Phylogenetic Analysis of SARS-CoV-2 Isolated from Pregnant Iraqi Women

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Abstract: *The aims of 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 pregnant 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 indicated a possible similarity flanked by Iraqi and Chinese incoming strains.*

Keywords: COVID-19, RT-PCR, Molecular diagnosis, mutations, Iraq

1. Introduction

The pandemic of the corona virus disease 2019 (COVID-19) which caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2), a extremely transmissible and pathogenic corona virus has been representing a huge threats to the word [1-3]. The SARS-CoV-2 pandemic likely first emerged in China in late 2019 and by march 2022 it reached 2.3 million cases with about 25.000 deaths in Iraq (WHO, 2022) [4]. Corona viruses belong to Coronaviridae family with single strand of positive sense RNA, genome of about 30 kb in length [5]. It was registered in many avian hosts as well as in various mammals including mice, bats, dogs, etc., In December 2019, Chinese governmental health authorities announced that groups of patients with pneumonia of an unknown reason in Wuhan, Hubei Province, China [6, 7]. The pathogen was a new coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was diagnosed by local hospitals using a surveillance mechanism for "pneumonia of unknown etiology [8-10]". The pandemic spread rapidly, and more than 28 million confirmed cases and nearly 900, 000 deaths were reported in just an 8 month period [11]. The rapid viral spread raised interesting questions about the way its evolution is driven during the pandemic [12]. From the SARS-CoV-2 genome, 16 nonstructural proteins (nsp1-16), 4 structural proteins (spike [S], envelope [E], membrane [M], and nucleoprotein [N]), and other proteins essential to complete the replication cycle have been translated [13]. The aim of this study was to conduct a large evolutionary analysis to demonstrate the human epidemic and evolutionary changes in specific genomic sites of SARSCoV.2 in pregnant Iraqi women [14].

2. Materials and methods

Sample collection
Thirty five pregnant patients receiving therapy for symptoms such the runny nose, fever, fatigue, and hacking were sampled. At the Women's and Children General Hospital in Al-Diwaniyha, Iraq, the COVID-19 testing research laboratory conducted each test. The nasopharyngeal swab was extracted from the Viral Transported Media (VTM; Biobase, China). 35 tests were gathered and examined in an effort to identify COVID-19. All tests were stored at 4 degrees Celsius and quickly transported on ice in a divided box to the laboratory.

Extractions of RNA
All swabs were processed for RNA extraction using a system and kit made in China by AeHealth and Lifotronic, respectively.200 ul of VTM were mixed with magnetic beads in the lyse buffer and added to the sample to lyse the cells and release the nucleic contents. This process was run in an incubator with agitation at 65 C. Second, magnetic beads were adsorbed and transported to the second stage (the washing stage) for 3 minutes in order to eliminate all dirt. The magnetic beads were transferred to elusion buffer and stirred there for 5 minutes in order to remove the RNA from them. RNA was collected and kept at-20 C in a tube. In this research The work processes were followed to all safety protection rules [15].

RT-PCR and gene sequencing procedures
To obtain genetic information, RNA samples were extracted from clinical specimens following the manufacturer's guidelines, utilizing the viral RNA separation kit provided by "FAVORGEN Biotech Corporation, Taiwan. " The quality of the extracted RNAs was evaluated spectrophotometrically using a NanoDrop instrument from Thermo Fisher Scientific Inc., Waltham, MA, USA. The Luna universal one-step RT-PCR Kit was employed for complementary DNA (cDNA) synthesis. The primers listed in table (1) as described in reference [4] were used for the cDNA synthesis process.

Table 1: DNA primers applied for amplification of RdRp along with S genes of SARS-CoV-2

Gene	sequences Primer	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

The amplification of the spike (S) protein and RNA-dependent RNA polymerase (RdRp) genes was carried out by employing specific primers indicated in table (1) (27). Both the RNA-dependent RNA polymerase (RdRp) gene, with a length of 344 base pairs, and the spike (S) protein, with a length of 158 base pairs, were successfully amplified using the conventional PCR technique [16].

