

Molecular study of the SARS-CoV-2 new variants in Iran

Morteza Motazakker¹, Morteza Bagheri^{1*}

Department of Genetics, Cellular and Molecular Research Center, Cellular and Molecular Medicine Research Institute, Urmia University of Medical Sciences, Urmia, Iran

Abstract

English:

Introduction: Various strains of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been identified worldwide such as lineages B.1.1.7 in the United Kingdom in December 2020, B.1.351, B.1.1.54, B.1.1.56 and C.1 in South Africa in 2020, and P.1 in Brazil during November 2020. We developed a multiplex real-time PCR assay for the molecular diagnosis of the SARS-CoV-2 new variants in Iran.

Materials and methods: In this cross-sectional study, 210 RNA samples were extracted from nasopharyngeal swabs using the GenePure Plus fully automatic Nucleic Acid Purification System model NPA-32+ (Hangzhou Bioer Technology Co., Ltd. (BIOER)) by magnetic beads method. The multiplexed real-time PCR assay was conducted to detect new strains of SARS-CoV-2.

Results: Among 210 patients diagnosed with SARS-CoV-2, 48 individuals were selected for the assessment of the presence or absence of the B.1.1.7 (United Kingdom), B.1.351 (South Africa), P.1 (Brazil), B.1.526 (New York State) and B.1.375 (United States) variants. The B.1.1.7 variant was detected in 58.34% of the samples analysed. However, the B.1.351 (South Africa), P.1 (Brazil), B.1.526 (New York State) and B.1.375 (United States) variants were not identified in our study.

Conclusion: SARS-CoV-2 B.1.1.7 UK Variant was the predominant strain from February 2020 to May 2021. In the case of other lineages, it is necessary to carry out the Sanger sequencing for clarification.

Keywords

B.1.1.7 • B.1.351 • P.1 • B.1.526 • B.1.375

Studiu molecular al noilor variante SARS-CoV-2 din Iran

Rezumat

Romanian:

Introducere: Diferite tulpini de coronavirus 2 cu sindrom respirator acut sever (SARS-CoV-2) au fost identificate la nivel mondial, cum ar fi liniile B.1.1.7 din Regatul Unit în decembrie 2020, B.1.351, B.1.1.54, B.1.1.56 și C.1 în Africa de Sud în 2020 noiembrie. Am dezvoltat un test PCR multiplex în timp real pentru diagnosticarea moleculară a noilor variante SARS-CoV-2 în Iran.

Material și metode: În acest studiu transversal, 210 probe de ARN au fost extrase din tampoane nazofaringiene utilizând GenePure Plus complet automat Nucleic Acid Purification System model NPA-32+ (Hangzhou Bioer Technology Co., Ltd. (BIOER)) prin metoda marelui magnetice. Testul PCR multiplexat în timp real a fost efectuat pentru a detecta noi tulpini de SARS-CoV-2.

Rezultate: Dintre 210 pacienți diagnosticați cu SARS-CoV-2, 48 de persoane au fost selectate pentru evaluarea prezentei sau absentei variantelor B.1.1.7 (Regatul Unit), B.1.351 (Africa de Sud), P.1 (Brazilia), B.1.526 (Statul New York) și B.1.375 (Statele Unite). Varianta B.1.1.7 a fost detectată în 58,34% din probele analizate. Cu toate acestea, variantele B.1.351 (Africa de Sud), P.1 (Brazilia), B.1.526 (Statul New York) și B.1.375 (Statele Unite) nu au fost identificate în studiul nostru.

Concluzie: SARS-CoV-2 B.1.1.7 Varianta UK a fost tulpina predominantă din februarie 2020 până în mai 2021. În cazul altor linii, este necesar să se efectueze secvențierea Sanger pentru clarificare.

