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Molecular And Phylogenetic Investigation Of SARS-COV-2 In South Iraq

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Abstract

The aims of the this study were to explore as well as follow down the SARS-CoV-2 patients in Iraq using modern molecular and phylogenetic.

We have studied thirty five definite cases of COVID-19 patients for the phylogenetic evaluation of the SARSCoV-2 in Iraq. The main genes of spike (s) and “RNA-dependent RNA polymerase (RdRp) genes” were got amplified via one-step RT-PCR then sequenced based on Sanger sequencing method. Bioedit and Mega bioinformatics software were applied for both sequences alignments and phylogenetic relationship building up frame .

The results showed that the main age of the patients enrolled in the present study was 42.45 $\pm$ 12.13 SD years and 64.5 percent were male.

The results indicated a possible similarity flanked by Iraqi and Chinese incoming strains.

Polymorphic regions in the investigated regions of the two genes by neighbor-joining as well as maximum likelihood method of RNA-dependent RNA polymerase (RdRp) and S genes revealed possibility of differences among Iraqi isolates and Wuhan isolate.

Conclusion: We discovered that the Iraqi SARS-CoV-2 isolates are closely linked to the Chinese and standard genomes through a groundbreaking phylogenetic investigation of these isolates. Additionally, there were several variations between the isolates from Wuhan and those from Iraq.

1-Introduction

A positive RNA virus known as “severe acute respiratory syndrome coronavirus-2” is the cause for the enduring coronavirus pandemic (COVID-19) started in 2019(1, 2).

(SARS-CoV-2) Approximately a year later the WHO declared SARS CoV-2 to be pandemic, a lot number of variations have been notorious(3). The Orthocoronavirinae subfamily of the Coronaviridae is where the SARS-CoV-2 virus is found(4). Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus are the four genera that make up this subfamily(5-7). The Alphacoronavirus and Betacoronavirus families are home to the vast majority of clinically significant Coronaviridae(8-11).

A globally contagious virus called SARS-CoV-2 that is easily transmissible might cause 1.9 million fatalities and at least 88 million

cases would occur(12). The genetic code of the virus is encoded in its RNA, and it often mutates(13). The development of the virus is a very crucial concept to comprehend(14). By comprehending the nature of mutation, potential medications may be created(15). The establishment for fast determination of possible SARS-CoV-2 infection is converse record polymerase chain response (RT-PCR)(16). To raise the indicator from the targeted virus in a test, these assays use a forward primer, a reverse primer, and a appropriate probe(17) . Concerning SARS-CoV-2 coronavirus variations include B.1.1.7 (501Y.V1), B.1.351 (501Y.V2), and B.1.1.28.1 (501Y.V3; also known as P.1)(18). The original COVID-19 case to be reported in Iraq was made public on February 24, 2020(19). The World Health Organization (WHO) stated out that the SARS-CoV-2 coronavirus pandemic is a “Public Health Emergency of International Concern” (PHEIC) on January 30, 2020(20). During Week 13, the Iraqi Ministry of Health (MOH) recorded 41,140 new COVID19 infections, which is a 6.76 percent rise from week 12 and 251 new fatalities, which is a 22.44 percent increase from the week before(21). The southern governorates of Al-Qadisyah, Wasit, Dhi-Qar, and Misan had the highest COVID 19 case reporting for WK24, with an overall positive rate of 11%.(13).

The aim of The current work is to apply the molecular and phylogenetic approaches to explore and monitor SARS-CoV-2 in Iraqi Coronavirus patients. As a result, we believe that the outcomes of this study will contribute to filling gaps in the research on COVID-19 in Iraq.

2-Materials and method

Sample collection

Patients getting therapy with symptoms such as diarrhea, fever, fatigue,

and coughing were sampled(22). All samples were processed at the COVID-19 testing laboratory at the Al-Hussein Teaching Hospital in Nasiriyah, Iraq. Viral Transported Media (VTM; Biobase, China) was used to obtain the NP swab from the patient. Thirty five samples were collected and analyzed in order to identify COVID-19(23). All samples were kept at 4 degrees Celsius and quickly transferred to the lab on ice in a separate box.

RNA Extractions

All swabs were processed for RNA extraction using an extraction system made by AeHealth in China and a kit made by Lifotronic in China. In order to lyse the sample's cells and liberate their nucleic contents, 200 ul of VTM were combined with magnetic beads in the lyse buffer and then added(24). This process was carried out in an incubator at 65 C with agitation. Second, to remove all debris, magnetic beads were adsorbed and moved to the second phase (the washing stage) for 3 minutes(25). In order to separate the RNA from the magnetic beads, they were transferred to elution buffer and mixed for 5 minutes. RNA was collected and stored in a tube at -20 C. In this investigation, all safety protection guidelines were adhered to the work protocols(26) .

