ISSN: 0067-2904

N Gene Mutations of SARS-CoV-2 and their Association with Variants and Pathogenicity among Iraqi Patients

Raghad KH. Maeh[1,2*], Hula Y. Fadhil[2]
[1]*Applied science department, Biotechnology division, University of Technology.*
[2]*Biology department, College of Science, University of Baghdad, Baghdad, Iraq.*

Received: 11/9/2024 Accepted: 18/2/2025 Published: 28/2/2026

Abstract:
Global spreading happened for a novel coronavirus called SARS-CoV-2 that emerged in 2019. The viral genetic diversity studies help us to comprehend the virus's transmission, pathogenesis, and epidemiology. The main aim of this study is to improve the dataset of Iraqi SARS-CoV-2 mutation, monitor virus spread and evolution, and identify possible mutation patterns. As seen from this study, all the sequenced positive samples had mutations in variable regions of N gene. It was discovered in 23 local isolates that 18 were Omicron associated with moderate infections, accounting for 61.1%. The remaining isolates comprised three Alpha and two Beta variants, all associated with severe infection, caused mutation leading to alteration of amino acids and nuclear proteins including transition transversion deletion etc. In addition, there is an altered coiled structure presented by the Alpha variant. The phylogenetic tree demonstrates higher similarity with other local isolates and very little diversity between references and worldwide isolates. In conclusion, the analysis of the N gene variable region brings important data on mutations that are useful for detecting variants and developing new detection assays against coronavirus-causing COVID-19 disease, referred to as SARs-Cov2. These findings may contribute to improved treatment options for people suffering from COVID-19 and vaccine development.

Keywords: Iraqi population, N gene, mutation, COVID-19

طفرات N gene للسارس كوفيد 2 ومصاحبتها للتغايرات والامراضية بين المرضى العراقيين

رغد خويطر مايح[2,1*] , حلا يونس فاضل [2]
[1]قسم العلوم التطبيقية,فرع التقنيات الاحيائية,الجامعة التكنلوجية
[2]قسم علوم الحياة,كلية العلوم,جامعة بغداد,بغداد العراق

الخلاصة:
حدث انتشار عالمي لفايروس كورونا يسمى السارس كوفيد 2 في عام 2019 . لذلك هذا الفايرس يجب أن يراقب بجد ويتم تتبع أي نوع من التغييرات بما في ذلك الطفرات التي تحدث بين المرضى الذين ثبتت إصابتهم. تساعدنا دراسات التنوع الجيني الفيروسي على فهم انتقال الفيروس وإمراضيته ووبائيته.تهدف هذه الدراسة الى التحري عن التغايرات الجينية في منطقة N gene للسارس كوفيد 2 في العزلات العراقية .وجد في هذه الدراسة جميع العزلات الموجبة تمتلك طفرات في منطقة التغاير N gene فكانت 23 عزلة محلية Omicron 18, كانت متصاحبة مع الاصابة المعتدلة بنسبة 61.1%. اما العزلات المتبقية فكانت ثلاثة الفا

*Email: raghadnoor79@gmail.com

واثنين بيتا متصاحبة مع الحالات الحادة . هذه الطفرات التي تسبب تغيير في الاحماض الامينية هي من نوع deletion, transition transversion الخ. بالاضافة الى ذلك وجد تغيير في تركيب البروتين في العزلة نوع الفا . وجد تشابه كبير في الشجرة الجينية مع باقي العزلات المحلية واختلاف بسيط بين references والعزلات العالمية . في الخلاصة تحليل منطقة التغاير N gene يعطي معلومات مهمة عن الطفرات للكشف عن التغايرات وتطوير طرق كشف جديدة ضد فايروس كورونا المسبب لمرض كوفيد 19 والمسمى بالسارس كوفيد 2 وهذا سوف يساعد في تطوير العلاج للناس المصابين بالكوفيد 19 وتطوير اللقاحات.