Cuvinte-cheie

B.1.1.7 • B.1.351 • P.1 • B.1.526 • B.1.375

*Corresponding author: Morteza Bagheri

E-mail: mortezabagheri@umsu.ac.ir

Open Access. © 2024 Motazakker and Bagheri, published by Sciendo

 This work is licensed under the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 License.

Introduction

COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). Totally, 704,753,890 cases infected with this disease and 675,619,811 cases recovered and approximately 7,010,681 deaths stated, until 13 April 2024 in the world (Worldometer). In Iran, 7,627,186 cases infected with this disease and approximately 146,811 deaths stated, until 13 April 2024 (Worldometer). In US, total cases, deaths and recovered were 111,820,082, 1,219,487 and 109,814,428, respectively (Worldometer). Globally, numerous variants of SARS-CoV-2 have been found such as lineages B.1.1.7 in the United Kingdom in December 2020, B.1.351, B.1.1.54, B.1.1.56 and C.1 in South Africa in 2020 (2) and P.1 in Brazil during November 2020 (3). These lineages are defined regarding several mutations in the spike protein (4). The transmission rates of new variants depend to the mutation in the receptor-binding site of spike protein (4). It has been estimated the effects of upcoming mutations on SARS-CoV-2 evolution (5). The evolution of SARS-CoV-2 is driven by the frequency at which mutations are created and spread in the world (6). The SARS-CoV-2 genome consists of a positive-sense single-stranded RNA with approximately 30,000 nucleotides and its replication is done by RNA-dependent RNA polymerase (RdRP) and a related exoribonuclease (ExoN). The SARS-CoV-2 encodes 16 non-structural proteins of nsp1-16, structural proteins of spike (S), envelope (E), membrane (M) and nucleocapsid (N) and accessory proteins of ORF (3a, 6, 7a, 7b, 8 and 10) (7). The nature of SARS-CoV-2 transcription facilitates the creation of several kind of mutations (8). The structural proteins of S, M and N of the SARS-CoV-2 as surface glycoproteins are liable for receptor binding. D614G mutation in the SARS-CoV-2 S protein was found in more than 95% of 28,000 spike gene sequences. Presence of D614G mutation in the SARS-CoV-2 S protein gene leads to an aspartic acid (A) to glycine (G) shift within the amino acid position 614 of the protein of S1 subunit of the SARS-CoV-2 spikes. This mutation greatly influences the infectivity of the SARS-CoV-2 by stabilising the mutation (9). The SARS-CoV-2 virus that found in Britain and South Africa in December 2020, recognised as B.1.1.7 (Alpha). The B.1.1.7 coronavirus lineage is dissimilar to the D614G strain regarding numerous aspects. The B.1.1.7 coronavirus lineage spreads faster than other strains and increase the prognosis of the disease and the risk of mortality (10, 11). Several number of mutations were reported in the B.1.1.7 coronavirus strain, including 13 non-synonymous mutations, six synonymous mutations and four deletions (12). One of the most famous changes within the B.1.1.7 strain is N501Y that results in a conversion of asparagine (N) to tyrosine (Y) at amino-acid site 50,112 (13). This mutation is located in the spike glycoprotein's receptor-binding domain

