Iraqi Journal of Biotechnology, 2022, Vol. 21, No. 2, 39-45

Alteration of M and N Genes of Corona Virus 2 (SARS-CoV-2)

Amer M. Kradi[1], Abdul Hussein M. Al-Faisal[2], Ahmed M. Turki[3]

[1] Ministry of Health/ Anbar Hospital

[2] Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

[3] College of Science/ Anbar University

Received: 1/6/2022 Accepted: 21/8/2022 Published: December 20, 2022

Abstract: One hindered fifty swabs and whole blood were collected from mild, moderate and sever Covid-19 patients (as well as apparently Healthy control) who admitted to Anbar Hospital and Anbar Health department from the period of August, 2020 to March, 2021 to identify the strain of viruses using two regions from N and M genes and the role of *ACE*-2 SNP rs 228666 and gene expression of *ACE*-1 and *ACE*-2 genes in disease severity. The locations of the coronavirus M and N genes were screened by direct sequencing from 15 patients with mild Covid-19 disease and 15 patients with severed Covid-19 disease for each gene. The sequences of that detected in patients were directly compared with virus reference sequences (http:NCBI Reference Sequences: (MZ397170.1 , ON406016.1 and OV237507.1). The comparison between subjects, reference sequence and Sequence analysis of the M and N gene locations were summarized in table (2). It was concluded that there were five novel deletions of the gene N in two severed Covid-19 patients and two deletions of the M gene in one severed Covid-19 patient.

Keywords: Coronavirus, *ACE2*, M and N Genes.

Corresponding author: (Email: alkradi1975@gmail.com).

Introduction

Corona Virus Disease 2019 (COVID-19) is an infectious disease caused by SARS-CoV-2, an RNA virus with a crown-like appearance, and spreads rapidly all over the world. It transmits from human-to-human mainly *via* respiratory system (1). COVID-19 appears as asymptomatic disease or shows mild symptoms in the majority of patients (about 80%) (2,3). In clinical evaluation, fever, cough, dyspnea, myalgia, and fatigue are the most common symptoms among mildly symptomatic patients. Moreover, uncommon symptoms, including headache, sputum production, hemoptysis, and diarrhea, have been reported in SARS-CoV-2 infection (4,5). However, the remained proportion of the patients experience severe complications within a short time after infection, such as acute respiratory distress syndrome (ARDS), Disseminated Intravascular Coagulation (DIC), sepsis followed by organ failure, and death (6,7). Complete genome sequencing of viruses is an essential tool for the development of diagnostics and vaccines, studying virus

pathogenicity and virulence, tracking evolutionary paths and studying the genetic association between viruses and their hosts (8). The rapid spread of SARS-CoV-2 across countries calls to questions on whether its evolution is mutation driven. It is reported that genomes with new variations are emerging as the virus moves across diverse environmental conditions (9). Variations at the nucleotide level which is proposed to be one of the most significant measures of viral evolvement, also contributes to the adaptation of the virus in every condition it finds itself thus creating a balance between its genetic information and genome variability (10,11).

Materials and methods

Nasal swabs were taken from COVID-19 patients from the Central Health Laboratory of the Anbar Health Department, and the samples were divided into three groups (mild infection, moderate infection, and severe infection). After confirmation of infection with Covid-19, these swabs containing VTM were taken.

Total RNA extraction

Principle Nucleic acid extraction kit magnetic beads (full automated system).

Kit of cDNA synthesis (AE311-02)

Table (1): Thermal cycler steps of conditions cDNA Reverse Transcription.

Condition	Step 1	Step 2	Step 3	Step4
Temperature	25 ºC	42°C	85 ºC	4 ºC
Time	10 min	30 min	5 min	------

Covid-19 gene primers

All primers were supplied by Bioner company as a lyophilized product of different picomols concentrations and resuspension using deionized water to reach a final concentration for 10 picomols / µl of suspension, each starter solution is individually attended at a concentration of 10 Picomol, taking 10 Picomol of the storage solution for each initiator and a guest to 90 picomol of ionized distilled water, then thoroughly mixed and stored in the refrigerator until use. The solutions of trunk primers were kept at a temperature of 20°C. Mixing and homogenizing the storage solution was taken into account after being removed from the ice before use table (2).