Introduction

The COVID-19 virus, initially found in Wuhan City, Hubei Province, China, in December 2019, has gone global [1]. SARS-COV-2 was the causative agent identified using laboratory diagnosis and by sequencing the genome with the help of next-generation sequencing (NGS)[2]. Within the Betacoronavirus genus, SARS-CoV-2 is part of the Sarbecovirus subgenus and one of the largest RNA viral genomes in the Coronaviridae family. It is an enveloped, non-segmented positive sense RNA virus with a genome about 30 kb long [3]. The initial whole-genome sequencing of SARS-CoV-2 isolates from affected patients within China showed high sequence identity amongst each other and RaTG13 bat Coronavirus strain [4]. Several investigations have been conducted on geographic distribution analysis and genetic diversity of SARS-CoV-2 using whole-genome sequencing (WGS) and categorizing strains by the Center for Disease Control CDC [5]. Such mutations shown by the presence of RNA viruses have various implications for diagnosis approaches, therapeutic strategies, and vaccine performance [6]. This particular study intends to analyze mutations in the N gene variable regions for previously sequenced coronavirus isolates that tested positive. Various mutations, such as transversion, transition, and deletion, were observed in local isolates. Transitions include changing two-ring purines (A, G) or one-ring pyrimidines (C, T), while transversions involve substituting purine with pyrimidine bases, leading to the exchange of one-ring and two-ring structures. Nucleocapsid has been shown to be crucial in viral diagnostics and the development of new vaccines [7]. It upholds the viral envelope within the structure of the viral genome and plays a vital role in viral budding, assembly, and the response of host cells during infection [8]. Besides, N gene is highly conserved across CoVs, having lower mutation rates compared with S-protein, which is more stable. Furthermore, N-protein serves as a significant target for the response of T-cells, thereby making it a probable candidate against newer strains of COVID-19 in next-generation vaccine studies. The nucleocapsid is a significant viral gene/protein concerning diagnostics (nucleic acid and antigen detection) and new vaccine design. Its function is to keep the genome structure inside the envelope and plays a significant role in viral budding, assembly, and the cellular response of the host to infection with the virus [9]. The main aim is to improve the dataset of Iraqi SARS-CoV-2 mutation, monitor virus spread and evolution, and identify possible mutation patterns. It is essential to understand the genetic diversity of the virus within Iraq for public health intervention efforts, and it can help formulate more effective therapies and preventive approaches specifically for the Iraqi population. Progressive research is required to distinguish more objectives of vaccines that are relatively resistant to genetic variety.

Materials and Methods

Study population

The study involved 30 patients with COVID-19 disease (moderate and severe) at Baghdad Teaching Hospital. The College of Sciences' Research Ethics Committee at the University of Baghdad, Iraq, approved the study protocol (Ref. No.: CSEC/0922/0084). The patients were swabbed nasally after a period of between four to five days at the hospital. Viral RNA was extracted using QIAamp Mini kit-Viral RNA. Real-time reverse transcriptase

polymerase chain reaction (rRT-PCR) identified SARS-CoV-2 by employing a commercial kit (AccuPower® SARS-CoV-2 Multiplex RT-PCR Kit, Bioneer, Korea) as per the manufacturer's protocol for amplification and positivity determination. Additionally, chest CT scans were obtained alongside other clinical information to confirm the diagnosis. The presence of SARS-CoV-2 variants was investigated by examining mutations in gene spike (S-gene) using AccuPower® SARS-CoV-2 Variants ID RT-PCR Kit (Bioneer, Korea), which identified Beta, Alpha, Gamma, and wild type variants based on specific mutations in the kit. The identification of the Omicron variant involved employing the TaqPath COVID-19 PCR test (TaqPath COVID-19 CE-IVD RT-PCR Kit, Thermo Fisher, Germany). Infections were described as SGTF (S Gene Target Failure Assay) when a patient's TaqPath COVID-19 PCR test was positive, and the ORF1ab or nucleocapsid gene targets had a cycle threshold of 36 or lower with non-detectable S gene [10]. There are two groups of patients, severe and moderate (31 and 68). WHO Interim Guidance recognized Criteria: 1- moderate (patient infected with pneumonia and without severe pneumonia) 2- severe (pulse oxygen saturation (SpO2) ≤ 93% or the rate of respiration ≥ 30 breaths/min, severe respiratory distress).