(RBD) and reduces receptor-binding affinity on human ACE2 protein (13, 14). The symptoms of B.1.1.7 strain are as same as others, which include fever, continuous cough, chest pain, loss of taste and smell, headache, diarrhoea and skin rash (15). On 24 November 2021, lineage B.1.1.529 was defined as Omicron and harbours 15 mutations in the receptor-binding domain (RBD) of the spike protein. The Omicron variant was similar to the Alpha (B.1.1.7), Beta (B.1.351) and Gamma (P.1) variants, regarding some mutations (16). About 34 mutations were reported in Omicron compared to the normal S protein (16). The SARS-CoV-2 S protein contains S1 and S2 subunits, which are divided into two and five primary subdomains, respectively (17). The SARS-CoV-2 S protein facilitates coronavirus entrance into the host cells through binding to a receptor on the host cell surface via S1 subunit. Then viral and host membranes are fused by the S2 subunit (18). The mutations in SARS-CoV-2 B 1.1.7 lineage include ORF1ab [C3267T(T1001I), C5388A(A1708D), T6954C(I2230T), 11288-11296 deletion (SGF 3675-3677 deletion)], spike [21765-21770 deletion (HV 69-70 deletion), 21991-21993 deletion (Y144 deletion), A23063T(N501Y), C23271A(A570D), C23604A(P681H), C23709T(T716I), T24506G(S982A), G24914C(D1118H)], Orf8[C27972T(Q27stop), G28048T(R52I), A28111G(Y73C)] and N [28280 GAT->CTA(D3L), C28977T(S235F)] (19). Karbalaie Niya et al. (20) discovered a high frequency of the B 1.1.7 lineage of the SARS-CoV-2 variant after February 2020 in Iranian COVID-19 patients. Also, they reported some rare mutations of M177I, I100C, I100T, L452R, N679K, Q173H, Y145H, A222V and H49Y. In this context, evaluating the regional variants of SARS-CoV-2 is crucial. Various techniques have been employed globally for the diagnosis of SARS-CoV-2. The gold standard among these is Sanger sequencing of particular genes. The present study conducted a multiplexed TaqMan real-time PCR assay for the molecular diagnosis of the SARSCoV2 new variants in Iran.

Materials and methods

Sampling of SARS-CoV-2

Research Ethics Committees of Urmia University of Medical Sciences approved the project (IR.UMSU.REC.1399.384). In this cross-sectional study, we used nasopharyngeal swab or oropharyngeal swab and the lower respiratory tract samples obtained from SARS-CoV-2-positive patients from February 2020 to May 2021, across multiple geographical regions of West-Azerbaijan province of Iran. The present examination was conducted at the Cellular and Molecular Research Center, Cellular and Molecular Medicine Research Institute, Urmia University of Medical Sciences, Urmia, Iran. Totally, 186 samples were referred to the centre by health centres

and hospitals for the molecular detection of new SARS-CoV-2 variants. Total RNA was extracted from the clinical samples using the GenePure Plus fully automatic Nucleic Acid Purification System model NPA-32+ (Hangzhou Bioer Technology Co., Ltd. (BIOER)) by magnetic beads method via Sansure Biotech Inc. (Changsha, Hunan Province, P. R. China) (reference number: S1002E).

Primers/probes

Three sets of primers and probes including the SARS-CoV-2 nucleocapsid (N1-FAM) (forward primer: 5'-gac ccc aaa atc agc gaa at-3', reverse primer: 5'-tct ggt tac tgc cag ttg aat ctg-3', probe: fam-accccgcatctacgttggggacc-bhq1), spike 69–70 deletion (Spike-HEX) (forward primer: 5'-tca act cag gac ttg ttc tta cct-3', reverse primer: 5'-tgg tag gac agg gtt atc aaa c-3', probe: hex-ttccatgtatatactgtctctggga-bhq1) and ORF1a 3675–3766 deletion (ORF1a-Cy5) (forward primer: 5'-tgc ctg cta gtt ggg tga tg-3', reverse primer: 5'-tgc tgt cat aag gat tag taa cac t-3' and probe: cy5-gttgtctggttttaagctaaaagactgtg-bhq2) were used for detection of SARS-CoV-2 UK, South African and Brazilian variants (21).

Multiplexed TaqMan real time-PCR assay

The multiplexed TaqMan real time-PCR assay was performed as previously described by Vogels et al. (21). The multiplexed real-time PCR reaction was performed on a Mic qPCR cycler (Bio Molecular Systems) with the cycling conditions including a reverse transcription (RT) step at 50°C for 20 min, primary denaturation at 95°C for 15 min, followed by denaturation at 95°C sec for 12 s and annealing at 55°C for 30 s (42 cycles). Interpretation of the results was based Table 1.

