History of Medicine, 2023, 9(1): 1553–1557
DOI: 10.17720/2409-5834.v9.1.2023.191

Diagnosis and Genome Sequence with Aphylogenetic tree of SARS-COV-2isolated from a COVID-19 patients in all Iraqi Hospitals

Mohammed issa mohsin[1], Hani Mousa Badr[2], Nada Noori Younis[3], Manal Abid Ahmed[4], Durad Qassim Jassim[5], Elham A. Kalaf[6], Nawras A. Tawfeeq[7], Yousif Tahssen Yousif[8], Farah Ahmed Mohammed[9]

[1, 2,4,5,6,7,8,9]Ministry of Health- CPHL/Iraq
[3]Ministry of Health- CPHL/Iraq
E.mail: nada_650@yahoo.com

***Correspondence author:** Nada Noori Younis (nada_650@yahoo.com)

Received: 20 January 2023 **Accepted:** 15 April 2023

Citation: Mohsin M I, Badr H M Younis N N, Ahmed M A, Jassim D Q Kalaf E.A, Tawfeeq N A, Yousif Y T, Mohammed F A (2023) Diagnosis and Genome Sequence with Aphylogenetic tree of SARS-COV-2isolated from a COVID-19 patients in all Iraqi Hospitals. History of Medicine 9(1): 1553−1557. https://doi.org/10.17720/2409-5834.v9.1.2023.191

Abstract

Coronavirus is a new pandemic disease which has emerged in Wuhan, China, and then spreads around the world. The cases number of the COVID-19, that have been daily reported in Iraq, has risen slowly. However, no confirmed study has been undertaken to evaluate the situation of the COVID-19 concerning the confirmed cases, deaths cases, and recovered. Nasopharyngeal and throat Swabs fluid were collected from(500) patients who has been diagnosed as positive for COVID-19 from all hospitals of the governorates of Iraq, males (250) and females(250) ,all specimens wereenrolled from The Central Public Health Laboratory in Baghdad, Iraqi Ministry of Health, in period from April 2021 to April 2022. for pupose of diagnosed using Real-Time PCR with ahigh significant percentage for all governorates 9.029 and the number of males 195, and by 57.86%and the number of females was 142 with a ratio of 42.14. Then confirmed the type of infection by doing sequences and find mutations in the Illumina device to (337) the number of injuries was distributed among 289 types of Omicron and 48 Delta with a significant percentage 8.335.A phylogenetic analysis was done using MolecularEvolutionary Genetics Analysis version 7.0 (MEGA 7.0) to examine similarities and differences between theIraq genome sequence and 20 SARS-CoV-2 genomesequence downloaded from GenBank and the GISAIDdatabaseto compared with neighboring countries.

Keywords

SARS-COV-2. Genome Sequence. COVID-19patients. SARS-COV-2isolated. Phylogenetic tree.

Coronavirus is one of the major viruses which primarily affecting the respiratory system in human (1). However, Coronaviruses have been also diagnosed in animals and can cause a range of severe diseases such as gastroenteritis and pneumonia (2,3). Previous coronavirus outbreaks have been reported, including severe acute respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome (MERS-CoV), which is described as a significant public health threat (4). In 2002, coronavirus infections (SARS-CoVs) spread in Guangdong, south China, causing high fever, breathlessness and pneumonia, and rapidly spread to various regions around the world.

The infection has spread in 26 countries, resulting about 8096 cases and 774 deaths (5,6).

Whereas MERS-CoV was first detected in Saudi Arabia in 2012. The disease has mild respiratory symptoms that can lead to acute respiratory syndrome and death. 2494 cases were infected by the virus, of which 858 died in more than 25 countries (7−8). In December 2019, Atypical

unkown pneumonia was first recorded in Wuhan city, Hubei province. Patients have showed high fever (more than 38 C□), dry cough, malaise, and breath difficulties. The infection has been linked to the seafood market of Wuhan, China and named COVID-19 (9−10). It spread rapidly to other Far East Asian nations, then to the Middle East and Europe. In severe cases the disease causes pneumonia, septic shock, metabolic acidosis and bleeding (11). The incubation period has been estimated from 5 - 14 days and may vary from patient to patient according to age and infection history (12).