Phylogenetic analysis
Phylogenetic analysis of PCR products purified according to the manufacturer's recommendations using the High Purity PCR Product Purification Kit (Roche Diagnostic GmbH, Mannheim, Germany). The ABI 3730 XL sequencer was also utilized for two-way sequencing. Raw data were clipped and processed with the popular bioinformatics program CLC workbench 5 and the Basic Local Alignment Finder (BLAST) [17].

3. Results

The research participants had an average age of 35 years with a standard deviation of 7.4 years,. The mean values for white blood cell count, D-Dimer, and C-reactive protein (CRP) were 16.56, 7.98, and 1000 cells/mm3, respectively. CRP measurements, as an indicator of inflammation, were qualitatively evaluated. The patients exhibited a positive CRP with an average value of 72.3233 and a standard deviation of 13.71. Furthermore, sequencing analysis was conducted on 10 out of the 35 samples. The obtained sequences were subjected to multiple sequence alignments as described above.

The analysis of the phylogenetic tree (Figures 1 and 2) revealed single nucleotide polymorphisms (SNPs) in the examined regions of the spike and RNA-dependent RNA polymerase (RdRp) genes. Additionally, in the neighbor-joining phylogenetic analysis, similarities were observed between the acquired sequence from Wuhan, Hubei Province, China, and the Iraqi COVID-19 sequences [18]. Moreover, the neighbor-joining phylogenetic analysis indicated that the Iraqi sequences and the sequences from China exhibited different mutations in the RdRp gene (Fig.2). The examination of the spike gene also produced similar results (Fig.1). The phylogenetic analysis demonstrated distinct mutations in both genes [19-22].

4. Discussion

Based on the examination of COVID-19 incomplete sequences, it has been determined that this illness is prevalent throughout the Middle East, including Iraq. SARS-CoV-2's highly infectious nature suggests that it has the potential for fast transmission. The goal of this pilot study in Iraq was to analyze and monitor the SARS-CoV-2 virus in COVID-19 patients utilizing molecular and phylogenetic analyses [23]. This study considers the relevance of boosting public knowledge about the transmission of SARS-CoV-2 as well as phylogenetic analyses [24].