RT-PCR and genes sequencing

Gene sequencing and RT-PCR RNA were extracted from clinical specimens in accordance with the manufacturer's instructions using the separation of viral RNA with kit of “FAVORGEN Biotech Corporation, Taiwan”. All extracted RNAs were spectrophotometrically assessed for

extraction quality using a NanoDrop-Thermo Fisher Scientific Inc., Waltham, MA, USA. The Luna universal- one step RT-PCR Kit was used for complementary DNA (cDNA) synthesis were done using primers showed in table (1) according to (27).

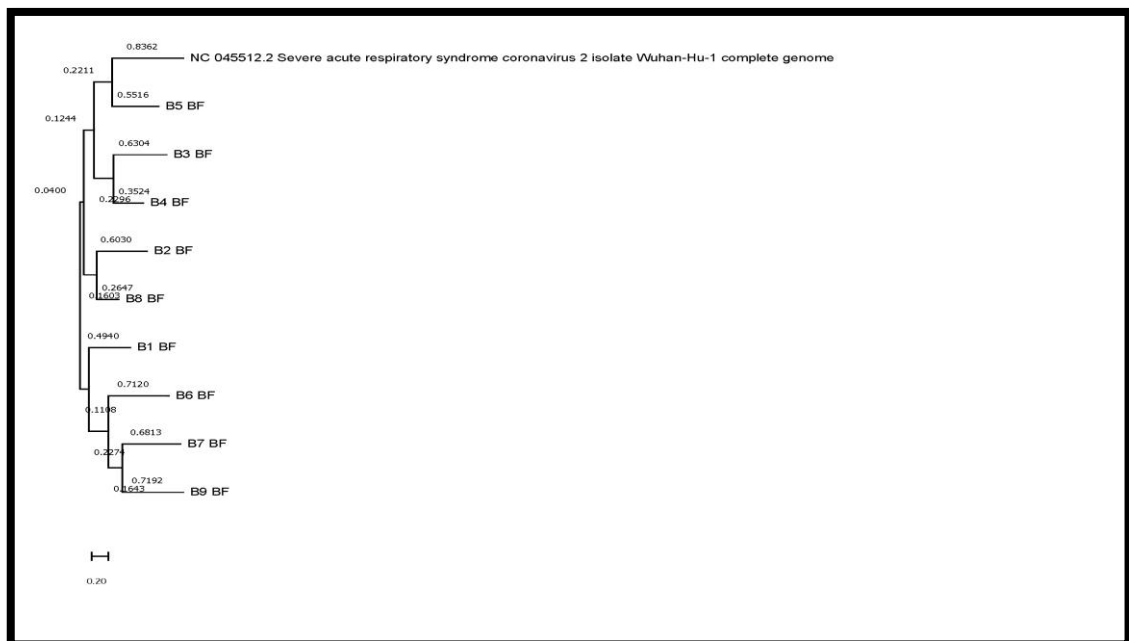
Table (1) DNA oligonucleotides applied for amplification of RdRp along with S genes of SARS-CoV-2(27)

gene	Primer sequences	Amplicon length
RdRP	F: 5'CAA GTG GGG TAA GGC TAGA CTTT'3 R:5'ACTT AGG ATA AT CCC AACC CAT'3	344b p
Spike	F:5'CCTACTAAATTAAATGATCTCTGCTTTACT'3 R:5'CAAGCTATAACGCAGCCTGTA'3	158b p

