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Molecular detection and Phylogenetic study role of ORF1ab in SARS-COV2 on patients in Hilla city

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Abstract---The Covid-19 pandemic, which is happening all over the world, needs serious attention. Covid-19 initiated by an RNA type virus can cause more severe problems later on due to its ability to mutate. CoV genomes code for a ORF1a / ORF1ab polyprotein and four structural proteins widely studied as major drug targets. The genomes also contain a variable number of open reading frames (ORFs) coding for accessory proteins that are not essential for virus replication, but appear to have a role in pathogenesis. This study was conducted on 50 nasopharyngeal swabs from Morjan teaching Hospital using RT-PCR and PCR for ORF1ab gene amplification after that, five samples were subjected the phylogenetic analysis. The results are confirmed by the ORF1ab analysis shows that the ORF1ab vary and mutated and have a number of mutation tht could affect it similarity between countries in aimed of detection our local isolate were had a 100 identities with United State concluding that the extreme effect of virus transmission and it is mobile genetic holding.

Keywords---, SARS-COV2, mutation, phylogenetic study.

Introduction

Coronaviruses (CoVs) infect animals and humans, causing respiratory and intestinal diseases. SARS-CoV (severe acute respiratory syndrome coronavirus), MERS-CoV (Middle East respiratory syndrome coronavirus), and SARS-CoV-2

(which causes the disease) were not considered highly pathogenic to humans until the last two decades, when three outbreaks of highly transmissible and pathogenic coronaviruses, including SARS-CoV (severe acute respiratory syndrome coronavirus), MERS-CoV (Middle East respiratory syndrome corona COVID-19) (Cui *et al.*, 2019). Coronavirus biology has improved as a result of extensive research on human coronaviruses. Coronaviruses are members of the Coronaviridae family of the nidovirales order. Phylogenetic analysis revealed that SARS-CoV-2 clusters with SARS-CoV in the Sarbecovirus subgenus, but MERS-CoV belongs to the Merbecovirus subgenus. (of the International, 2020). All members of the coronavirus family are enveloped viruses that possess long positive-sense, single-stranded RNA genomes ranging in size from 27 to 33 kb. The coronavirus genomes encode five major open reading frames (ORFs), including a 5' frameshifted polyprotein (ORF1a/ORF1ab) and four canonical 3' structural proteins, namely the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins, which are common to all coronaviruses(Ashour *et al.*, 2020).

Material and Method

Steps in the Polymerase Chain Reaction

Extraction of RNA from Nasopharyngeal Samples

Using a nucleic acid extraction kit, viral RNA was isolated from nasopharyngeal samples (Cat. No. H 200201 Zybico Inc- Germany) It's a magnetic bead technique, which was carried out in accordance with the following production instructions: 500 µl of extraction reagents I, 4µl magnetic bead solution, and 15µl Proteinase K were mixed with 500 µl of working solution and 200 µl of sample, then mixed well and lysed at 55°C for 4 minutes and absorbed after 1 minute on the magnetic separator, the supernatant was discarded Fifty l of elution buffer was eluted at 80°C for 2 minutes before being absorbed by Reserve the supernatant by running it through a magnetic separator for 30 seconds.Six hundred l of extraction reagent II was added, thoroughly mixed, and lysed at 55C° for 4 minutes, then absorbed by magnetic separator for 1 minut the supernatant was discarded. RNA convert to the cDNA according to the PrimeScript™ RT reagent Kit

Polymerase chain reaction gene amplification of severe acute respiratory syndrome Coronavirus-2

By reverse transcriptase-polymerase chain reaction, one pair of primers was utilized to amplify a segment of SARS COV-2 ORF ab1 as mention in the table 1.