Results

Among 210 patients diagnosed with SARS-CoV-2, 48 individuals were selected to assess the presence or absence of the B.1.1.7 (United Kingdom), B.1.351 (South Africa), P.1

(Brazil), B.1.526 (New York State) and B.1.375 (United States) variants. The B.1.1.7 variant was detected in 58.34% of the samples analysed. However, the B.1.351 (South Africa), P.1 (Brazil), B.1.526 (New York State) and B.1.375 (United States) variants were not identified in our study.

Discussion

The high expanding rate of new SARS-CoV-2 variants, such as SARS-CoV-2 UK, South African and Brazilian, has been considered a new challenge for clinicians and researchers. The amplified communication rates of these variants depends on new mutation in the spike protein–receptor binding site (22). In addition, ethnicity greatly influences the SARS-CoV-2 pandemic and susceptibility to SARS-CoV-2 infection (23). Current investigation as a single-centre study was to access the SARS-CoV-2 UK, South African and Brazilian variants in west Azerbaijan of Iran using multiplex TaqMan real-time PCR. The findings of this study revealed that the B.1.1.7 strain was the predominant variant. An assessment of the SARS-CoV-2 variants indicated a significant prevalence of the B.1.1.7 lineage of the SARS-CoV-2 variant among Iranian COVID-19 patients following February 2020 (20, 24). The results of the present study, in addition to other studies, show that the B.1.1.7 SARS-CoV-2 lineage was the most prevalent variant of COVID-19 in Iran from February 2020 to May 2021 in Iran (20, 24). The B.1.1.7 SARS-CoV-2 lineage was responsible for most of the new cases of COVID-19 in many European countries (25). The higher transmission rate of B.1.1.7 lineage depends on the mutation of a single asparagine (N) to tyrosine (Y) of the receptor-binding motif (RBF) at position 501 (N501Y). As indicated, the B.1.1.7 variant shows about an eight-fold increase in affinity for angiotensin-converting enzyme-2 (ACE-2) to initiate entrance into host cells in humans (13). The increased infectiousness of B.1.1.7 is associated with its ability to evade host immunity (13). The B.1.1.7 lineage is recognised via 18 amino acid modifications and three deletions within the spike protein (26). The genomic analyses indicated that the D614G mutation was observed more frequently among Iranian COVID-19 patients during the specified period. Given that SARS-CoV-2 strains can potentially escape neutralising antibodies and reducing their effectiveness, identifying these mutants would enable the swift implementation of control measures designed to reduce the effects of the disease. While the sensitivity of the RT-PCR test may be influenced by sampling errors, it has remained the cornerstone of COVID-19 diagnosis. The use of Sanger sequencing and real-time PCR techniques is very valuable for determining new species in different groups. In comparison to Sanger sequencing, the multiplexed RT-qPCR

Table 1. Interpretation of the results in this study.

Result	N1-FAM	ORF1a-Cy5	Spike-HEX
Potentially B.1.1.7 (United Kingdom)	CT ≤ 35	Undetected	Undetected
Potentially B.1.351 (South Africa), P.1 (Brazil), or B.1.526 (New York State)	CT ≤ 35	Undetected	CT ≤ 35
Potentially B.1.375 (United States)	CT ≤ 35	CT ≤ 35	Undetected
Other lineages	CT ≤ 35	CT ≤ 35	CT ≤ 35
Inconclusive	CT > 35 or undetected	Any value	Any value

'Other lineages' means required the other molecular diagnostic tests to clarify.

technique is a highly effective and cost-efficient approach for identifying variants and managing the spread of infection. The research emphasizes the significance of ongoing genomic surveillance to track the emergence of new variants and their effects on public health. In the present study, we had limitation regarding Sanger sequencing instrument for the examination of tested samples.

Conclusion

Our findings indicated that the B.1.1.7 strain was the most prevalent variant. Furthermore, the B.1.351 (South Africa), P.1 (Brazil), B.1.526 (New York State) and B.1.375 (United States) strains were not found in West Azerbaijan (Iran). In the case of other lineages, it is necessary to carry out the Sanger sequencing for clarification.

