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Original Article

Genetic Sequence of Coronavirus Strains Isolated from Iraqi Patients and their Relationship with some Liver Enzymes and Interleukins

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Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which is a positive-sense single-stranded RNA virus from the genus *Betacoronavirus* causes COVID-19 (coronavirus disease 2019). According to daily reports issued by the Iraqi Ministry of Health, the SARS-COV-2 was firstly detected in Al-Najaf city in February 2020 and identified in the Central Public Health Laboratory (CPHL) in Baghdad, Iraq. The outcomes of this study were based on 100 nasopharyngeal swaps and venous blood samples from hospitalized patients in Al-Kindy and CPHL. Patients were assigned to five groups (Asymptomatic, Mild, Moderate, Severe, and Deceased) based on disease severity as indicated by World Health Organization (WHO). The positive samples were identified by real-time quantitative polymerase chain reaction (RT-PCR) and subjected to some liver enzyme assays and interleukins measurements, and the correlation with the genetic sequence was determined by Illumina Miseq technology. Liver enzymes levels of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) showed statistically significant differences, especially between the deceased groups. Interleukins (IL-10, IL-18, and TNF- α) significantly differed among groups. This study revealed that three isolates belonging to the original strain isolated from Wuhan (A19) and characterized by their virulence caused severe symptoms and led to admission to isolation hospitals and intensive care units, and the last two isolates of (UK alpha V1) appeared in Iraq in early 2021. These strains which were less virulent than the Wuhan strain spread faster and appear in moderate and asymptomatic patients.

Keywords: SARS-COV-2, Genetic sequence, AST, ALT, LDH, TNF- α, Il -10, Il- 18

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly contagious and can be transmitted by asymptomatic infections (1). This virus has a distinct base sequence that separates it from other species, and it is genetically distinct enough from SARS-CoV-1 (79% genetic similarity) and Middle East respiratory syndrome coronavirus (MERS-CoV) (50% genetic similarity) to be introduced as a new virus. Phylogenetic studies place it in the *Betacoronavirus* genus, subgenus *Sarbecovirus*, of the *Coronaviridae* family (2). On February 11, 2020, the World Health Organization (WHO) declared SARS-CoV-2 to be coronavirus disease 2019 (COVID-19) which led to intensive care unit (ICU) admission in a large number of critically ill patients.

Total mortality for COVID-19 individuals, according to documented clinical parameters, ranges from 2%-5%, and this rate can be significantly higher among the elderly (1). The mortality rate in Wuhan city, which was

the epicenter of the pandemic, peaked at around 7% in the early stages (3). Although the majority of confirmed COVID-19 cases were mild at first, several developed severe respiratory failure, metabolic acidosis, septic shock, and acute respiratory distress syndrome, or even died. The early detection of risk factors in critically ill patients may allow for more appropriate supportive care and, as a result, a reduction in mortality (4). The reverse transcription-polymerase chain reaction (RT-PCR) from a throat swab or nasopharyngeal swab is the gold standard for diagnosis (5).

The innate immune response is the first line of defense against viral infections. Pattern recognition receptors on host dendritic cells recognize viral genomic DNA or RNA in infected cells, triggering the release of cytokines and chemokines3, which attract immune cells, such as macrophages, neutrophils, and T-cells to the infection site to help fight the virus (6). Individual cytokines perform specific roles depending on their source and target cells; therefore, the nature of the immune response is determined by that (7). Pro-inflammatory cytokines, such as type I/II interferons, Interleukin-1 (IL-1), Interleukin 6 (IL-6), and tumor necrosis factor-α (TNF-α), play a key role in the early response, whereas anti-inflammatory molecules, such as IL-10, are produced during long-term infection to keep inflammation under control and preserve immunological homeostasis (8).

In fact, some researchers suggest that IL-10 may be over-expressed in anti-SARS-CoV-2 immunity, with levels higher in elderly patients with regard to a "hyper-inflammatory response," likely due to reduced T-cell receptors in these patients (9). The IL-10 levels were found to be higher in COVID-19 patients, as compared to those with S`ARS-CoV or MERS, as they were in other cytokines (10). Studies have indicated that IL18 serum levels were higher in COVID-19 patients, in comparison with those in healthy people. Moreover, they hypothesized that this and other cytokines were elevated due to the activation of T-helper 1 (Th1) and T-helper 2 (Th2) cells. Serum IL-18 levels have been also found to be elevated in patients with SARS-CoV or MERS (10, 11). Serum TNF- levels have been demonstrated to be elevated in COVID-19 patients, with higher levels associated with more serious disease. Similar findings were recorded in COVID-19 patients, pointing to an inverse association between TNF levels and T-cell counts (12).