Table 1
The new designed PCR primers for E and N genes of SARS-COV2

Primer	Sequence (5'-3')	Product Size
ORF1ab gene	F CGGATGGCTTATTGTTGGCG	639pb
	R ACAAC TCCGGATGAACCGTC	

PCR condition used shown in table 2

Table 2
PCR condition for detection and amplification ORF1ab

PCR Master mix	Volume
DNA template	5 μ L
ORF1ab gene Forward primer (10pmol)	1 μ L
ORF1a gene Reverse primer (10pmol)	1 μ L
PCR water	13 μ L
Total volume	20 μ L

The presence of amplification was confirmed using agarose gel electrophoresis after polymerase chain reaction amplification. The requirements for extracting DNA as shown in figure 1

Phylogenetic analysis and standard sequencing

The PCR result was shipped to Macrogen in Korea for Sanger sequencing utilizing ABI3730XL, automated RNA sequences. <https://dna.macrogen.com>. The sequencing study was created in collaboration with the in the basic local alignment search tool, standard genes and local isolates are searched by (BLAST) program, which you can find at the National Center for Biotechnology. <http://www.ncbi.nlm.nih.gov> (NCBI - National Center for Biotechnology Information). The process of evolution Molecular Evolutionary Genetics Analysis was used to conduct the research.

Results

Patients and clinical isolates

A total of (50) samples were taken from patient who suffering from SARS-COV2, during the period from September 2021 to December 2021, who admitted to Morjan Hospital were used (for ORF1ab gene of SARS-COV2 study). Alignment of Sequences from Severe Acute Respiratory Syndrome-2 with a Reference Isolate. After electrophoresis on 1.5 percent agarose gel electrophoresis stained with Ethidium bromide, the sequences of nucleotides of 5 local isolates from nasopharyngeal to amplify ORF1ab gene area of SARS-COV-2 were analyzed. M: 100bp ladder marker, specific band, were shown in figure 1 which was used in sequence analysis.

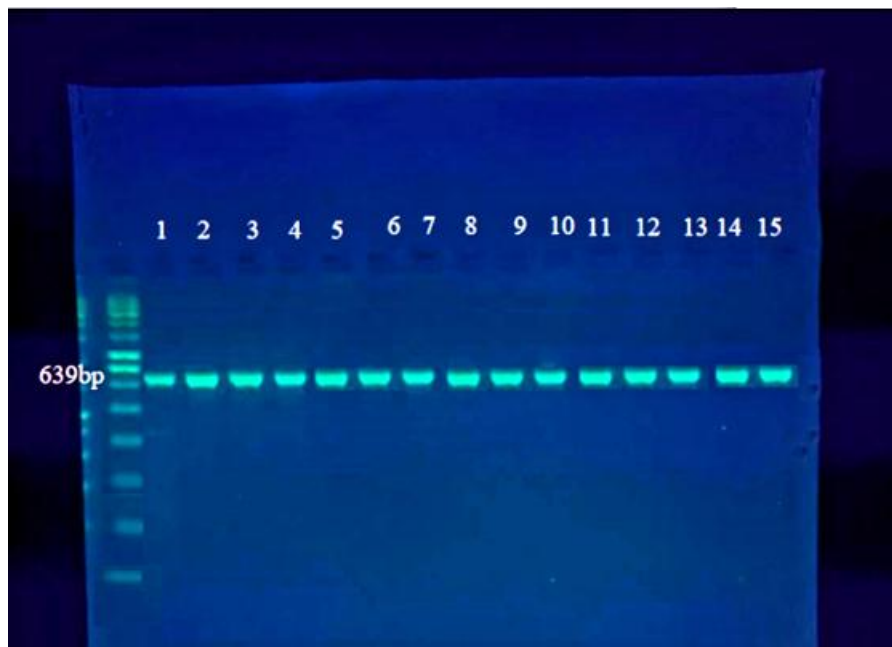


Figure 1. An agarose gel electrophoresis demonstrating PCR product size (639bp) analysis for the SARS-COV2 ORF1ab gene

The results of ORF1ab gene amplification in COVID-19 samples were fractionated on 1.5 percent agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. The nucleotide sequence was generated by amplification of ORF1ab genes in five isolates of ORF1ab, which were then compared using Blast software, translated to a specific amino acid, and produced a substantial difference. Using the NCBI site's GenBank library (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>), alignment was performed against reference isolates (OL539215.1, OL525506 and OL538734). All of the local isolates from patients had nucleotide sequence identities with the reference strain that ranged between 99 and 100 percent appeared in the table 4. Isolates were registered after correspondence with the National Center for Biotechnology Information (NCBI - National Center for Biotechnology Information) and were assigned an accession number shown in the table3.

