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Assessment and Molecular Docking of SARS-CoV-2 NSP3 and NSP12 Mutants in Iranian Patients in Golestan Province

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Abstract

Background: Molecular analysis of SARS-CoV-2 genome is important to predict viral pathogenicity. In addition to transmission, replication is a key factor in pathogenicity of the virus. Notably, mutations in non-structural proteins (NSP3 and NSP12) can affect host immune response and viral replication. Therefore, this study was conducted to investigate different mutations of SARS-CoV-2 NSP3, and NSP12 during different waves of COVID-19 infection.

Methods: We recruited 57 NGS sequences including 8 NGS sequences from Golestan SARS-CoV-2 RNA samples, obtained as part of clinical testing in different referral centers of Iran. After obtaining sequences from the global initiative on sharing all influenza data (GISAID), and evaluating and processing data, all sequences were aligned to the Wuhan variant genome (NC_045512.2) using MEGA6. The HDOCK server was used for molecular docking.

Results: In NSP3, mutations in positions (nts 315, 545, 2666, 3264) were more frequent and among them mutation in positions including nt 545 (aa182) and nt 2666 (aa889) were associated with an increase in codon usage. In the term of NSP12, mutations in positions such as nts 406 (aa137), 965 (aa323), 1233, 1653, 1836, 2733 were more frequent. The molecular docking results showed more affinity in some variants of NSP3 and NSP12 as well.

Conclusion: This study has assessed mutation in SARS-CoV-2 Nsp3, and NSP12 which are viral protease, and viral polymerase (RdRp). The mutations reported in this study may help this virus to replicate faster and evade the pharmaceutical agents which target viral polymerase activity and be very effective in viral pathogenesis. In addition, this study highlights the importance of ongoing genomic variation studies to be performed on SARS-CoV-2 variants.

Key Words: COVID-19, Golestan, Iran, Molecular Docking, Mutation, NSP3, NSP12, SARS-CoV-2 Genome.

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1- INTRODUCTION

COVID-19, an infectious disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), appeared in Wuhan, China in late December 2019 and became a global health crisis (1). After two years, COVID-19 infection is still a public health priority in the world. The development of different types of vaccines was the only way to diminish the outbreak (2). Still, the effectiveness of different vaccines is under investigation, because of recurrent mutations in the genome of SARS-CoV-2 (3). Although the majority of the important mutations are within the spike gene, Non-structural proteins (NSPs) such as NSP3, and NSP12 are critical targets for designing new, universally developed drugs to control different effects of cytokine storm (4).

The SARS-CoV-2 RNA-dependent RNA polymerase (RdRp: NSP12) plays an important role in the viral RNA synthesis, and therefore, because of its conserved sequence, it is the main target for drug production (5). So far, a wide range of viral RdRp inhibitors, nucleoside analogues (NA), have been approved. However, due to the viral exonuclease (ExoN: nsp14), SARS-CoV-2 is able to remove embedded nucleoside analogues and thus resistance too many of these available antiviral drugs is a main challenging issue for NA drug design (6). Another viral nonstructural protein, NSP3, which is the viral protease, has the most mutations among SARS-CoV-2 non-structural proteins (7). Previous studies have suggested that mutations in the NSP3 gene may affect virus replication and pathogenesis. On the other hand, some studies have shown the association between the drug resistance of the virus and mutations in NSP3, and NSP12 (8).

Phylogenetic analysis has shown that SARS-CoV-2 is closely related to RTG13 bat coronaviruses with 96% identity, 79% identity with SARS-CoV and 50% identity with MERS-CoV); and they have a common ancestor (9). The mechanism of pathogenesis in this virus is not completely clear, but studies have shown that inflammation and production of various cytokines, especially inflammatory cytokines, are the main causes of pathogenesis (10). The polymerase enzyme errors cause many mutations during the replication cycle of this virus. These mutations in virus genes have clear effects on transmission, escape from the immune system and increase the virulence of the virus. Although, some mutations lead to the weakening of the virus (11).

The genome of SARS-CoV-2 is a non-segmented positive-sense single stranded RNA with a length of 27-32 kb. Its genome encodes several proteins, consisting of structural, non-structural, and accessory proteins (12). Viral structural proteins (S, M, N, E) have key roles in viral assembly and especially viral spike is important in the viral infection cycle and in the development of effective vaccines in the battle against global pandemic (13). Non-structural proteins (NSPs 1-16) have key roles in viral genome replication and evasion from the immune system; and in association with accessory proteins, they are important in viral survival, infectivity, cytokine storm, and transmission between the hosts (14).