Patients with severe COVID-19 who are frequently bedridden and have irregular coagulation activity should be given special attention in terms of the risk of pulmonary embolism (13). The studies to date have found evidence that increases in lactate dehydrogenase (LDH) levels correlate with increased tissue damage and inflammation. The LDH is a biomarker that is highly predictive of COVID-19 mortality risk (14). Patients with severe COVID-19 infection are more likely to have abnormal liver function test indicators (Aspartate aminotransferase (AST), Alanine aminotransferase (ALT)). The data support the idea that COVID-19 patients with liver impairment are more likely to have serious consequences (15).

2. Materials and Methods

2.1. Study Design

According to Bradford and Slavin (1940), 100 nasopharyngeal swaps (Sigma Virocult Company, UK) were obtained with viral transport medium from 100 patients who were hospitalized in Al-Kindy in the isolation hall and ICU, as well as the Central Public Health Lab (CPHL). The samples were stored frozen at -70°C for RNA extraction and PCR, in addition to the venous blood samples that were distributed in serum gel tubes and EDTA tubes for serum and plasma preparation, respectively. Patients were classified as asymptomatic, mild, moderate, severe, and critical cases depending on WHO guidelines from October 2020 to February 2021. A specially designed questionnaire was used for patient case information detection.

2.2. Reveres Real-Time Polymerase Chain Reaction Detection and Genome Sequencing

The QIAamp Viral RNA Mini Kit (Qiagene, Germany) was used to extract viral RNA according to

the manufacturer's instructions. The isolated RNA was stored at -70°C until it was needed. The SARS-CoV-2 Multiplex Real-Time RT-PCR Kit (Bioneer/Korea) was used to perform qualitative multiplex RT-PCR detection of the E gene, RdRp, N gene, and internal positive control. The following steps are included in the PCR running program :Reverse transcription, 50 C0, 20 min, 1 cycle, Pre-denaturation, 95 C0, 5min, 1 cycle, Touchdown, 95 C0, 5 sec, 5 cycles and 60 C0, 30 sec, 5 cycles, Denaturation, 95 C0, 5 sec, 40 cycles, Annealing and extension, 58 C0, 30 sec, 40 cycles. Five samples were sent for genomic strain sequencing, asymptomatic and mild cases were associated with the 20I (Alpha, V1) strain, while moderate, severe, and deceased cases were associated with the 19A strain.

2.3. Phylogenetic Analysis

The phylogenetic analysis was performed using MEGA software (version 20) using the neighbor-joining analysis method with 1,000 bootstrap replications. Phylogenetic tree compared the Five Iraqi SARS-CoV-2 with 10 reference sequences. The reported Iraqi SARS-CoV-2 sequences revealed many silent and missense mutations across the genome, when compared to the Wuhan (LR757996) reference, and the correlation with the genetic sequence was determined by Illumina Miseq technology.

2.4. Immunological Assay

Serum was separated from blood collected in serum gel tube to determine the Enzyme-Linked Immunoassay Sorbent Assay (ELISA) to detect Interleukins level in patients' serum (IL-10, IL-18, and TNF-α) using BT LAB Kit provided by (Bioassay/Technology Laboratory, China).

2.5. Liver enzymes (Alanine Transaminase, Aspartate Transaminase, and Lactate Dehydrogenase) Determination

Serum was separated from blood collected in a serum gel tube to determine the LDH, AST, and ALT levels in patients' serum using (Roche Diagnostic cobasC311/Germany).

2.6. Statistical Analysis

The data were analyzed in SPSS software (version 20) using the t-test to assess the significant differences between the means and the Person's correlation to examine the correlation between parameters. A *P*-value of less than 0.05 was considered statistically significant.

3. Results and Discussion

The outcomes of this study were based on 100 patients distributed over five groups with different frequencies: severe (41%), mild (22%), asymptomatic (15%), moderate (13%), and deceased cases (9%) as displayed in figure 1.