SARS-CoV-2 N gene Detection

Positive samples in fast rRT-PCR for SARS-CoV-2 were selected for testing in conventional one-step RT-PCR, and subsequences were tested for sequencing. N gene conserved region amplification product size accounted for approximately 889 base pairs, using specific primers that included forward primer-5′ -ATG TCTGATAATGGACCCCAAA3` and reverse primer 5′CAGTTCCTTGTCTGATTAGTTCC3`. This product was used for detection and sequencing as directed by this study through Amplifx software with reference to NC_045512.2. For preparing RNA template amplification solution through conventional RT-PCR SuperScript TM III Platinum TM one-step RT–PCR kit from Invitrogen USA was applied .The reaction mixture (25 µl) after optimization contained 12.5 µl of reaction buffer (5x), 0.5 µl of SuperScript TM III RT/Platinum TM Taq mix, 0.8 µl of each primer (mention the concentration), and 0.4 µl of nuclease-free, and 10 µl of RNA template, the amplification program comprised an incubation at 50 ºC for 30 minutes to synthesize cDNA and then denaturation at 94ºC for 3 minutes.

The cycling stage involved incubation at 94 ºC for fifteen seconds, annealing at forty-five degrees Celsius for half a minute, and extension at seventy-two degrees Celsius for thirty seconds. Lastly, there was an extension step that took place at seventy two degrees Celsius, lasting seven minutes.

Gel electrophoresis

Improved RT-PCR results were isolated through electrophoresis on a 1.5% agarose gel using TBE buffer (1X). The staining with 4% ethidium bromide dye solution revealed specific primer target bands, as well as ladder bands (100 bp). In every case, both positive and negative controls were implemented. Accurate results were identified by the lack of false-positive results in the negative control tests and by the positive control sample showing positive results when viewed under UV light at 350 nm.

Sequencing of coronavirus samples

The sequencing of human coronaviruses was done using the conventional RT-PCR product (23 samples) and the forward primer for the N gene product (889 bp) on the genetic analyzer (Applied Biosystems) at Macrogen Company, USA. The Basic Local Alignment Search Tool (BLAST) program online was utilized for homology search, employing blastx and blastn algorithms found on the National Center for Biotechnology Information (NCBI) website at (http://www.ncbi.nlm.nih.gov). The phylogenetic tree for classifying the SARS-CoV-2

species was built by utilizing the neighbor-joining method with 1000-bootstrap in MEGA version 11.0 software.

A significance level of 90 was applied as a bootstrap value to demonstrate the importance of the clusters. The findings were cross-referenced with the GenBank database using the ExPASY-translate tool for amino acid sequence and motifs and with blastx; the CDS regions identified in blastn were also compared with ORF-Finder (https://www.ncbi.nlm.nih.gov/orffinder). The quality of the nucleotide sequence analysis was evaluated using Snap gene software.

Results

The result in Figure 1 showed the amplification of a coding region of SARS-CoV-2 positive samples with an amplicon's size of 889 bp.