Molecular analysis of SARS-CoV-2 genome is important to predict viral pathogenicity. In addition to transmission, replication is a key factor in pathogenicity of the virus, and mutations in NSP3 and NSP12 can affect evading the immune system and viral replication. So far, different variants of this virus have been registered in Iran. In the present study, viruses isolated from patients in Golestan, Iran, were sequenced using RNAseq method. This study was conducted to investigate different mutations of SARS-CoV-2 non-structural genes (NSP3,

NSP12) during different waves of COVID-19 infection and their effects on NSP3 and NSP12 functions.

2- MATERIALS AND METHODS

2.1 Specimen recruitment

We recruited 57 NGS sequences including 8 NGS sequences from Golestan SARS-CoV-2 RNA samples, obtained as part of clinical testing in different referral centers of Iran. After obtaining sequences from the global initiative on sharing all influenza data (GISAID), evaluating and processing data, all sequences were aligned to the Wuhan variant genome (NC_045512.2) using MEGA6. All patients were referred between March 2020 and September 2021, with clinical presentations of COVID-19 disease, confirmed by real-time RT-PCR assay at those corresponding local centers.

2.2 Lineage assignment

Besides 57 sequenced samples included in the current study, 7 SARS-CoV-2 reference variants from GISAID were subjected to lineage assessment. We used Pangolin v2.0.7 (17), to assess the lineages present in Iranian SARS-CoV-2 samples.

2.3 Phylogenetic analysis

MEGA6 was used to construct a phylogenetic tree and to estimate the most recent common ancestor (TMRCA) (17). Eventually, MEGA6 was used to estimate TMRCA and construct a Bayesian phylogenetic tree of 57 sequences, plus Wuhan and other variants as reference.

2.4 Molecular docking

After obtaining protein PDBs from I-TASSER and applying mutations to the main PDBs by Swiss model, the HDOCK server was utilized for protein-protein docking and predicting the interaction sites between viral NSP3 and the cellular G4S and also viral NSP12 and cellular RBM41. Additionally, using 3Drefine, energy minimization on protein structures was performed.

3- RESULTS

3-1. Identified NSP3 and 12 Mutations

In the present study we identified the variations in the polypeptide sequence of NSP3, and NSP12 and then we compared the Iranian SARS-CoV-2 sequences with the reference sequence reported from Wuhan, China, and other variants Alpha, Beta, Gamma, Lambda, and Delta. In NSP3, mutations in positions (nts 315, 545, 2666, 3264) were more frequent and among them mutation in positions including nt 545 (aa182) and nt 2666 (aa889) were associated with an increase in codon usage as listed in **Table 1**. In the term of NSP12, mutations in positions such as nts 406 (aa137), 965 (aa323), 1233, 1653, 1836, 2733 were more frequent as listed in **Table 2**. All mutations in NSP3 and NSP12 which were involved in amino acid alterations were included in the tables.

3-2. Molecular docking outcomes

The docking results for Wuhan NSP3 and G4S (ArylSulfatase B) protein showed a docking score of -266, while for Gorgan (Golestan province, Iran) 532, 2021, and 8781 the docking scores were -276, -266, and -285, respectively. Moreover, for Wuhan NSP12 and RBM41 protein, the outcomes showed a docking score of -322, while for Gorgan 75, 2021, and 532 the docking scores were -323, -286, and -309, respectively (**Table 3** and **Fig. 1**). These scores indicate that the affinity of NSP12 to RBM41 was lower for Gorganian variants (except for Gorgan75) compared to the Wuhan variant. In the case of NSP3, Gorganian variants had higher affinity to G4S compared to Wuhan virus. The LG score and Maxsub represent that the structure is of very good quality.