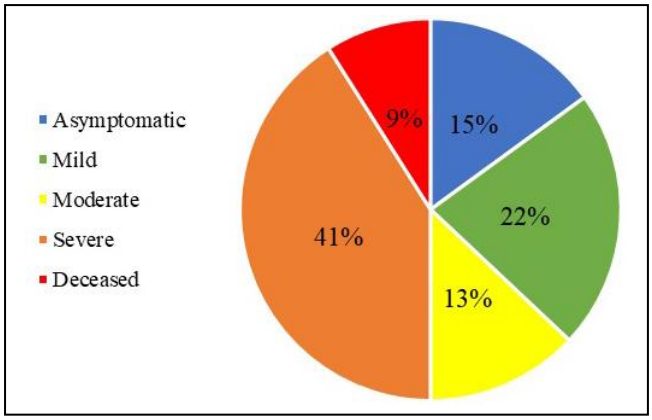

Figure 1. Disease severity distribution among 100 COVID-19 patients

All three parameters, including IL-10, IL-18, and TNF-α showed a statistically significant difference between groups with different disease severity as depicted in figure 2.

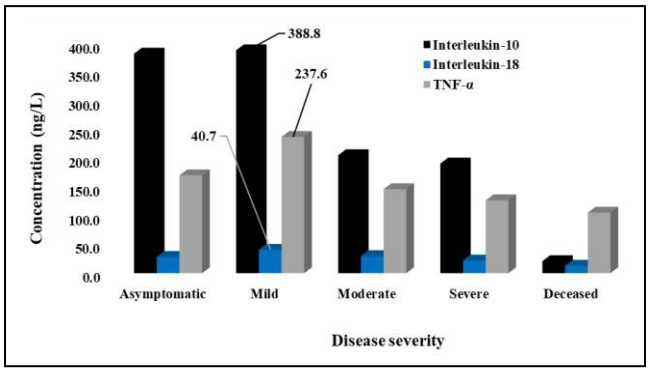

Figure 2. Differences in IL-10, IL-18, and TNF-α among COVID-19 patients with different levels of disease severity

The IL-10 was highest in "mild" and "asymptomatic" cases, demonstrating a progressive decrease with an increase in disease severity. The linear regression showed a strong inverse relationship (R^2=0.90) with disease severity. The IL-10 is used as a measure of disease severity. The COVID-19 patients admitted to the ICU had significantly greater plasma levels of IL-10, as compared to those who were not, according to Huang, Wang (16). The production of IL-10 in the early stages of the disease was associated with the severity of the condition, according to a follow-up clinical examination involving 71 COVID-19 patients from Beijing, China (53 moderate, 18 severe, and 18 controls). Several studies have pointed to elevated levels of Interferon-gamma (IFN-γ) in patients with COVID-19 (16-18).

One research indicated that CD4+ T cells generated less IL-18 in patients with severe COVID-19 than those with mild COVID-19, which might be explained by the reduced quantity and functional depletion of T cells in severe COVID-19 patients. Consequently, it was proposed that the greater levels of IFN are due to macrophages rather than T cells (19). The TNF-α level in the blood has been found to be increased in severe COVID-19 cases in recent studies (20). The TNF- α levels in the blood were found to be greater in certain patients who had been infected with the coronavirus in the respiratory system (21). On the other hand, no significant increase was observed in serum concentrations of TNF-α in moderate and severe COVID-19 patients, signifying that the reaction of the host immune system is likely to be different from SARS-CoV-2 due to infections from other pathogens. The LDH levels showed statistically significant differences and there was a strong positive linear relation (R^2=0.90) with increasing disease severity as illustrated in figure 3.