Table (2): Primer sequences of M and N genes.

Primer	Primer sequence (5-3)	Product length (bp=base pairs)	Reference
M *Gene*	**F** TGTAGGCTTGATGTGGCTCA	346	This study
	R GCCAATCCTGTAGCGACTGT		
N *Gene*	**F** TGAAAGATCTCAGTCCAAGATGG	362	This study
	R CAAAGCAAGAGCAGCATCAC		

There are two PCR tubes for each sample, one tube for each gene, (M, N genes) . The detection of quantity based on fluorescent power of Sybr green. The reaction mix was composed of the component with their quantity as mentioned in Table (3) below.

Table (3): Components of quantitative real-time PCR used in M and N genes genes.

Component	Volume per 20µl Reaction
qPCR Master Mix, (SYBR)	10 µl
Forward Primer	1 µl
Reverse Primer	1 µl
cDNA Template	5 µl
Nuclease-Free Water	to 20 µl

PCR tubes were spined to remove the bubbles and to collect the liquid (1 minute at 2000 g), then the program for Real-Time PCR was set up with indicated thermocycling protocol as shown in Table (4).

Table (4): RT-PCR cycling program.

Loop's steps	Temperature °C	Time	Number of cycles
Initial denaturation	95	3min	1
denaturation	95	20 sec	40
annealing extension	55	1min	
Melt Curve	55-95		1

Results and discussion

All nucleotide sequences of the genes M and N of corona virus 2 (SARS-CoV-2) were downloaded from Gene bank http: //www.ncbi.nih.gov /nuccora/ MZ397170.1 and ON406016.1 and aligned together using blast program. The locations of the corona virus M and N genes were screened by direct sequencing from 15 patients with mild Covid-19 disease and 15 patients with severed Covid-19 disease for each gene. The sequences of that detected in patients were directly compared with virus reference sequences (http: NCBI Reference Sequences: (MZ397170.1, ON406016.1 and OV237507.1).

The comparison between subjects, reference sequence and Sequence analysis of the M and N gene locations were summarized in table (4-1). The results revealed that there were five novel deletions of the gene N in two severed Covid-19 patients and two deletions of the M gene in one severed Covid-19 patient. These results suggested that completely different translation occurred after these deletions (Table 5) and (Figure 1) which leading to change the amino acids sequencing which could be the severity of the disease due to them. Our results agreed with those of (12), who identified a high rate of mutations and the formation of new malevolent forms of the virus, which impacts the severity of the virus in the severity of infection. While other discovered a link between mutations and mortality, the effects of this mutation must be addressed in terms of virulence, pharmacological treatments, and how to combat these changes that occur in this virus (13, 14).

Our findings are also consistent with what that obtained by (15, 16) who discovered a rapid alterations and frequent mutations at the protein gene level in the N and M genes and their function in amplifying and spreading infection. Cumulative viral genetic alterations on the N and S genes had a substantial impact on the severity of infection in a study conducted in the

United States of America (17). R203K and G204R mutations in the N gene and M (C26750T) or ORF1b (M1499I or G17964T) mutations in the M gene are indicative of flexibility and the potential to improve the rate of transmission rather than altering virulence (18). While (19) discovered three alterations in the novel strain of N (K417 N, E484 K and N501Y) in South Africa, which led to 56000 thousand deaths as a result of the severity of the infection that resulted from this triple mutation in the virus.

The levels of mutation frequency at the N and M protein levels were detected by (20) by resulted in an increase in the virus's efficiency. A major mutation in deletions in strains harboring S:69-70 and S:144 of B.1.1.7 (501Y.V1), resulting in the mutation's rapid dissemination (21). Mutations of N E484K and E484K have also been shown to induce a considerable spread of infection to the virus (22). The increase in mutations also makes treating the virus and using vaccines that target this virus challenging, and these mutations are one of the most significant barriers to developing antiviral medications and vaccines (23).