Table-1: Identified NSP3 Mutations

NSP3 mutation cite	Sequences	Nucleotides	Amino acid	Codon Usage
370	Sari 8054	C-T(CCA-TCA)	124:P-S	16.9-12.2
374	Sari 6970, 8054, Shiraz 5557, Tabriz 765, Tehran 2081, 4686	C-T(TCA-TTA)	125:S-L	12.2-7.7
545	Alpha, mu, Ahvaz 7387, 7337, 7522, Esf363, Shiraz 3125, 718, Alpha Ahvaz, Alpha ghom, AlphaSari, Alpha Tehran, Tehran551, Gorgan 532	C-T(ACT-ATT)	182:T-I	13.1-16
829	Gamma	C-A(CCT-ACT)	277:P-T	16.9-15.1
1088	Isfahan 363	T-C(CTC-CCC)	363:L-P	19.6-19.8
1106	Gamma	C-T(TCA-TTA)	369:S-L	12.2-7.7
1141	Tehran 686	G-A(GAA-AAA)	381:E-K	29-24.4
1226	Gorgan 2021	A-G(GAT-GGT)	409:D-G	21.8-10.8
1273	Sari 6971	G-A(GAA-AAA)	425:E-K	29-24.4
1280	Lambda	C-T(ACT-ATT)	427:T-I	12.1-16
1369	Alpha	A-G(ATC-GTC)	457:I-V	20.8-14.5
1459	Delta, delta Bushehr, delta Yazd	G-T(GCT-TCT)	487:A-S	18.4-15.2
1802	Isfahan 4567	C-T(GCT-GTT)	601:A-V	18.4-11
2030	Gamma	C-T(ACA-ATA)	607:T-I	15.1-7.5
2033	Tehran 676	C-T(CCT-CTT)	678:P-L	17.5-13.2
2378	Beta Hormozgan	C-T(TCA-TTA)	793:S-L	12.2-7.7
2508	Beta, Esfahan 4567, Betahormozgan	G-T(AAG-AAT)	836:K-N	31.9-19.1
2666	Alpha, Ahvaz 7387, 7337, 522, Es f363, Shiraz 3125, Alpha Ahvaz, Sari, Ghom, Tehran, Tehran 3551, Gorgan532	C-A(GCT-GAT)	889:A-D	18.4-21.8
2880	Tabriz 765	T-A(TTT-TTA)	960:F-L	17.6-7.7
2926	Gamma	A-C(AAA-CAA)	976:K-Q	24.4-12.3
3008	Tehran 5676	C-T(ACT-ATT)	1003:T-I	13.1-16
3156	Gorgan 8781	C-A(AAC-AAA)	1052:N-K	19.1-24.4
3563	Tabriz 765	C-T(ACC-ATC)	1188:T-I	18.9-20.4
3630	Sari 7247	G-T(AAG-AAT)	1210:K-N	31.9-17
3670	Tabriz 768	G-C(GAT-CAT)	1224:D-H	21.8-10.9
3680	Delta, delta yazd, delta Bushehr, Gorgon 8781	C-T(CCA-CUA)	1227:P-L	16.9-7.2
3769	Tehran 5661	A-G(ATA-GTA)	1256:I-V	7.5-7.1
3851	Gorgan 2021, Bushehr Delta, Shiraz 5558	C-T(TCT-TTT)	1284:S-F	15.2-17.6
3877	Isfahan 363	G-A(GAA-AAA)	1293:E-K	29-24.4
3914	Shiraz 5558	C-T(ACT-ATT)	1305:T-I	13.1-16
4040	Alphago	C-T(ACU-AUU)	1347:T-I	13.1-16
4091	Isfahan 364	C-T(ACU-AUU)	1364:T-I	13.1-16

4232	Alfa, mu, Ahvaz 7387, 7337, 522, Gorgan 532, Sari 8054, Esfahan 363, Shiraz 3125, 4718, Alpha Ahvaz, Sari, Ghom, Tehran, Tehran 3551	T-C(ATA-ACA)	1411:I-T	7.5-15.1
4367	Shiraz 5558	A-G(AAT-AGT)	1456:N-S	17-12.1
4397	Sari 7247	C-T(TCT-TTT)	1466:S-F	15.2-17.6
4402	Delta, Lambda, Bushehr delta, Yazd delta	C-T(CCT-TCT)	1468:P-S	17.5-15.2
4702	lambda	T-G(TTT-GTT)	1568:F-V	17.6-11
5020	Tehran 5676	A-G(AGT-GGT)	1674:S-G	12.1-10.8
5021	Shiraz 4719	G-T(AGT-ATT)	1674:S-I	12.1-16
5045	Sari 8353	T-C(ATC-ACC)	1682:I-T	20.8-18.9
5076	Shiraz 5557	G-T(AAG-AAT)	1692:K-N	31.9-17
5147	Sari 8563	C-T(TCA-TTA)	1716:S-L	12.2-7.7
5204	Sari 6970	C-T(GTA-GCA)	1735:A-V	7.1-15.8
5390	Tehran 4686	C-T(GCA-GTA)	1797:A-V	15.8-7.1
5791	Tabriz 767	G-T(GTT-TTT)	1931:V-F	11-17.6