Several studies have revealed that COVID-19 can be transmitted between humans via nasal droplets and direct contact in both symptomatic and asymptomatic patients (13). No vaccine or effective medication currently available to prevent or cure COVID-19 infections; however,some preventive health measures can help to resolve primary complications in patients (14). On 16 March 2020, the disease affected more than 150 countries and territories around the world.Over the past few months there has been a significant increase in COVID-19 cases. In Iraq, the first confirmed case of COVID-19 has been reported in Najaf province for the Iranian student came from Iran on 24February 2020, followed by 4 cases from one family in Kirkuk province on 25 February, they have also a travel history to Iran. An additional case was recorded on 27February in Baghdad, for a patient who recently visited Iran. (15). 74 confirmed cases and 8fatalities have been reported across Iraq as of 12 March 2020 (16). The confirmed cases jumped to 1415 on 16 April 2020, with 78 fatalities were recorded (17). By 24 May 2020, the confirmed cases of COVID-19 reached 4469 and reported 160 deaths, while 2738 patients recovered from the infection (18). Here, we aim to describe a comprehensive, epidemiological study of all casesdiagnosed in Iraq by 24 May 2020. We hope our study will alert the community to the risk ofthis novel coronavirus, in order to prevent a second wave of the virus infections.

Materials and Methods

Patients´ Samples

Nasopharyngeal and throat swab fluid (5-10 ml) samples were collected from (500) patients who has been diagnosed as positive for COVID-19 and grouped into male (250) and Female (250), all specimens were enrolled from

The Central Public Health Laboratory, Iraqi Ministry of Health, in period from April 2021 to April 2022.

Detection by molecular techniques

RNA extraction from nasopharyngeal and throat swab samples

The RNA was extracted from patients has been diagnosed as positive for COVID-19 by using QIAamp viral RNA Mini kit, the RNA quality and integrity were assessed by using Qubit RNA HS (High Sensitivity) Assay Kits that make RNA quantitation easy and accurate then Reading by Qubit TM4 Fluorometer (Invitrogen /Thermo Fisher Scientific/ Singapore).

Polymerase chain reaction (PCR)

The specimen confirmed by a quantitative reverse transcriptase polymerase chain reaction, qRT- PCR by using TaqPath™ 1-step Multiplex Master Mix (applied biosystems/ Lithuania) with specific primers for the S-gene, N-gene and ORF1ab Assays of SARS-COV-2, to confirm the presence of virus in sample after transport, with a cycle threshold (Ct) value below to 25 in National Influenza Center in CPHL According to our diagnostic criteria, this sample remained positive since the qRT-PCR are considered positive when the Ct value is below 34, corresponding to a high viral load, were transferred in biohazard containers (Tyco healthcare Kendall company) to the specific place for treatment before disposal . (337) of specimen has been chosen and Only samples that meet a Ct < 25 were transferred high purity samples were selected for sequence processing that collected during (April 2021 to April 2022).

SARS-CoV-2 Whole Genome Sequencing

The selected samples were further characterized by next-generation sequencing (NGS) using the Ampliseq illumina sequencing (Illumina, U.S.A). Using whole genome Sequencing became interesting to study the genetic polymorphism of these viruses. According to viruses from the first epidemic wave, the total number of mutations along the genomes compared to the Wuhan-Hu-1 reference genome varied from eight mutations (strain code Djibouti/CNSS00390-IHU1031243473/2020) up to 21 mutations (strain code Djibouti/CNSS00326-IHU1031243462/2020). These mutations lead to a number of amino acid substitutions varying from 4 to 11 (19).

Results and Discussion

The ongoing SARS-CoV-2 epidemic is causing concernaround the world due to its extreme

contagiousness. Coronaviruses contain the largest known RNA viruses (26.4 to31.7 kb) (20-21). R N A viruses often have extremely high mutation rates, making them very virulent and resulting in the development of new species and changes in the mortality rate and symptoms of host (14-7).