In terms of influencing the secondary structure of amino acids by exchanging valine for alanine and proline in terms of mutation and leucine replacement, resulting in a loss of protein structural integrity (24, 25). Amino acids are also vital in the regulation and stability of enzymes. As a result, changes in amino acids can cause a change in the virus's functions in terms of severity or a loss of virulence characteristics. A single amino acid mutation in the virus for isolates from Vietnam and India, both strains showed two mutations to reduce protein stability (26). Also, (27) discovered significant alterations in amino acid residues by removing the amino acid identified in the Arizona mutation. The mutation rate is important in the formation of a new variation.

Table (5): Deletions detected in M and N genes of corona virus 2 (SARS-CoV-2) in severed Covid-19 patients.

Gene	No. of patient	Symptoms	Wild type code and amino acid	Deleted base	Site	Type of mutation	Gene Bank No.
N	1	Severed	CTT	--TT	c.28609 del C	Deletion-Frame shift	MZ397170.1
			GGT	--GT	c.28618 del G	Deletion-Frame shift	
			AGC	AG--	c.28851 del C	Deletion-Frame shift	
	1	Severed	AGT	A--T	c.28770 del G	Deletion-Frame shift	ON526062.1
			GCT	--CT	c.28845 del G	Deletion-Frame shift	
M	1	Severed	CTA	C--A	c.26898del T	Deletion-Frame shift	MZ397170.1
			TAC	T--C	c.27093 del A	Deletion-Frame shift	

Table (6): The effect of mutations on M and N genes of corona virus 2 (SARS-CoV-2).

Gene	Wild type code and amino acid	Deleted base	New code and amino acid	Site	GenBank No.
N	CTT L leucine	--TT	TTC UUC phe	c.28609 del C	MZ397170.1
	GGT G Glycine	--GT	GTG GUG val	c.28618 del G	
	AGC S serine	AG--	AGA Arg	c.28851 del C	
	AGT S serine	A--T	ATT AUU iie	c.28770 del G	ON526062.1
	GCT A alanine	--CT	CTC CUC leu	c.28845 del G	
M	CTA L lucine	C--A	CAG Leu	c.26898 del T	MZ397170.1
	TAC Y tyrosin	T-C	TCC UCC ser	c.27093 del A	