Tabe-2: Identified NSP 12 Mutations

NSP12 Mutation cite	Sequences	Nucleotides	Amino acid	Codon Usage
181	Gorgan 2021	G-T(GAT-TAT)	62:D-Y	21.8-12.2
406	Sari 6012, 6848, 6970, 8054, 8563, Shiraz 5557, Tabriz 765, Alpha Tehran 5533, 2081, 4686, Alpha Sari	G-T(GGT-TGT)	137:G-C	10.8-10.6
551	Isfahan 2026	C-T(GCT-GTT)	185:A-V	18.4-11
965	Gorgan 2020 C and Gorgan 2021, 532 T	C-T(CCT-CTT)	323:P-L	17.5-13.2
1202	Ahvaz 3673	C-T(ACT-ATT)	402:T-I	13.1-16
1210	Ahvaz 3674	G-A(GUU-AUU)	405:V-I	11-16
1583	Sari 7247	C-T(GCA-GTA)	529:A-V	15.8-7.1
1891	Gamma	A-G(ATT-GTT)	632:I-V	16-11
2008	Delta, Bushehr delta, Yazd delta	G-A(GGT-AGT)	671:G-S	10.8-12.1
2451	Shiraz 4719	G-T(ATG-ATT)	818:M-I	22-16
2494	Sari 8353	G-A(GAU-AAU)	833:D-N	21.8-17

Table-3: Docking scores and other results of molecular docking

Sequences	Docking score	LG score	Maxsub
Nsp3-Gorgan 532	-276	6.26	0.53
Nsp3-Gorgan 2021	-266	6.26	0.53
Nsp3-Gorgan 8781	-285	6.26	0.53
Nsp3 Wuhan	-266	6.26	0.53
Nsp12- Gorgan 75	-323	5.2	0.47
Nsp12-Gorgan2021	-286	5.2	0.47
Nsp12- Gorgan 532	-309	5.2	0.47
NSp12wuhan	-322	5.3	0.48

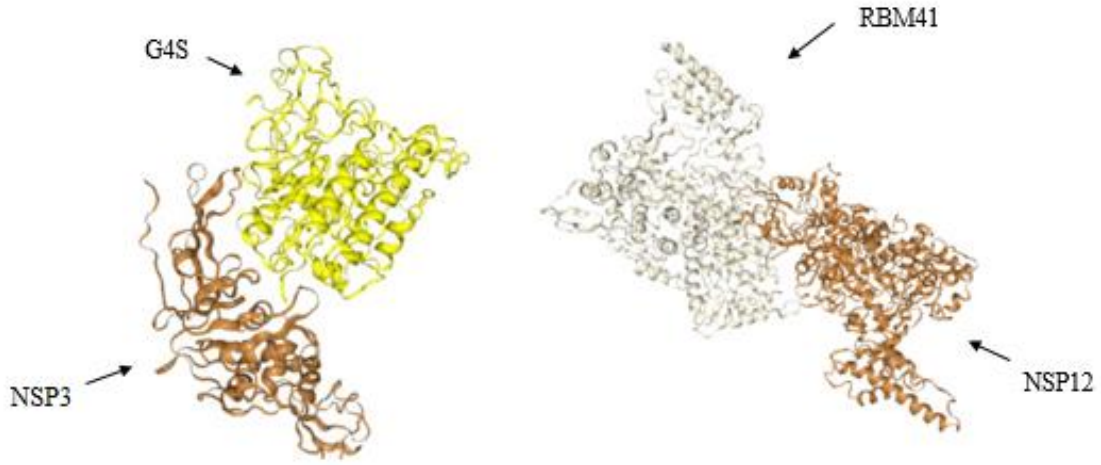

Fig. 1: The HDOCK results and the binding position of NSP3 and NSP12 with their host targets G4S and RBM41 which are involved in neutrophil migration and transcription, respectively. Energy minimization makes the structures stable in their interactions.

3-3. Phylogenetic analysis outcomes

The phylogenetic analysis result is presented in **Fig. 2**. The majority of Gorgan sequences was similar to Ahvaz, Tehran and Tabriz (in Iran) and also was related to Wuhan. The different Gorgan sequences were Gorgan 532 and Gorgan 2021. The Gorgan 532 sequence was closer to Ghom and Ahvaz and Gorgan 2021 was closer to Sari and Tabriz. These outcomes may be caused by passengers from these cities to Gorgan or vice versa. Also, of 8 Gorgan sequences, 6 were from B4 lineage, 1 was B.1.1.7 and 1 was B1.

