020;55(4):105946.
12. Nasir N, Habib K, Iffat Khanum, Khan N, Muhammad Z, Mahmood S. Clinical characteristics and outcomes of COVID-19: Experience at a major tertiary care center in Pakistan. The Journal of Infection in Developing Countries. 2021;15(04):480-489.
13. Hadfield J, Megill C, Bell S, Huddleston J, Potter B, Callender C et al. Nextstrain: real-time tracking of pathogen evolution. Bioinformatics. 2018;34(23):4121-4123.
14. Umair M, Ikram A, Salman M, Khurshid A, Alam M, Badar N et al. Whole-genome sequencing of SARS-CoV-2 reveals the detection of G614 variant in Pakistan. PLOS ONE. 2021;16(3):e0248371.
15. Tamim S, Trovao N, Thielen P, Mehoke T, Merritt B, Ikram A et al. Genetic and evolutionary analysis of SARS-CoV-2 circulating in the region surrounding Islamabad, Pakistan. Infection, Genetics and Evolution. 2021;94:105003.