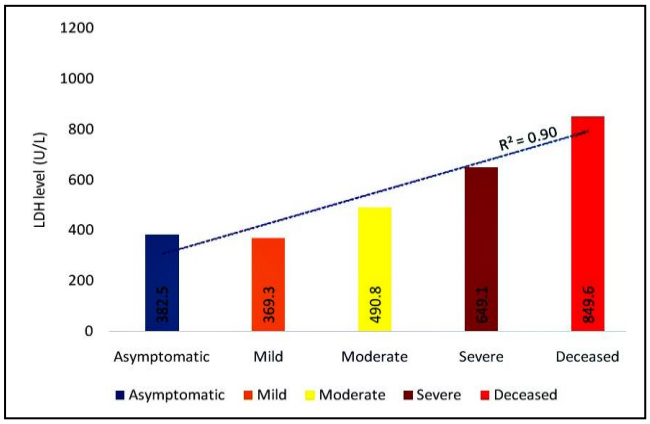

Figure 3. Lactate dehydrogenase levels among 100 patients with different severities of disease

The LDH concentration was significantly increased among the risk variables. Several studies have linked COVID-19 severity to higher LDH levels. COVID-19 is thought to produce direct liver harm as a result of viral hepatitis and immunological interactions involving intrahepatic cytotoxic T cells and Kupffer cells (22). Han, Zhang (23) proposed that either LDH is significantly correlated with lung damage and illness severity or the myocardial and liver damage caused by COVID-19 is attributable to the direct damage of the virus to the targeted organ rather than hypoxia caused by lung injury. It was also demonstrated that increasing lactate production by LDH causes a rise in immune suppressive cells and a reduction in cytolytic cells, both of which are strongly associated with disease severity (24). Multiple organ injury and failure appear to play a more critical role in this path of physiology in impacting clinical outcomes in COVID-19 individuals with elevated LDH levels. The ALT and AST differed significantly in all groups.

Both enzymes had strong polynomial relation with disease severity increasing gradually from asymptomatic to mild to moderate, dropping slightly for severe cases before sharply rising again in deceased cases as depicted in figure 4.

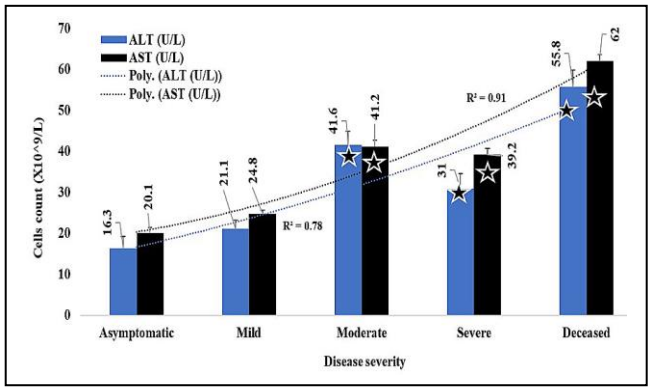

Figure 4. ALT and AST levels among 100 patients with different severities

Several hospital-based investigations have found evidence of liver damage in COVID-19 patients, with elevated levels of the liver enzymes (AST and ALT) ranging from 14%-53 % higher than normal (25). Furthermore, liver biopsy specimens from a dead COVID-19 patient revealed moderate micro-vesicular steatosis, modest lobular, and portal activity, indicating that SARS-CoV-2 was involved in liver damage (26). In another investigation, ALT and AST levels were shown to be significantly greater in deceased patients, as compared to those in recovered ones; moreover, AST levels were increased in 52% of deceased patients and 16% of those who survived (27). The levels of ALT and AST revealed a statistically significant rise in severe COVID-19 patients, compared to mild patients in a retrospective analysis (28).

Five samples were sent for genomic strain sequencing, asymptomatic and mild cases were associated with the 20I (Alpha, V1) strain, while moderate, severe, and deceased cases were associated with the 19A strain. The largest gene, ORF1ab, is made up of overlapping open reading frames that code for the polyproteins PP1ab and PP1a. The polyproteins are broken into NSP1-16, which are nonstructural proteins. The A-1 ribosomal frame shifting event is required for the generation of the longer (PP1ab) or shorter (PP1a) protein. Among the proteins discovered are papain-like proteinase (NSP3), 3C-like proteinase (NSP5), RNA-dependent RNA polymerase (NSP12, RdRp), helicase (NSP13, HEL), endoRNAse (NSP15), 2'-O-Ribose-Methyltransferase (NSP16), and other nonstructural proteins.

Nonstructural proteins in SARS-CoV-2 are involved in viral transcription, replication, proteolytic processing, suppression of host immunological responses, and host gene expression suppression. According to Gene ID: 43740578, updated on Nov. 22, 2021, by NCBI, about 12 single nucleotide polymorphisms (snp) were obtained in the ORF1ab region: three of them were missense and occurred in Iraq. Baghdad SARS-CoV-2 isolate 19A-S632-26.2020 in gene bank position to A/T 8274, T/G 17405, and T/C 20865, which were also missense, occurred in Iraq. Baghdad SARS-CoV-2 isolate 19A-S683-50.2020 in gene bank position A/T 2852. These mutations all occurred in patients infected with the old version, the Wuhan stain.