4- DISCUSSION

The number of COVID-19 cases increases every day. Its related mortality rate varies among different regions, and this is attributed to several factors, including genetic basis, community age, and viral mutations (10, 15). Since universal vaccination is currently considered the ultimate key to disease elimination, the emergent genetic variants might undermine the efficacy of the desired therapeutic interventions-ongoing effort to develop effective medicines (16). Recently, location-dependent species such as Delta show the weak sides of vaccination (17). Because of the widespread availability of NGS tools and the online sharing of genome information, mutations, and variants are routinely tracked and a large number of full-length genome information from various countries affected by the pandemic are available. In this study we obtained 8 whole genome sequences from GISAID for Gorgan to compare with other sequences of Iran and Wuhan sequence.

Generally, mutations are more frequent among RNA viruses than DNA viruses, primarily because of a less competent proofreading system. Notably, the virus mutagenic capability is related to the non-structural proteins including viral protease and RdRp, which shows the significance of mutation in these proteins (18).

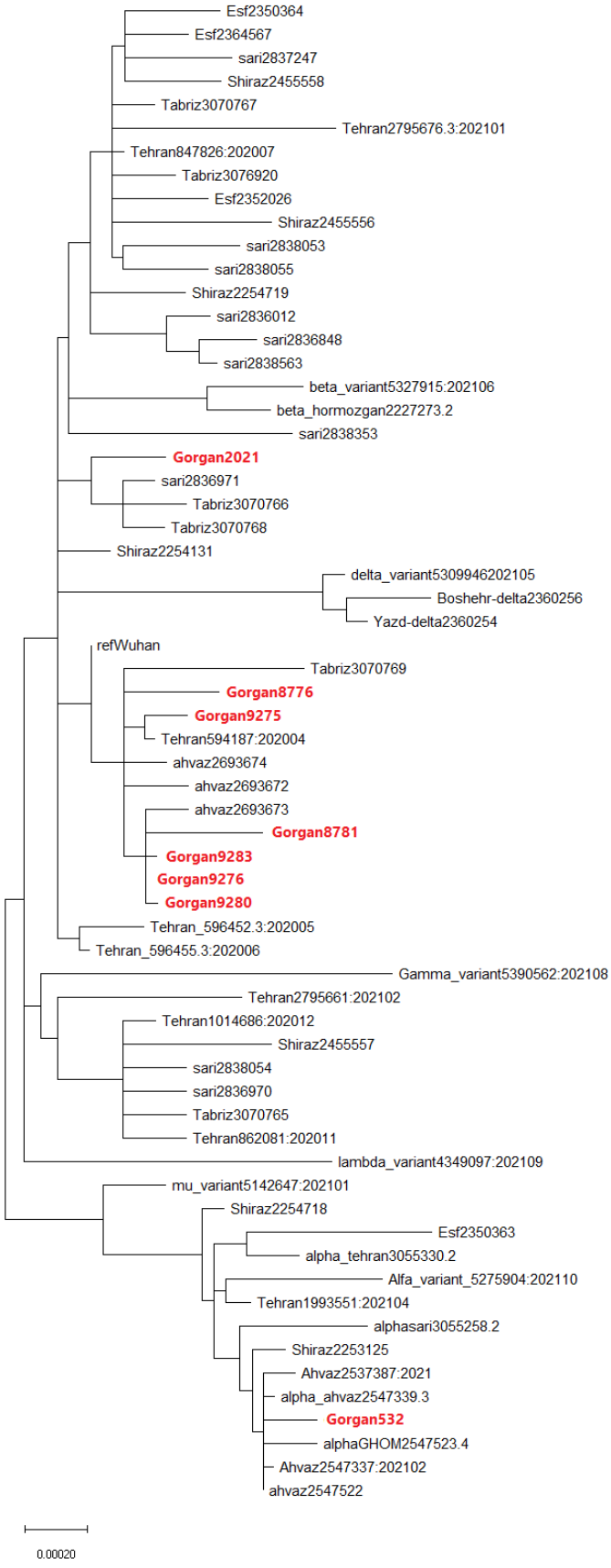

Fig. 2: Phylogenetic tree for SARS-CoV-2 variants

Interestingly, it was reported that viral strains with RdRp mutations exhibited a mutation rate three times higher than those without an RdRp mutation. Rapid generation of new SARS-CoV-2 variants is observed due to the individual infection of different ages and genetic compositions (19). In the current study we found several mutations in Gorgan and other cities in NSP3 and NSP12 sequences which can affect their interactions with host proteins.