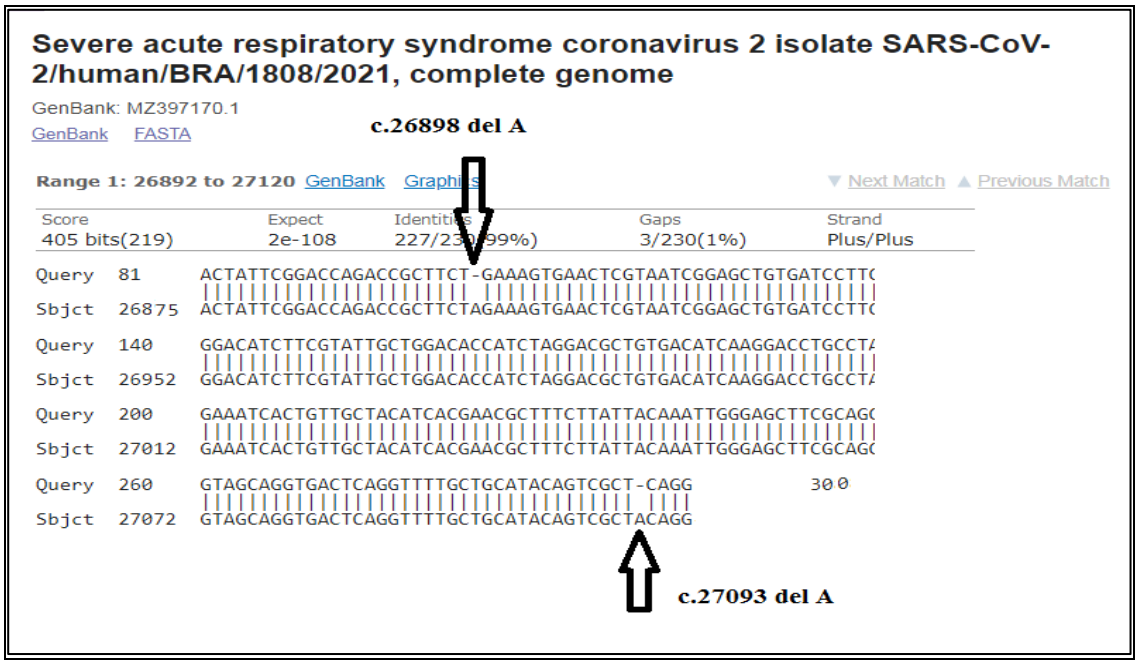

Figure (1): Pairwise with dots alignment of reference protein (MZ397170.1) and analyzed sequence protein (M) showing point (c.26898 del T, c.27093 del A) of protein variation.

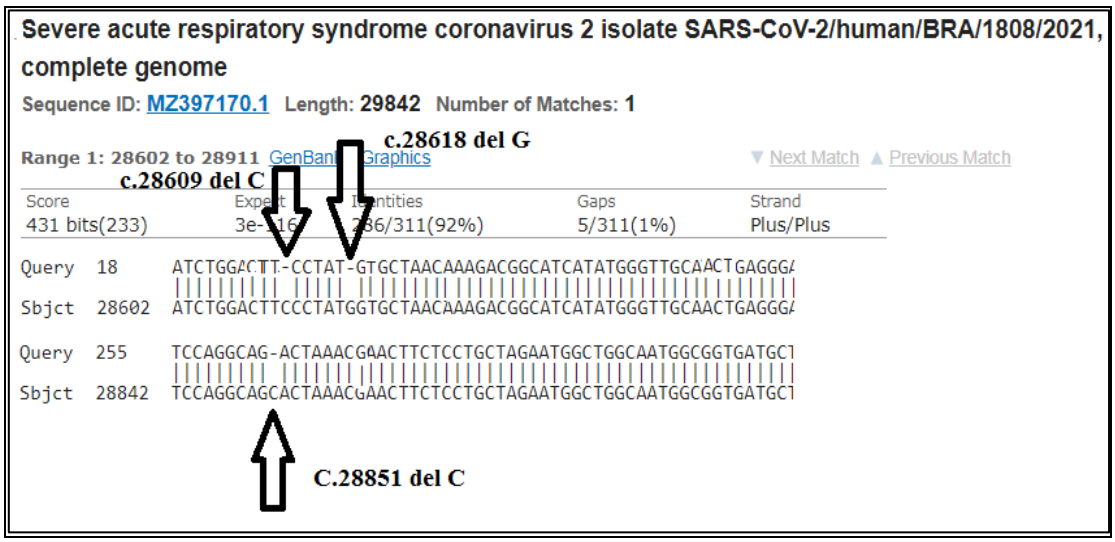

Figure (2): Pairwise with dots alignment of reference protein (MZ397170.1) and analyzed sequence protein (N) showing point (c.28609 del C, c.28609 del C, c.28851 del C) of protein variation.