In the 20I (Alpha v1) strain, single nucleotide polymorphisms (snp) were obtained in the ORF1ab region: six of which belonged to the sample Iraq, Baghdad SARS-CoV-2 20I (Alpha, V1)-S691-76.202, three of which were missense in gene bank position G/C 9276, C/T 18040, and T/A 20542, and the three others were silent in gene bank position T/C 10227, C/T 19854, and T/C 20703. The last sample which had two single nucleotide polymorphisms (snp) was obtained in the ORF1ab region that occurred in Iraq. Baghdad SARS-CoV-2 20I (Alpha, V1)-S725-59.2021 in gene position to G/C 9276, which were also missense, as displayed in table 1.

The S protein, a glycoprotein, is a homotrimer that is required for the development of infection. The S protein monomers have a molecular weight of approximately 140 kDa and comprise 1, 273 amino acids. The main purpose of S protein is binding to the Angiotensin-Converting Enzyme 2 (ACE2) receptor and then entering the host cell. The location between 21290 and 25384 lengths is 3822 nt (29). There is a polymorphism in nucleotide shifting three in original one in gene bank position G/A 23380, G/A 233,581, and G/A 24,850, two of which were missense on silent,

and eight mutants frequently between alpha v1 strain in gene bank four positions in T/C 21621, T/G 24506, C/G 24914, and T/G 25362 belong to the Iraqi sample. Baghdad SARS-CoV-2 20I (Alpha, V1)-S691-76.2021 and the other four in gene position T/C 21621, T/G 24506, C/G 24914, and T/G 25362 belong to the sample Iraq. All eight mutants of Baghdad SARS-CoV-2 20I (Alpha, V1)-S725.-59.2021 were missense as depicted in table 2.

The M gene is significant since it codes for both matrix and membrane proteins; moreover, it has a length of 666 nt, ranging from 26398 to 27063. There is a polymorphism in nucleotide shifting, three in original one in gene bank position T/A 26708, G/A 26904, and C/T 27014, two silent and one missense, as displayed in table 3.

Table 1. Detection of mutant ORF1ab SARS-CoV-2 isolated in different regions of Iraq

No.	Location of gene bank	Nucleotide change	ID. of sample	Amino acid change	Predicted effect	Type of mutation
1	A/T 8274	AAT>TAT	Iraq. Baghdad SARS-CoV-2 19A-S632-	N/Y	Missense	Transversion
2	T/G 17405	TTA>GTA	26.2020	L/V	Missense	Transition
3	T/C 20865	TTT>TCT	Sever	F/S	Missense	Transition
4	A/T 2852	GAA>GTA	Iraq. Baghdad SARS-CoV-2 19A-S683-50.2020 Died	E/V	Missense	Transversion
5	G/C 9276	TGG>TCG	Iraq. Baghdad SARS-CoV-2 20I (Alpha, V1)-S691-76.2021 Asymptomatic	W/S	Missense	Transversion
6	T/C 10227	CTC>CTT		L/L	Silent	Transition
7	C/T 18040	TCT>GCT		S/A	Missense	Transversion
8	C/T 19854	GAC>GAT		D/D	Silent	Transition
9	T/A 20542	TCC>ACC		S/T	Missense	Transversion
10	T/C 20703	TAT>TAC		Y/Y	Silent	Transition
11	G/C 9276	TGG>TCG	Iraq. Baghdad SARS-CoV-2 20I (Alpha, V1)-S725-59.2021 Mild	W/S	Missense	Transversion
12	G/C 10055	GAG>GTG		E/V	Missense	Transversion

Table 2. Detection of mutant S gene SARS-CoV-2 isolated in different regions of Iraq