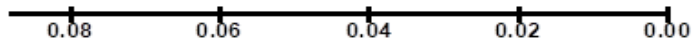
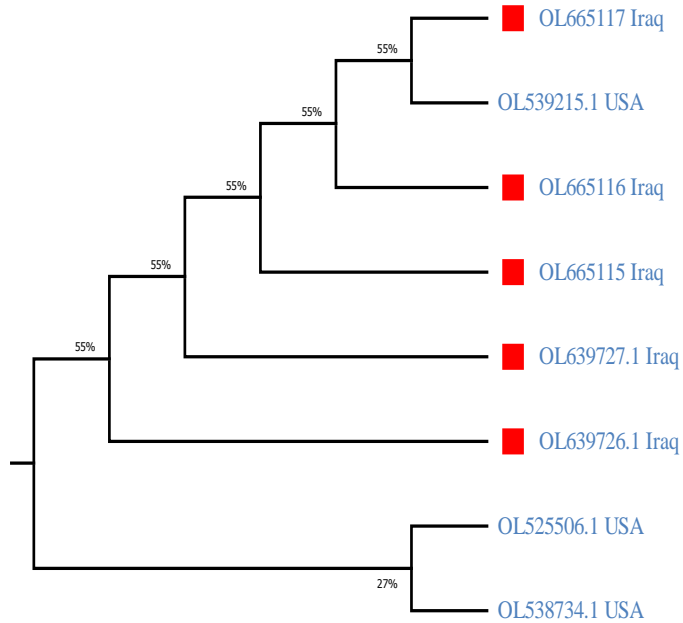


Figure 3. (NCBI - National Center for Biotechnology Information): Phylogenetic tree analysis for international isolates based ORF1ab gene partial sequence by using Test UPGMA tree (Unweighted Pair Group Method with Arithmetic Mean).

Where the results show that the local SARS-COV2 (ORF1ab (OL665117 and OL665116) close related to USA isolates (ORF1ab), the local OL639726.1) close related to USA OL525506, OL538734.1 (Tamura *et al.*, 2013).

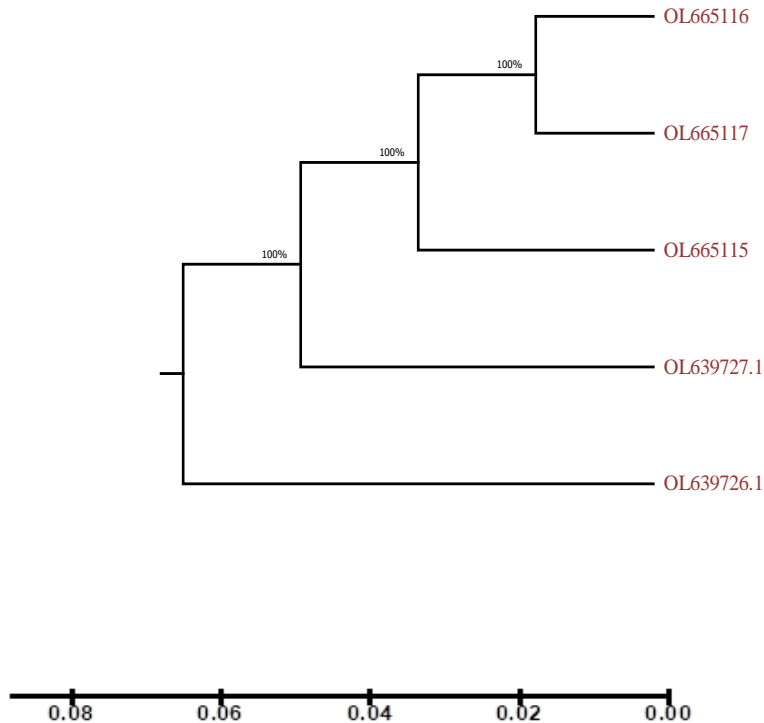


Figure 4. Phylogenetic tree analysis for local isolates based ORF1ab gene partial sequence by using Test UPGMA tree (Unweighted Pair Group Method with Arithmetic Mean).