For genome analysis, our isolated genome sequence of thevirus strain was uploaded to the NCBI databases accessionnumber MW633517 on February 25, 2021. SARS-CoV-2isolate sequences were aligned to a reference sequence (NC 045512.2) from Wuhan to identify mutations and thelocation of the mutations was predicted using the programClustalW.

The demographic study depended upon the information collected about sex, number of infection in all Hospitals of Iraq and type of Corona virus, Figure (1) show the distribution of patients have CoV-19 in all Iraq about one year in study for(337)Patients with CoV-19.

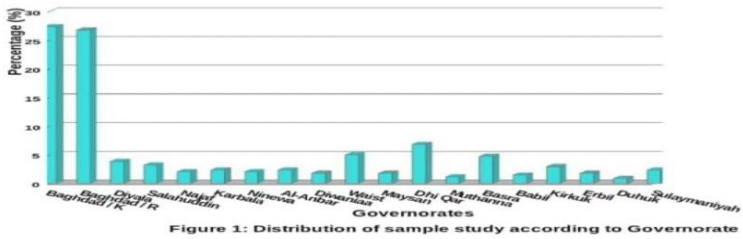

Figure 1: Distribution of sample study according to Governorate

Figure (2) show the distribution of patients have CoV-19 in all Iraq about one year in study for(337)Patients with CoV-19according to sex with highly significant differences (P≤0.01) 9.029

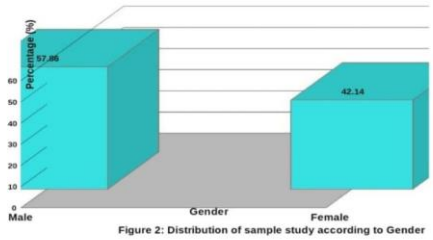

Figure 2: Distribution of sample study according to Gender

The number of males 195 , and by 57.86% and the number of females was 142 with a ratio of 42.14%.

The number of injuries was distributed among 289 types of Omicron and 48 Delta with a significant percentage 8.335. Figure (3) show the distribution of patients have CoV-19 in all Iraq about one year in study for(337)Patients with CoV-19 according to type of Coronavirus.

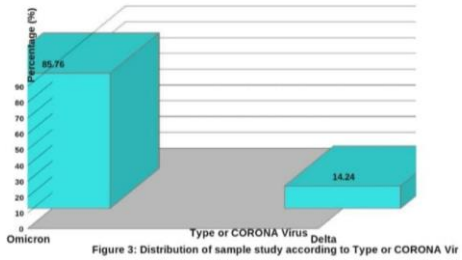

Figure 3: Distribution of sample study according to Type or CORONA Vir

All Patients (337) detectionby qRT-PCRspecimen has been chosen and Only samples that meet a Ct < 25 were transferred high purity samples were selected for sequence processing that collected during (April 2021 to April 2022), (Fig 4).