No.	Location of gene bank	Nucleotide change	ID. of sample	Amino acid change	Predicted effect	Type of mutation
1	G/A 23380	GGT > GAG	Iraq.Baghdad SARS-CoV-2 19A-	G/E	Missense	Transition
2	G/A 233581	CAT>CCT	S697-62.2020	H/P	Missense	Transition
3	G/A 24850	GTG>GTA	Moderate	V/V	Silent	Transition
4	T/C 21621	ATC>ACC	Iraq.Baghdad SARS-CoV-2 20I (Alpha, V1)-S691-76.2021 Asymptomatic	I/T	Missense	Transition
5	T/G 24506	TCA>GCA		S/A	Missense	Transition
8	C/G 24914	CAC>GAC		H/D	Missense	Transversion
9	T/G 25362	GTA>GGA		H/G	Missense	Transition
10	T/C 21621	ATC>ACC	Iraq.Baghdad SARS-CoV-2 20I (Alpha, V1)-S725.-59.2021 Mild	S/T	Missense	Transition
11	T/G 24506	TCA>GCA		S/A	Missense	Transition
12	C/G 24914	CAC>GAC		H/D	Missense	Transversion
13	T/G 25362	GTA>GGA		H/G	Missense	Transition

Table 3. Detection of mutant M gene in SARS-CoV-2 isolated in different regions of Iraq

No.	Location of gene bank	Nucleotide change	ID. of sample	Amino acid change	Predicted effect	Type of mutation
1	T/A 26708	TTT>GTT	Iraq.Baghdad SARS-CoV-2	F/V	Missense	Transversion
2	G/A 26904	GAG>GAA	19A-S697-62.2020	E/E	Silent	Transition
3	C/T 27014	TAC>TAT	Moderate	Y/Y	Silent	Transition

Both the genetic material (RNA) and the structural proteins required for virus entry into the host cell are included within virus particles. The infectious RNA of a virus encodes structural proteins that form viral particles, nonstructural proteins that drive virus assembly and reproduction while also controlling the host response to the virus and accessory proteins with unknown roles once inside the cell. The ORF7a is a viral accessory protein that is encoded. It is assumed to be a type I transmembrane protein based on its resemblance to other coronavirus proteins and has a length of 369 nucleotides (nt) ranging from 27,273 to 27,641 (29). There were seven polymorphisms in nucleotide shifting, one in the original one in gene bank position A/T 27449, missense belonging to the Iraqi sample. Baghdad SARS-CoV-2 20I (Alpha, V1)-S691-76.2021 in gene bank positions GC/TA 27541, CT/TA 27534, and G/T 28095, two missenses and one silent, and the other three shifting in Iraq. Baghdad SARS-CoV-2 20I (Alpha, V1)-S725.-59.2021in gene bank position GC/TA 27541, C/T 27568, and T/A 28086, also two missenses and one silent, as illustrated in table 4.

The N gene protein, nucleocapsid protein, contains two folds. The ORF9a consists of 31 amino acids, while ORF9b contains 98 amino acids and suppresses the IFN-I response (30). The N gene has a length of 1269 nt, ranging from 28,120 to 29,388. There are four polymorphisms in nucleotide shifting, two in original one in gene bank position Iraq. Baghdad SARS-CoV-2 19A-S697-62.2020 ORF9a in location A/G 28766, C/G 28768 (missense), and the other two shiftings belong to the UK strain in Iraqi samples. Baghdad SARS-CoV-2 20I (Alpha, V1)-S725.-59.2021 the two samples are from the same gene bank, ORF9b in position GAT/CTA 28281 (missense) as displayed in table 5.

The ORF3a is thought to be a viroporin-like protein with an ion channel activity that activates the inflammasome NLRP3. It could be involved in virus replication and pathogenesis as well. The ORF3a gene has a length of 828 nt, ranging from 25393 to 26220 nt (29). There were two polymorphisms in nucleotide shifting in the UK strain in Iraqi samples. Baghdad SARS-CoV-2 20I (Alpha, V1)-S725.-59.2021, the two samples are the same gene bank position G/T 25628 (missense) as shown in table 6.

In E gene, there were two polymorphisms in nucleotide shifting in the origin strain in the Iraqi sample. Baghdad SARS-CoV-2 19A-S632-26.2020 in gene bank position A/T 20865 (missense) and the other shifting in Iraq. Baghdad SARS-CoV-2 19A-S697-62.2020 in gene bank position A/T 26269 (missense), as demonstrated in table 7.