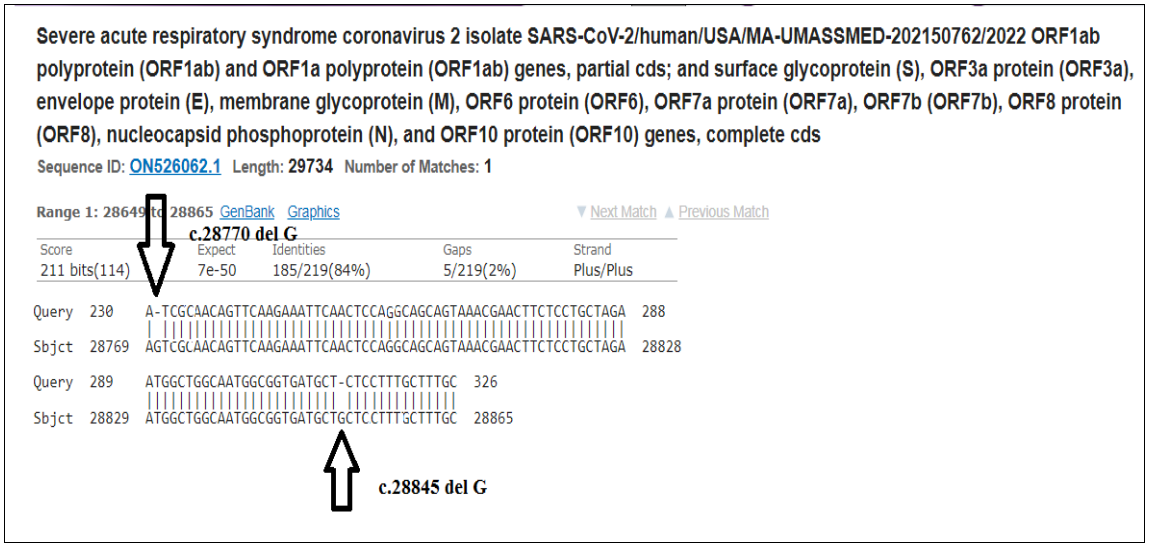

Figure (3): Pairwise with dots alignment of reference protein (ON526062.1) and analyzed sequence protein (N) showing point (c.28770 del G, c.28845 del G) of protein variation.

References

1. Tay, M. Z.; Poh, C. M.; Rénia, L.; MacAry, P. A. and Ng, L. (2020). The trinity of COVID-19: immunity, inflammation and intervention. Nature reviews. Immunology, 20(6): 363–374.
2. Huang, C.; Wang, Y.; Li, X.; Ren, L.; Zhao, J.; Hu, Y. *et al*. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet (London, England), 395(10223): 497–506.
3. Wu, Z. and McGoogan, J. M. (2020). Characteristics of and Important Lessons from the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases from the Chinese Center for Disease Control and Prevention. JAMA, 323(13): 1239–1242.
4. Vargas-Gandica, J.; Winter, D., Schnippe, R.; Rodriguez-Morales, A. G.;Mondragon, J.; Escalera-Antezana, J. P.and Paniz-Mondolfi, A. (2020). Ageusia and anosmia, a common sign of COVID-19? A case series from four countries. Journal of NeuroVirology, *26*(5), 785-789.
5. Yang, W.; Cao, Q.; Qin, L.; Wang, X.; Cheng, Z.; Pan, A. *et al*. (2020). Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19): A multi-center study in Wenzhou city, Zhejiang, China. The Journal of infection, 80(4): 388–393.
6. Campeau, L.; Thistlethwaite, F.; Yao, J. A.; Hobbs, A. J.; Shahriari, A., Vijh, R. and Zbar, A. (2022). Transmission dynamics of SARS-CoV-2 in British Columbia's largest school district during the second half of the 2020–2021 school years. Canadian Journal of Public Health, 113(5), 653-664.
7. Miesbach, W. and Makris, M. (2020). COVID-19: Coagulopathy, Risk of Thrombosis, and the Rationale for Anticoagulation. Clinical and applied thrombosis/hemostasis : official journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis, 26: 107-149.
8. Maurier, F.; Beury, D.; Fléchon, L.; Varré, J. S.; Touzet, H.; Goffard, A. *et al*. (2019). A complete protocol for whole-genome sequencing of virus from clinical samples: Application to coronavirus OC43. Virology, 531, 141–148.
9. Pachetti, M.; Marini, B.; Benedetti, F.; Giudici, F.; Mauro, E.; Storici, P. *et al*. (2020). Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. Journal of Translational Medicine, 18(1): 179.
10. Lauring, A. S. and Andino, R. (2010). Quasispecies theory and the behavior of RNA viruses. PLoS pathogens, 6(7):101-105.
11. Domingo E. (2000). Viruses at the edge of adaptation. Virology, 270(2): 251–253.
12. Wabalo, E. K.; Dubiwak, A. D.; Senbetu, M. W. and Gizaw, T. S. (2021). Effect of Genomic and Amino Acid Sequence Mutation on Virulence and Therapeutic Target of Severe Acute Respiratory Syndrome Coronavirus-2 (SARS COV-