Not only viral enzymes are key factors in the viral replication, but also are the target elements of many therapeutic agents used in the management of COVID-19. Redes Vir, Favipiravir, Sofosbuvir, Ribavirin, Galidesivir, Pimodivir, Baloxavir marboxil, and Beclabuvir are all RdRp inhibitors studied in this case and have revealed variable effectiveness (20). The key point in applying drugs is that they must be targeted to the highly conserved regions of the NSP3, and NSP12 to avoid drug resistance. So far, some mutations are known to be associated with drug resistance. For example, in a study by Mohammadi et al., it was revealed that mutations including F480L, D484Y, and V557L in RdRp protein can be associated with resistance to Remdesivir. Thus, it may be helpful to administer several different drugs (combination therapy) for different viral proteins in the infection management (21). In this study, neither of these mutations were observed in RdRp sequences.

In the present study and in the case of the RdRp, the P323L mutation corresponding to mutation 14408 is located far from the RNA binding domain, which lies within the finger-palm-thumb domains and, therefore, any direct effect of the mutation on the ability of the mutated RdRp to bind RNA should be excluded. However, the mutation could exert its effect allosterically. Since allosteric effects are impossible to be inferred by static crystal structures, simulation techniques can be employed to further elucidate the effect of the mutation on the dynamics of the interface domain and the NiNAR, fingers, and palm domains that are in direct contact with the domain where the mutation is found. Moreover, experiments on the stability of the P323L variant are needed, since changes in the stability of the mutated nsp12 could alter its cellular concentration, which would subsequently affect the replication rate of the virus within the host cell. In a study by Mutlo et al., P323L mutation of RdRp was found close to the interaction site with Nsp8 protein which can affect their polymerase activities (22). Alam et al. showed that P323L mutation of RdRp can alter attachment affinity to the NSP3 (Ubl1) (23). One of the most prevalent mutations in this study was the NSP3:C3037T synonymous mutation. In a study, Zeng et al. has reported significant epistatic connections of NSP3:C3037T with RdRp and spike mutations (24).

On the other hand, the NSP3 is thought to neutralize the host interferon (IFN) response, a key immune system antiviral signaling pathway (25). By interaction with host G4S, NSP3 can suppress SRS-A which is a leukotriene and subsequently regulates neutrophil migration and also can interfere with its IFN production and inflammation response. The NSP3 is capable of directly cleaving IRF3, leading to reduced IRF3 activation and IFN-I release (26). Therefore, docking results showed that mutations in NSP3 can cause an increased suppressive effect on IFN response or reduce its effect on IFN production. In a study by Tomaszewski et al. it is reported that although the synonymous mutation F106F in NSP3 is not detectable in its phenotype but it can be effective in escape from the host immune system (27). In our study, we found this mutation in several Gorgan sequences. Interestingly, molecular docking clearly showed the effects of each

variant in the term of interaction to the host cells. This study showed that mutations in positions (nts 315, 545, 2666, 3264) were more frequent in NSP3 and among them mutation in positions including nt 545 (aa182) and nt 2666 (aa889) were associated with an increase in codon usage which is associated with an increase in translation rate. Also, molecular docking scores indicate that affinity of NSP12 to RBM41 was lower for Gorgan variants (except for Gorgan75) compared to Wuhan variants. In the case of NSP3, Gorgan variants had higher affinities to G4S compared to Wuhan virus.

5- CONCLUSIONS

This study has assessed mutations in SARS-CoV-2 NSP3 and NSP12 which are viral proteases and RdRp. The mutations reported in this study may help this virus to replicate faster, evade immune responses and the pharmaceutical agents which target protease and RdRp activities. In addition, this study highlights the importance of ongoing genomic variation studies to be performed on the emerged SARS-CoV-2 variants for management, control and treatment of COVID-19.

6- ETHICS CONSIDERATIONS

All experimental protocols of the Ethics Committee of Golestan University of medical sciences were observed, and the committee approved this study (code: IR.GOUMS.REC.1398.386).

7- CONFLICT OF INTEREST

None.

8- FUNDING

None.

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