The five Iraqi sequences retrieved ranged in length from 29,541 to 29,825 nucleotides and encompassed the whole coding area and over 99.1% of the genome. The sequences that have been duplicated and sequences with missing parts have been eliminated as well. The 10 selected sequences came from 10 different nations across the globe. Figure 5 demonstrates that Iraqi SARS-CoV-2 sequences were spread in separate clusters independently of each other, with strains primarily from Europe and Asia.

Table 4. Detection of mutant ORF7a gene in SARS-CoV-2 isolated in different regions of Iraq

No.	Location of gene bank	Nucleotide change	ID. of sample	Amino acid change	Predicted effect	Type of mutation
1	A/T 27449	ACA>TCA	Iraq.Baghdad SARS-CoV-2 19A-S697-62.2020 Moderate	T/S	Missense	Transversion
2	GC/TA 27541	GCT>GTA	Iraq.Baghdad SARS-CoV-2 20I	A/V	Missense	Transition
3	CT/TA 27534	GCT>GTA	(Alpha, V1)-S691-76.2021	A/V	Missense	Transition
4	G/T 28095	CTT>CTA	Asymptomatic	L/L	Silent	Transition
5	GC/TA 27541	GCT>GTA	Iraq.Baghdad SARS-CoV-2 20I	A/V	Missense	Transition
6	C/T 27568	CAA>TAA	(Alpha, V1)-S725.-59.2021	Q/Z	Missense	Transition
7	T/A 28086	CTT>CTA	Mild	L/L	Silent	Transversion

Table 5. Detection of mutant N gene in SARS-CoV-2 isolated in different regions of Iraq

No.	gene	Location of gene bank	Nucleotide change	ID. of sample	Amino acid change	Predicted effect	Type of mutation
1	ORF9a	A/G 28766	AAA>AGG	Iraq.Baghdad SARS-CoV-2 19A-S697-62.2020 Moderate	K/R	Missense	Transition
2	ORF9a	C/G 28768	CAT>CCT		H/P	Missense	Transversion
3	ORF9b	GAT/CTA 28281	GAT>CTA	Iraq.Baghdad SARS-CoV-2 20I (Alpha, V1)-S691-76.2021 Asymptomatic	D/L	Missense	Transition
4	ORF9b	GAT/CTA 28281	GAT>CTA	Iraq.Baghdad SARS-CoV-2 20I (Alpha, V1)-S725.-59.2021 Mild	D/L	Missense	Transition

Table 6. Detection of mutant ORF3a gene SARS-CoV-2

No.	Location of gene bank	Nucleotide change	ID. of sample	Amino acid change	Predicted effect	Type of mutation
1	G/T 25628	GTT>GAT	Iraq.Baghdad SARS-CoV-2 20I (Alpha, V1)-S691-76.2021 Asymptomatic	V/D	Missense	Transition
2	G/T 25628	GTT>GAT	Iraq.Baghdad SARS-CoV-2 20I (Alpha, V1)-S725.-59.2021 Mild	V/D	Missense	Transition

Table 7. Detection of mutant E gene SARS-CoV-2

No.	Location of gene bank	Nucleotide change	ID. of sample	Amino acid change	Predicted effect	Type of mutation
1	A/T 20865	AGC>GGC	Iraq.Baghdad SARS-CoV-2 19A-S632-26.2020 Sever	S/G	Missense	Transversion
2	A/T 26269	AGC>GGC	Iraq.Baghdad SARS-CoV-2 19A-S697-62.2020 Moderate	S/G	Missense	Transversion

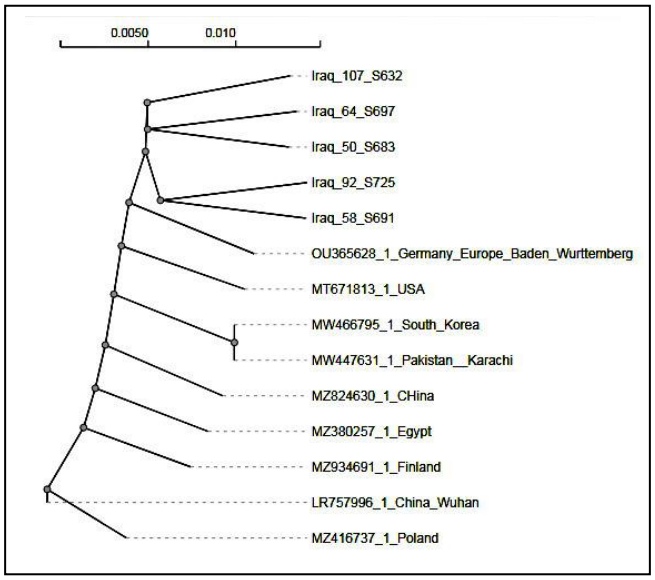

Figure 5."Phylogenetic tree comparing the Five Iraqi SARS-CoV-2 with 10 reference sequences"