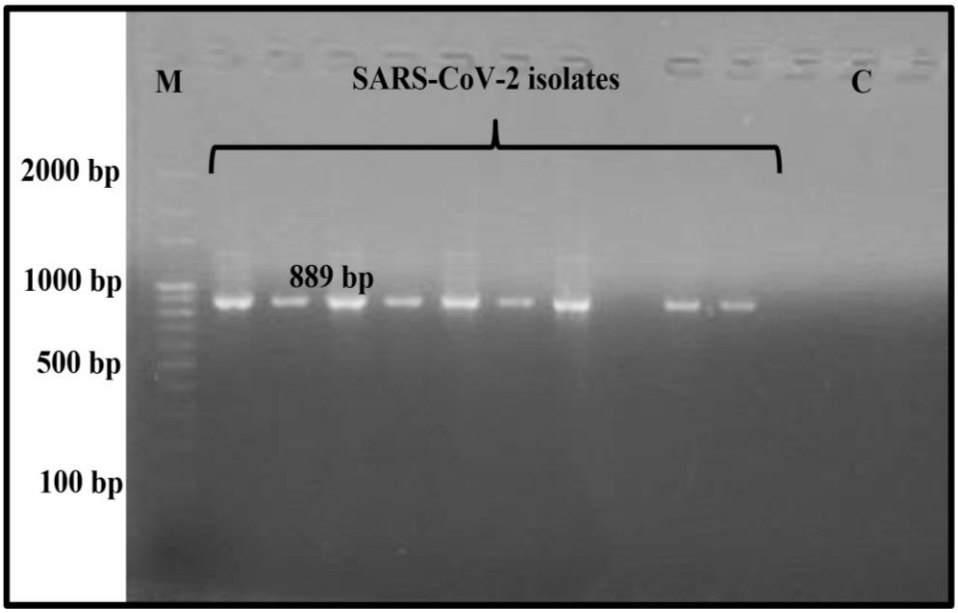

Figure 1: The amplification of N gene for SARS-CoV-2 positive isolates. The amplicon's size was about 889 bp. The amplicons were run on agarose gel 1.5% and visualized with Transilluminator, M: Marker 100 bp, C: negative control. The dye used was Ethidium bromide (EtBr), and the electrophoresis ran at 75V for 50 min.

Regarding the mutations ascertained, SARS-CoV-2 N gene sequence analysis results showed three variants, including Alpha, Beta, and Omicron, with 3, 2, and 18 local isolates, respectively. Figure 2 demonstrates that Omicron variant 11/18 (61.1%) was associated with moderate infections, while the remaining isolates Alpha and Beta variants 100% with severe infection.