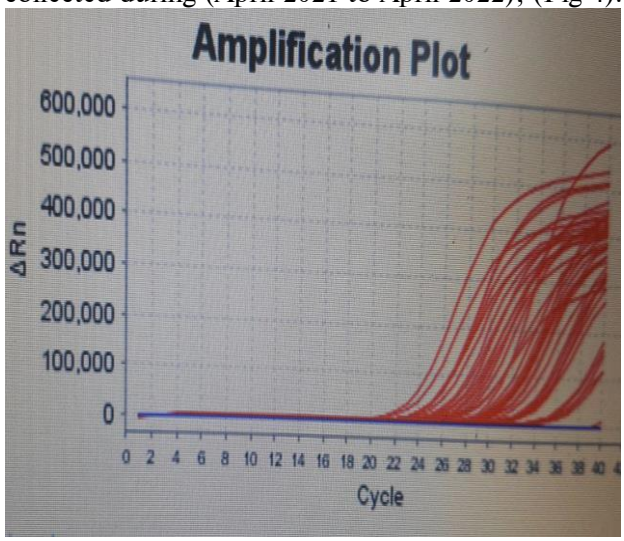

Figure (4) qRT-PCR Amplification plot of RNA Genome of Patients with CoV-19

Here we analyzed the mutations of eachgenes separately by comparing with hCoV-19/Wuhan/WIV04/2019 in GISAID.Based on mutation analysis, our isolate from Iraq contained 25 various silent and missense mutationsNine mutations were highlighted as unique in theviral SARS-COV-2 isolate compared to the Wuhan referencesequence (Fig. 5).

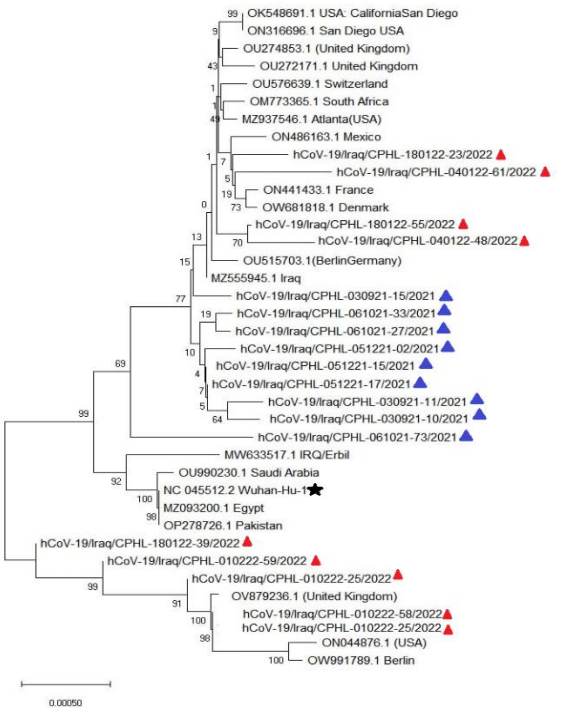

Fig. (5) Phylogenetic tree of SARS-CoV2 full-length genomes constructed by MEGA X. The Neighbor joining method was used with 1,000 bootstrap replicate. The tree contains 18 SARS-CoV2 Iraqi sequences of these study compared to the reference (marked by black star) sequence and some other sequences from GeneBank. The blue triangle and red triangle indicates Iraqi isolates (into tow wave 2021, 2022 respectively).

In this study, we reported the circulation of distinct clades of SARS-CoV2 during the tow waves in Iraq. showed important mutations in the different parts of the genome which all were analyzed and compared with circulating variants worldwide. The effects of important mutations were discussed here. more than two thirds of the SARS-CoV2 genome with 21,290 nucleotides at the 5' end which encodes 16 non-structural proteins (NSP1-NSP16). Among these NSPs, NSP3 had the highest number of mutations. NSP3 is important for virus replication and it can suppress host protein synthesis, then amino acid substitutions in NS3 deserve greater study in vitro.NSP6 is important for viral assembly, viral protein folding and replication. NSP6-L37F leads to asymptomatic transmission and reduced virulence which we can see in the most of theviruses detected during the 2st wave and one in the 2nd wave [22].In our research NSP12-P323L located in the NSP8 binding cleft [23] was detected after the first wave. NSP12-P323L is the most common detected substitution with increasing in occurrence over time [24].The S protein is crucial factor for the entry of SARS-CoV2 to the host cell which interacts with the angiotensin-converting enzyme 2 (ACE-2) receptor through its receptor binding domain (RBD) [25]. The S477N is the part of an epitope recognized by human neutralizing antibodies and located in the RBD. A study showed that S477N increases the affinity for the ACE-2 receptor [26]. Singh., et al. showed that S477N strengthen the binding of SARS--COV2 spike to the ACE-2 receptor [27]. In this study, two samples had S477N which both belonged to GR clade. Among the variations in the alpha variant, S-N501Y, is in the receptor binding site which was shown to increase the binding of SARS-CoV2 to the ACE-2 host receptor, leading to increased viral fitness and transmission [28−29].