Two samples from the five, asymptomatic and mild cases, were associated with the 20I Alpha, V1 the UK (B.1.1.7). This variation appears to have originated and/or first expanded in the South East of England, as announced on December 14, 2020. Multiple mutations in Spike are linked to Variant 20I (Alpha, V1), especially noteworthy: S: N501Y. The two oldest genomes of the B.1.1.7 strain were collected on 20 September 2020 in Kent and 21 September 2020 in Greater London. Infections with B.1.1.7 were identified in the United Kingdom until early December 2020. In comparison with the results of this study, it was found that this strain spread rapidly since it appeared in Baghdad while collecting samples in mid-January, and the symptoms of the disease were not as severe as in the original strain since it appeared among asymptomatic and mild groups.

The fast spread of this lineage highlights the need for more global genomic and epidemiological surveillance, as well as laboratory antigenicity and infectivity testing (31). The five Iraqi SARS-CoV-2 sequences were non-identical with 0.1% nucleotide divergence between each other. The reported Iraqi SARS-CoV-2 sequences revealed many silent and missense mutations across the genome when compared to the Wuhan (LR757996) reference. There were 52 SNP mutations in total, with 17 and 35 SNPs found in the non-structural and structural regions, respectively. Each sequence had a unique SNP profile (the number of SNPs found in each sequence varied from 2 to 12). The majority of SNPs in the structural area were observed in the S gene, which codes for the spike protein.

The phylogenetic analysis of typical sequences from 10 nations assigned the five Iraqi sequences to five groups, along with sequences from other countries. These findings pointed to several SARS-CoV-2 viral introductions in Iraq. According to Yin (2021), four primary genotypes may be identified based on the placements of SNPs (32). The SNPs allowed us to distinguish three distinct genotypes in the Iraqi sequences, based on their comparison with the Wuhan reference sequence. These SNPs were identified as the most frequently occurring mutations observed from the beginning of the pandemic among the four studied primary genotypes. The genome of SARS-CoV-2 is designated as LR757996 (32, 33).

The SNP mutations in the M, S protein, RNA polymerase, RNA primase, and nucleoprotein, which are important proteins for diagnosis and vaccine production, have been previously discovered (32, 33). Understanding the viral propagation and genetic development over time requires the analysis of SARS-CoV-2 sequences from the majority of afflicted countries in various stages of the COVID-19 epidemic. This exploratory inquiry revealed the results of the first SARS-CoV-2 whole genome sequencing from Iraq. We need more sequences from a variety of clinical and epidemiological settings in order to track the molecular epidemiology of SARS-CoV-2 and identify mutations within these sequences that cause a gain-of-function mutation.

The Iraqi variation of the Wuhan strain (A19) is more severe than the UK Alpha V1 strain, which matches the date of sample collection and the strains circulating at that time. The groups of original Wuhan strain (moderate, severe, and deceased) had a high rate of parameters, compared to the minor increase in groups (asymptomatic and mild) in alpha strains that started appearing in early 2021, indicating a link between biochemical parameters and SARS-CoV-2 variations. On the other hand, Interleukins (IL-10, IL-18, and TNF-) were demonstrated to differ statistically between groups with varying degrees of disease severity. Finally, there were statistically significant variations in plasma liver enzymes.

Authors' Contribution

Study concept and design: N. S. M.
Acquisition of data: R. A. S.
Analysis and interpretation of data: N. S. M.
Drafting of the manuscript: A. A. T.
Critical revision of the manuscript for important intellectual content: R. A. S.

Statistical analysis: A. A. T.
Administrative, technical, and material support: N. S. M.

Ethics

The research was carried out following the consent of the Ethics Committee of the College of Biotechnology-University of Technology.

Conflict of Interest

The authors declare that they have no conflict of interest.

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