Where the results show that all local were 100% similar to each other's (Tamura Stecher Peterson Filipski and Kumar, 2013). The UPGMA tree (Unweighted Pair Group Method with Arithmetic Mean) is put to the test. approach utilizing NCBI and MEGA 6 Software for alignment for all positive SARS COV-2 isolates (5 isolates in ORF1ab gene) using 3 reference isolates during phylogenetic tree The construction for all positive SARS COV-2 isolates. The sequencing of genes is quite similar to a global isolate from a different nation .As seen in, compatibility ranged from 99 to 100 percent Table 4, phylogenetic tree figure 3 and 4.

Discussion

To the best of our knowledge, this is the first SARS-COV-2 phylogenetic analysis investigation in Babylon governorate. A phylogenetic analysis of SARS-COV-2 RNA isolated from NPS revealed 50 different groups, with the ORF1ab gene found in isolates 5 were recorded with accession number table 3. These local isolates were aligned with 3 reference isolates using the Mega version 6 program (Figure 2). All of these isolates appear to be similar to global reference isolates from various countries around the world, with five local isolates of ORF 1ab gene clustered with global isolates from the United States, China, and Saudi Arabia,

this may help to understand why all of these global issues exist. Analyses of isolates from the same years and era, as well as the Iraq pandemic arrived from a different country Furthermore, in relation to the transmission route of SARS-COV-2 is exceedingly fast and, according to various sequencing analysis, the virus has a modest mutation rate.

The findings of this study are analogous to the findings of another study conducted in the United States to illustrate the genetic geographic distribution of SARS-COV-2 in Los Angeles, which discovered that 15% of isolates were very close to a reference isolate from the same city. China, and 85 percent comparable to the European reference isolate (Zhang *et al.*, 2020). (NCBI - National Center for Biotechnology Information, Tushir *et al.*, 2021) conducted a study and discovered that phylogenetic analysis of the SARS-COV-2 genomic sequence was compared to a reference isolate from a different country demonstrate that the Indian isolate is very closely connected to a neighboring isolate Bangladesh, Nepal, and China (Hong Kong) are only a few examples.

These discrepancies could be due to the usage of various genes and the size of the sample. The genes utilized to detect SARS-COV-2, which has a global distribution and affects every country on the planet, have a high level of stability and resistance various routes of transmission Several studies have been conducted to evaluate the genomic data. To determine the distinctive trait of SARS-COV-2, researchers looked at the characteristics of the entire worldwide population in various countries that assist health systems in comprehending epidemiology by (Aydiillo *et al.*, 2020) (Forster *et al.*, 2020).

In correlation, a study conducted by (Zhang and Yoo, 2016) on the first three weeks of the pandemic in Canada found that 35 percent of SARS-COV-2 nucleotide sequences in the Canadian population are similar to isolates from Asia and 42 percent are similar to isolates from Europe, and the authors suggest that the reasons for these similarities are due to most patients having a history of travel to these countries. Another study conducted by (Meza-Robles *et al.*, 2020) used one-step RT-PCR as an alternative method for diagnosing SARS-COV-2 in Mexico. They used four sets of primers targeting the ORF1ab gene, which is the most variable region between human SARS-COV-2 and bat coronavirus, to show that COV-2 is more closely related to bat coronavirus than SARS-COV-1 and MERS-COV during phylogenetic analysis.

Conclusion

Research outcome confirmed by ORF1ab analysis, which shows that ORF1ab varies and mutates, and has a number of mutations that could affect its similarity between countries in order to detect our local isolate, which had 100 identities with the United States, concluding that the virus has a severe effect on transmission and is genetically mobile.

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