Ethics

Research Ethics Committees of Urmia University of Medical Sciences approved the project (IR.UMSU.REC.1399.384).

Acknowledgements

We would like to thank the esteemed Vice Chancellor for Research and technology of Urmia University of Medical Sciences.

Funding

This work supported by Urmia University of Medical Sciences (Grant No: 2816).

Competing interests

The authors declare that there are no conflicts of interest.

References

1. Bagheri M, Hallaj T, Ansari L, Pakdel FG. Detection of coronavirus disease 2019 (COVID-19) by TaqMan real-time PCR in Iran. *Maedica (Bucur)*. 2023;18(3): 442–446. doi:10.26574/maedica.2023.18.3.442
2. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Detection of a SARS-CoV-2 variant of concern in South Africa. *Nature*. 2021;592(7854): 438–443. doi:10.1038/s41586-021-03402-9
3. Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, et al. Genomics and epidemiology of the P.1 SARS-CoV-2 lineage in Manaus, Brazil. *Science*. 2021;372(6544): 815–821. doi:10.1126/science.abh2644
4. Verma S, Patil VM, Gupta MK. Mutation informatics: SARS-CoV-2 receptor-binding domain of the spike protein. *Drug Discovery Today*. 2022;27(10): 103312. doi:10.1016/j.drudis.2022.06.012
5. Otto SP, Day T, Arino J, Colijn C, Dushoff J, Li M, et al. The origins and potential future of SARS-CoV-2 variants of concern in the evolving COVID-19 pandemic. *Current Biology*. 2021;31(14): R918–R929. doi:10.1016/j.cub.2021.06.049
6. Markov PV, Ghafari M, Beer M, Lythgoe K, Simmonds P, Stilianakis NI, et al. The evolution of SARS-CoV-2. *Nature Reviews. Microbiology*. 2023;21(6): 361–379. doi:10.1038/s41579-023-00878-2
7. V'kovski P, Kratzel A, Steiner S, Stalder H, Thiel V. Coronavirus biology and replication: implications for SARS-CoV-2. *Nature Reviews. Microbiology*. 2021;19(3): 155–170. doi:10.1038/s41579-020-00468-6
8. Peacock TP, Penrice-Randal R, Hiscox JA, Barclay WS. SARS-CoV-2 one year on: evidence for ongoing viral adaptation. *The Journal of General Virology*. 2021;102(4): 001584. doi:10.1099/jgv.0.001584
9. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, et al. Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 virus. *Cell*. 2020;182(4): 812–827.e19. doi:10.1016/j.cell.2020.06.043
10. Challen R, Brooks-Pollock E, Read JM, Dyson L, Tsaneva-Atanasova K, Danon L. Risk of mortality in patients infected with SARS-CoV-2 variant of concern 202012/1: matched cohort study. *BMJ*. 2021;372: n579. doi:10.1136/bmj.n579
11. Davies NG, Abbott S, Barnard RC, Jarvis CI, Kucharski AJ, Munday JD, et al. Estimated transmissibility and impact of SARS-CoV-2 lineage B. 1.1. 7 in England. *Science*. 2021;372(6538): eabg3055. doi:10.1126/science.abg3055
12. Singh J, Samal J, Kumar V, Sharma J, Agrawal U, Ehtesham NZ, et al. Structure-Function Analyses of New SARS-CoV-2 Variants B.1.1.7, B.1.351 and B.1.1.28.1: Clinical, Diagnostic, Therapeutic and Public Health Implications. *Viruses*. 2021;13(3): 439. doi: 10.3390/v13030439.
13. Yang TJ, Yu PY, Chang YC, Liang KH, Tso HC, Ho MR, et al. Effect of SARS-CoV-2 B.1.1.7 mutations on spike protein structure and function. *Nature Structural & Molecular Biology*. 2021;28(9): 731–739. doi:10.1038/s41594-021-00652-z
14. Yi C, Sun X, Ye J, Ding L, Liu M, Yang Z, et al. Key residues of the receptor binding motif in the spike protein of SARS-CoV-2 that interact with ACE2 and neutralizing antibodies. *Cellular & Molecular Immunology*. 2020;17(6): 621–630. doi:10.1038/s41423-020-0458-z