2). Infection and Drug Resistance, 14: 2187–2192.
13. Toyoshima, Y.; Nemoto, K.; Matsumoto, S.; Nakamura, Y. and Kiyotani, K. (2020). SARS-CoV-2 genomic variations associated with mortality rate of COVID-19. Journal of Human Genetics, 65(12): 1075–1082.
14. Pachetti, M.; Marini, B.; Benedetti, F.; Giudici, F.; Mauro, E., Storici, P.and Ippodrino, R. (2020). Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. Journal of Translational Medicine, 18(1), 1-9.
15. Laha, S.; Chakraborty, J.; Das, S.; Manna, S. K.; Biswas, S. and Chatterjee, R. (2020). Characterizations of SARS-CoV-2 mutational profile, spike protein stability and viral transmission. Infection, genetics and evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Iinfectious Diseases, N 85: 104-445.
16. Biswas, S. K., and Mudi, S. R. (2020). Spike protein D614G and RdRp P323L: the SARS-CoV-2 mutations associated with severity of COVID-19. Genomics and informatics, 18(4).
17. Kaushal, N.; Gupta, Y.; Goyal, M.; Khaiboullina, S. F.; Baranwal, M. and Verma, S. C. (2020). Mutational Frequencies of SARS-CoV-2 Genome during the Beginning Months of the Outbreak in USA. Pathogens (Basel, Switzerland), 9(7): 565-568.
18. Kiryanov, S.; Levina, T. and Kirillov, My. (2020). Spread of variants with gene N hot spot mutations in russian SARS-CoV-2 isolates. Bulletin of Russian State Medical University, (4), 21-26.
19. Hirotsu, Y., & Omata, M. (2021). Discovery of a SARS-CoV-2 variant from the P. 1 lineage harboring K417T/E484K/N501Y mutations in Kofu, Japan. Journal of Infection, 82(6), 276-316.
20. Hirotsu, Y., and Omada, M. (2021). Discovery of a SARS-CoV-2 variant from the P. 1 lineage harboring K417T/E484K/N501Y mutations in Kofu, Japan. Journal of Infection, 82(6), 276-316.
21. Hodcroft, E. B.; Domman, D. B.; Snyder, D. J.; Oguntuyo, K. Y.; Van Diest, M.; Densmore, K. H. *et al*. (2021). Emergence in late 2020 of multiple lineages of SARS-CoV-2 Spike protein variants affecting amino acid position 677. medRxiv: the Preprint Server for Health Sciences, (2).12-21.
22. Toovey, O.; Harvey, K. N.; Bird, P. W. and Tang, J. (2021). Introduction of Brazilian SARS-CoV-2 484K.V2 related variants into the UK. The Journal of Infection, 82(5): e23–e24.
23. Wise J. (2021). Covid-19: The E484K mutation and the risks it poses. BMJ (Clinical research ed.), 372-359.
24. Volz, E.; Hill, V.; McCrone, J. T.; Price, A.; Jorgensen, D.; O'Toole, Á. *Et al.* (2021). Evaluating the Effects of SARS-CoV-2 Spike Mutation D614G on Transmissibility and Pathogenicity. Cell, 184(1): 64–75.
25. Chand, G. B.; Banerjee, A. and Azad, G. K. (2020). Identification of novel mutations in RNA-dependent RNA polymerases of SARS-CoV-2 and their implications on its protein structure. Peer Journal, 8: 92-94.
26. Rehman, S.; Mahmood, T.; Aziz, E. and Batool, R. (2020). Identification of novel mutations in SARS-COV-2 isolates from Turkey. Archives of Virology, 165(12): 2937–2944.
27. Holland, L. A.; Kaelin, E. A.; Maqsood, R.; Estifanos, B.; Wu, L. I.; Varsani, A. *et al*. (2020). An 81-Nucleotide Deletion in SARS-CoV-2 ORF7a Identified from Sentinel Surveillance in Arizona (January to March 2020). Journal of Virology, 94(14): 711-720.