For both genes, neighbor-joining phylogenetic analysis revealed a considerable similarity between the sequences acquired from Iraqi patients and the sequences examined from the Wuhan strain NC 045512.2. Notably, the RdRp gene had the most similarities (Fig.2 [25]). Similarly, the sequencing analysis of the spike gene from the Wuhan strain NC 045512.2 revealed similarities with Iraqi isolates (Fig.1).
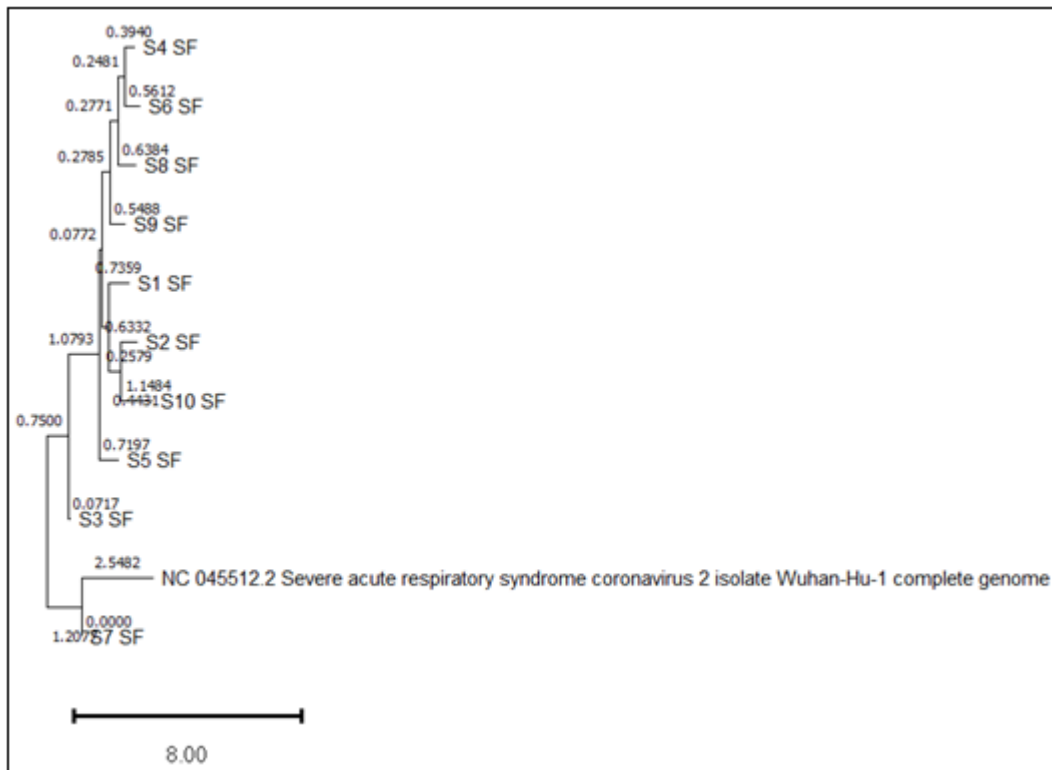
Figure 1: Phylogenetic tree constructed using the neighbor joining approach for a portion of the SARS-CoV-2 spike gene.
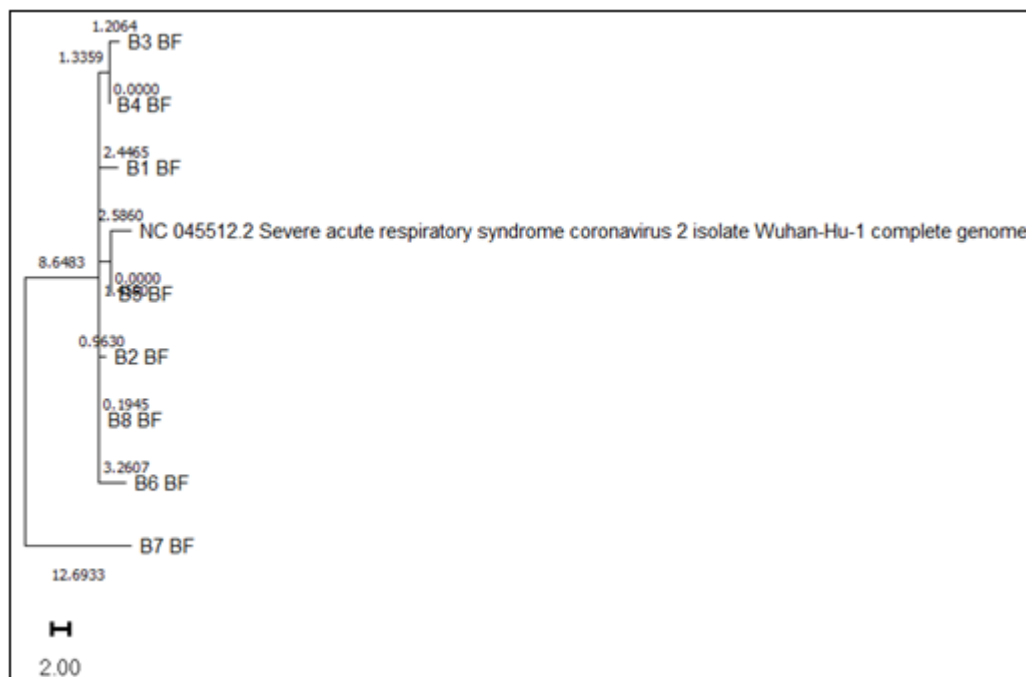

Figure 2: Phylogenetic tree constructed using the neighbor joining method for a portion of the SARS-CoV-2 RNA-dependent RNA polymerase (RdRp) gene

5. Conclusions

In conclusion, our phylogenetic analysis indicates that the Iraqi isolates exhibit similarities to the Chinese isolates.

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