Nucleoprotein was mainly expressed in the initial stages of infection, and is important in viral RNA transcription and replication. Nucleoprotein has been shown to affect some basic cellular processes, inflammatory responses to upregulate the expression of the proinflammatory factor COX2, and it inhibits the innate immune responses in the host cell [30]. Therefore,amino acid substitutions in the nucleoprotein might have significant effect in immune response.

In conclusion, we detected different lineages of SARS-CoV2 contributing to all wavesand showed that all viruses circulating during the 5th wave belonged to delta variant. We comparedthe mutations identified in our complete genomes study with those reported in GISAID.The findings of this study showed that with progression of the pandemic, the number of mutationswere considerably increased which showed the adaptive evolution of SARS-CoV2 inhuman to increase transmissibility. Therefore genomic surveillance is an important tool toscreen the progression of the COVID-19 pandemic.

References

Aldiabat K, Kwekha Rashid AS, Talafha H, Karajeh A (2018) Theextent of smartphones users to adopt the use of cloud storage.J Comput Sci 14(12):1588–1598. https:// doi. org/ 10. 3844/ jcssp.2018. 1588. 1598.

Alhayani BSA, llhan H (2021) Visual sensor intelligent module basedimage transmission in industrial manufacturing for monitoringand manipulation problems. J Intell Manuf 32:597–610. https://doi. org/ 10. 1007/ s10845- 020- 01590-1.

Alhayani B, Abbas ST, Mohammed HJ et al (2021) Intelligentsecured two-way image transmission using corvus corone moduleover WSN. Wirel Pers Commun. https:// doi. org/ 10. 1007/s11277- 021- 08484-2

Al-Bdery ASJ, Mohammad GJ, Hussen B (2020) Vancomycin andlinezolid resistance among multidrug-resistant Staphylococcusaureus clinical isolates and interaction with neutrophils. GeneRep 21:100804

Ali FA, Hussen BM, Zaki S (2020) Molecular detection of bla ctx-mgene among pseudomonas aeruginosa strains isolated from differentclinical samples in erbil city. Ann Trop Med Public Health 23:231

Al-Sa'ady AT, Mohammad GJ, Hussen BM (2020) Genetic relation and virulence factors of carbapenemase-producing Uropathogenic Escherichia coli from urinary tract infections in Iraq. Gene Rep 21:100911

Angeletti S, Benvenuto D, Bianchi M, Giovanetti M, Pascarella S, Ciccozzi M (2020) COVID-2019: the role of the nsp2 and nsp3 in its pathogenesis. J Med Virol 92(6):584–588

Banerjee S, Seal S, Dey R, Mondal KK, Bhattacharjee P (2021) Mutational spectra of SARS-CoV-2 orf1ab polyprotein and signature mutations in the United States of America. J Med Virol

93(3):1428–1435

Barzinjy AA, Aziz KF, Hussen BM, Qader SS, Hamad SM, Qader A et al (2021) Fear of COVID-19 as a precautionary measure to prevent the epidemic among the population of the Kurdistan Region/ Iraq: based on a questionnaire survey. Z Gesundh Wiss. 1–8

Chen J, Wang R, Wang M, Wei GW (2020) Mutations strengthened SARS-CoV-2 infectivity. J Mol Biol 432(19):5212–5226

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu D et al (2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill 25(3):2000045

Das JK, Sengupta A, Choudhury PP, Roy S (2021) Characterizing genomic variants and mutations in SARS-CoV-2 proteins from Indian isolates. Gene Rep 25:101044

Domingo E (2000) Viruses at the edge of adaptation. Virology

270(2):251–253

Duffy S (2018) Why are RNA virus mutation rates so damn high? PLoS Biol 16(8):e300000319-Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM et al (2021) SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev Microbiol 19(7):409–424

Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM et al (2021) SARS-CoV-2 variants, spike mutations and immune escape. Nat Rev Microbiol 19(7):409–424