15. Sumner MW, Xie J, Zemek R, Winston K, Freire G, Burstein B, et al. Comparison of symptoms associated with SARS-CoV-2 variants among children in Canada. *JAMA Network Open*. 2023;6(3): e232328. doi:10.1001/jamanetworkopen.2023.2328
16. He X, Hong W, Pan X, Lu G, Wei X. SARS-CoV-2 Omicron variant: characteristics and prevention. *MedComm*. 2021;2(4): 838–845. doi:10.1002/mco2.110
17. Xia X. Domains and functions of spike protein in sars-cov-2 in the context of vaccine design. *Viruses*. 2021;13(1): 109. doi:10.3390/v13010109
18. Li F. Structure, function, and evolution of coronavirus spike proteins. *Annual Review of Virology*. 2016;3(1): 237–261. doi:10.1146/annurev-virology-110615-042301
19. Naveca F, Nascimento V, Souza V, Corado A, Nascimento F, Silva G, et al. Phylogenetic relationship of SARS-CoV-2 sequences from Amazonas with emerging Brazilian variants harboring mutations E484K and N501Y in the Spike protein, 2021.
20. Karbalaie Niya M H, Tabibzadeh A, Yazdani S, Hormati A, Keshavarz M, Mousaei Ghasroldasht M, et al. Molecular Study of SARS-CoV-2 Variants in Iranian Patients: a Multi-Center Study. *Jundishapur Journal of Microbiology*. 2023; 16(7): e138090. doi:10.5812/jjm-138090
21. Vogels CBF, Breban MI, Ott IM, Alpert T, Petrone ME, Watkins AE, et al. Multiplex qPCR discriminates variants of concern to enhance global surveillance of SARS-CoV-2. *Plos Biology*. 2021;19(5): e3001236. doi:10.1371/journal.pbio.3001236
22. Gu H, Chen Q, Yang G, He L, Fan H, Deng YQ, et al. Adaptation of SARS-CoV-2 in BALB/c mice for testing vaccine efficacy. *Science*. 2020;369(6511): 1603–1607. doi:10.1126/science.abc4730
23. Vahidy FS, Nicolas JC, Meeks JR, Khan O, Pan A, Jones SL, et al. Racial and ethnic disparities in SARS-CoV-2 pandemic: analysis of a COVID-19 observational registry for a diverse US metropolitan population. *BMJ Open*. 2020;10(8): e039849. doi:10.1136/bmjopen-2020-039849
24. Rezaei Z, Asaei S, Sepehrpour S, Jamalidoust M, Namayandeh M, Norouzi F, et al. SARS-CoV-2 variants circulating in the Fars province, southern Iran, December 2020-March 2021: a cross-sectional study. *Health Science Reports*. 2023;6(6): e1373. doi:10.1002/hsr2.1373
25. Michaelsen TY, Bennedbæk M, Christiansen LE, Jørgensen MSF, Møller CH, Sørensen EA, et al. Introduction and transmission of SARS-CoV-2 lineage B.1.1.7, alpha variant, in Denmark. *Genome Medicine*. 2022;14(1): 47. doi:10.1186/s13073-022-01045-7
26. Zárate S, Taboada B, Muñoz-Medina JE, Iša P, Sanchez-Flores A, Boukadida C, et al. The alpha variant (B.1.1.7) of SARS-CoV-2 failed to become dominant in Mexico. *Microbiology Spectrum*. 2022;10(2): e0224021. doi:10.1128/spectrum.02240-21