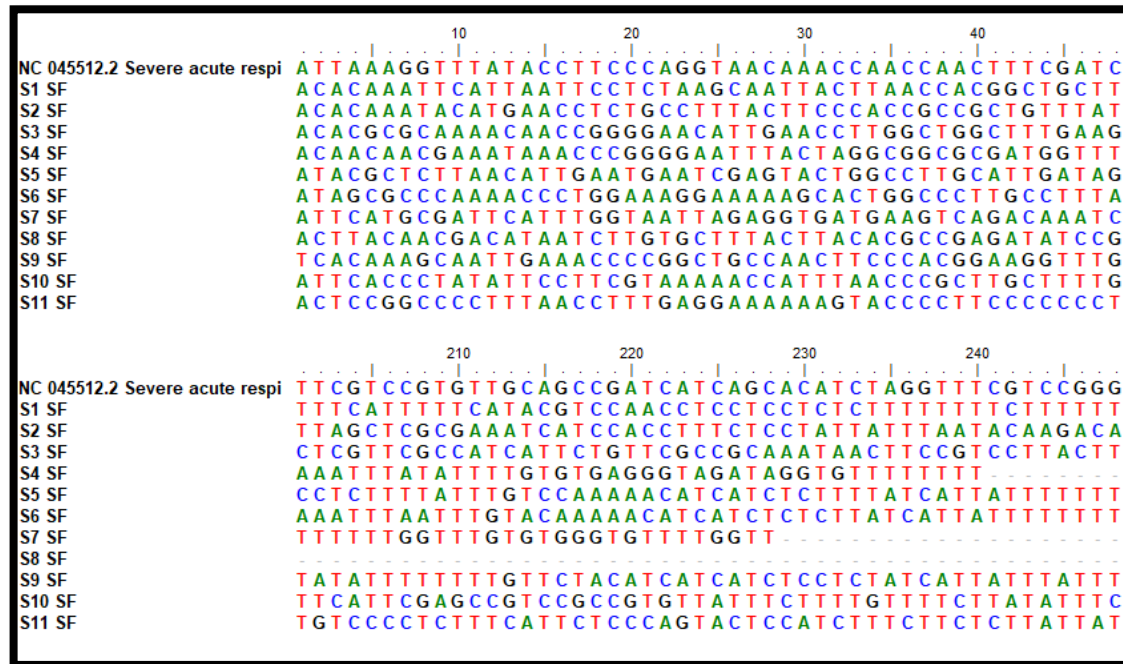
S protein and RNA-dependent RNA polymerase (RdRp) genes were amplified using specified primers as showed in table (1)(27). The RNA-dependent RNA polymerase (RdRp) gene, which has 344 base pairs, the spike (S) protein, which has 158 base pairs were all amplified using the traditional PCR technique(28).



Figuer (1): Phylogenetic tree for partial region of spike gene of the SARS-CoV-2 via neighbor joining method.



Figuer(2): Phylogenetic tree for partial region of RNA-dependent RNA polymerase (RdRp) gene of the SARS-CoV-2 by neighbor joining method.



Figuer(3): Partial MSA analysis for SNPs in spike gene showed polymorphisms



Figuer(4): Partial MSA analysis for SNPs in region of RNA-dependent RNA polymerase (RdRp) gene showed polymorphisms

The patients in this research had a mean age of 42.45 $\pm$ 12.13 SD years, and 64.5 percent of them as male. The white blood cell count mean, D-Dimer value, and CRP value were 15.46, 6.78, and 1000 cells/mm³, respectively. C-reactive protein measurements of the inflammatory indicators were evaluated qualitatively (CRP). Patients had a positive CRP with a value of 71.3233 $\pm$ 12.71 SD. Additionally, sequencing analysis were run on 10 out of 30 samples.

Multiple sequence alignments were done using the obtained sequences as showed above.

The MSA analysis could reveal the snps as in Figures 3 and 4. in the analyzed regions of the two genes spike and (RdRp), as furthermore, the acquired sequence from Wuhan strain in Hubei Province, China, and the Iraqi Covid-19 sequences exhibited similarities in the neighbor joining phylogenetic analysis, moreover, the neighbor joining phylogenetic analysis exposed that Iraqi sequences and that from China. Additionally, the S gene examination revealed the different results (Fig. 3). According to the phylogenetic investigation of the acquired sequence from Wuhan in Hubei province, China and Iraqi sequences for revealed the highest degree of similarity. Additionally, the neighbor joining phylogenetic analysis revealed that Iraqi sequences and that from China had different mutations for RdRp (Fig. 4). Additionally, the S gene examination revealed the same outcomes (Fig. 3) in which The results of the phylogenetic analysis showed different mutations also

Discussion

According to the COVID-19 partial sequences analysis, this sickness is widely spread in the Middle East, including in Iraq. Due of its high

contagiousness, the SARS-CoV-2 shows a strong would-be for transmission(25). In the current preliminary analysis in Iraq, we sought to examine and follow the SARS-CoV-2 in Iraqi COVID-19 patients via molecular and phylogenesis analysis , taking into account the significance of the public's awareness on the subject of the SARS-CoV-2 spreading and its phylogenesis analysis.

The neighbor joining phylogenetic analysis revealed that a high resemblance between Iraqi sequences for the two genes and the studied sequences from Wuhan strain NC 045512.2 (Additionally, the neighbor joining phylogenetic analysis revealed that the shared the highest similarities with RdRp (Fig. 2)(26, 29-31). Spike gene Wuhan strain NC 045512.2 sequence analysis revealed a similarity with Iraqi isolates sequences (Fig. 1)(32, 33).

Conclusion :

The Iraqi isolates have the similarities with the Chinese isolates, according to our phylogenetic analysis.

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