Hasan HS, Alhayani B et al (2021) Novel unilateral dental expander appliance (udex): a compound innovative materials. Comput Mater Continua 68(3):3499–3511. https:// doi. org/ 10. 32604/ cmc.2021. 015968

Hui EK (2006) Reasons for the increase in emerging and re-emerging viral infectious diseases. Microbes Infect 8(3):905–916

Hussen B, Qader S, Aziz K, Hazha J, Qader A, Jamal A (2021a) The assessment of lifestyle change due to COVID-19 pandemic in northern Iraq. Health Biotechnol Biopharma 4(4):43–50

Devaux, C. A., Ikram, O. M. A. R, Levasseur, A., Brechard, L.,Abdillahi Hassan, I., Salah Abdillahi, I., ... &Raoult, D. (2021). Whole genome sequencing of SARS-CoV-2 strains in COVID-19 patients from Djibouti shows novel mutations and clades replacing over time. Frontiers in medicine, 1444.

Woo PC, Lau SK, Lam CS, Lai KK, Huang Y, Lee P et al (2009)Comparative analysis of complete genome sequences of threeavian coronaviruses reveals a novel group 3c coronavirus. J Virol83(2):908–917.

Mihindukulasuriya KA, Wu G, St Leger J, Nordhausen RW, Wang D

(2008) Identification of a novel coronavirus from a beluga whaleby using a panviral microarray. J Virol 82(10):5084–5088.

Wang R, Chen J, Hozumi Y, Yin C, Wei G-W. Decoding asymptomatic COVID-19 infection and transmission. The journal of physical chemistry letters. 2020; 11(23):10007–15. https://doi.org/10.1021/acs.jpclett.0c02765 PMID: 33179

Mutlu O, Ugurel OM, Sariyer E, Ata O, Inci TG, Ugurel E, et al. Targeting SARS-CoV-2 Nsp12/Nsp8 interaction interface with approved and investigational drugs: an in silico structure-based approach. Journal of Biomolecular Structure and Dynamics. 2020:1–13.

Martin R, Li J, Parvangada A, Perry J, Cihlar T, Mo H, et al. Genetic conservation of SARS-CoV-2 RNA replication complex in globally circulating isolates and recently emerged variants from humans and minks suggests minimal pre-existing resistance to remdesivir. Antiviral research. 2021;

188:105033.https://doi.org/10.1016/j.antiviral.2021.105033 PMID: 33549572

Shatizadeh Malekshahi S, Yavarian J, Shafiei-Jandaghi NZ. Usage of peptidases by SARS-CoV-2 andseveral human coronaviruses as receptors: A mysterious story. Biotechnology and Applied Biochemistry.

2020.

Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KH, Dingens AS, et al. Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. Cell. 2020; 182 (5):1295–310. e20. https://doi.org/10.1016/j.cell.2020.08.012 PMID: 32841599

Singh A, Steinkellner G, Ko¨ chl K, Gruber K, Gruber CC. Serine 477 plays a crucial role in the interaction of the SARS-CoV-2 spike protein with the human receptor ACE2. Scientific reports. 2021; 11(1):1–11.

Mallon PW, Crispie F, Gonzalez G, Tinago W, Leon AG, McCabe M, et al. Whole-genome sequencing of SARS-CoV-2 in the Republic of Ireland during waves 1 and 2 of the pandemic. medRxiv. 2021.

Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B, et al. SARS-CoV-2 501Y. V2 escapes neutralization by South African COVID-19 donor plasma. Nature medicine. 2021; 27 (4):622–5. https://doi.org/10.1038/s41591-021-01285-xPMID: 33654292

Surjit M, Liu B, Kumar P, Chow VT, Lal SK. The nucleocapsid protein of the SARS coronavirus is capable of self-association through a C-terminal 209 amino acid interaction domain. Biochem Biophys Res Commun. 2004 May 14; 317(4):1030–6. https://doi.org/10.1016/j.bbrc.2004.03.154 PMID: 15094372