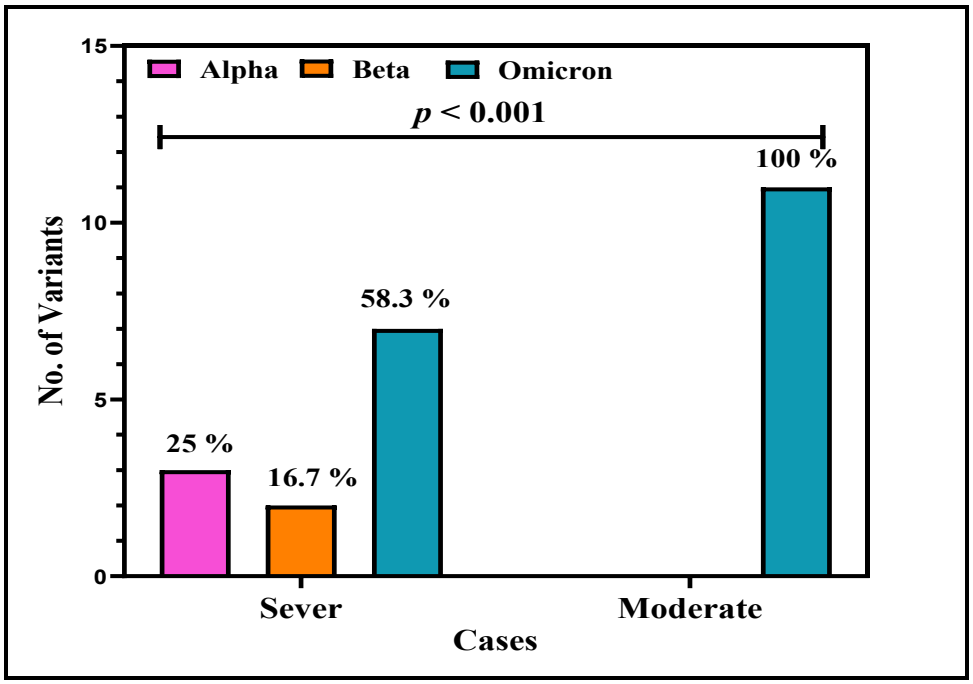

Figure 2: Variants of SARS-CoV-2 stratified N gene mutations and infection severity; *p*: Chi-Square test probability.

Identify mutations of SARS-CoV-2 local isolates stratified reference strains

To identify the viral genome and detect the virus , the nucleic acid of the PCR products was sequenced to check their specificity. To determine the genetic relatedness of isolates, nucleic acid sequencing for selected genomic regions of coronavirus was used. In this study, 23 local isolates were analyzed and compared with the reference strains available in the GenBank database in NCBI. Alignment Search Tool (BLAST), available at the NCBI, was used. The results of the alignment of amino acids were compared with data obtained from GenBank published ExPASY-translate tool and the output of blastx which are available at the NCBI. Sequence using alignment of amino acid sequences of reference strains, established to ensure the alignment of conserved amino acid motifs. The open reading frame was determined within the sequence region by the ORF finder at NCBI.

SARS-CoV-2 positive sequenced isolates in this study showed some variations in mutations in the N genes variable regions. Local isolates showed many types of mutation, which were transition, transversion, and deletion in the flank sense, as summarized in Table 1. Three strains called Alpha, two Beta, and the other Omicron have been discovered locally. The Alpha isolate exhibited 3 transversions (missense mutations) and 3 transitional mutations, whereas the Beta isolate displayed 1 transition (missense mutations), and the other Omicron isolate exhibited 3 transitions (missense mutation) and 3 deletions (Table 1).

Table 1: Type of Mutations, Location, and Amino acid change in sense translated region of Nucleocapsid N gene in Iraqi isolates of Severe acute respiratory syndrome coronavirus 2 Stratified to NCBI Reference Sequence: NC_045512.2

Isolate/ Variants	location of gene bank	Nucleotide change	No. of codon/location	Amino acid change	Predicted effect	Type of mutation
Alpha 1, 2, 3	GA 28280 CT CT 28754 AG C 28757 T G 28761 A G 28763 A A 28769 T	GAT > CTT CTC >AGC CCT > TCT AGG > AAG GGA > AGA ACT> TCT	D3L L201S P202S Q203K G204R T206S	Asparagine > Leucine Leucine > Serine Proline > Serine Glutamine > Lysine Glycine > Arginine Threonine > Serine	Missense Missense Missense Missense Missense Missense	Transversion Transversion Transition Transition Transition Transversion
Beta 1, 2	C 28887 T	ACT > ATT	T205I	Threonine > Isoleucine	Missense	Transition
Omicron 1 to 18	C 28311 T G 28370 Δ C 28367 Δ A 28364 Δ G 28761 A G 28763 A	CCC > CTC GAA > Δ CGC > Δ AGT > Δ AGG > AAG GGA > AGA	P13L E31Del R32Del S33Del R203K G204R	Proline > Leucine Glycine > Del Arginine > Del Serine > Del Arginine > lysine Glycine > Arginine	Missense Deletion Deletion Deletion Missense Missense	Transition Frameshift Frameshift Frameshift Transition Transition

RaptorX software was used (http:reptox.uchicago.eDu/predict) for drawing the protein structure (helixes and coils) and for protein conformational changes detection of three different samples and reference of SARS-CoV-2 taken from NCBI. Furthermore, the Alpha variant is modified in a coiled structure. There was a lack of similarity in the reference with Alpha local strain conformation of protein, as shown in Figure 3.

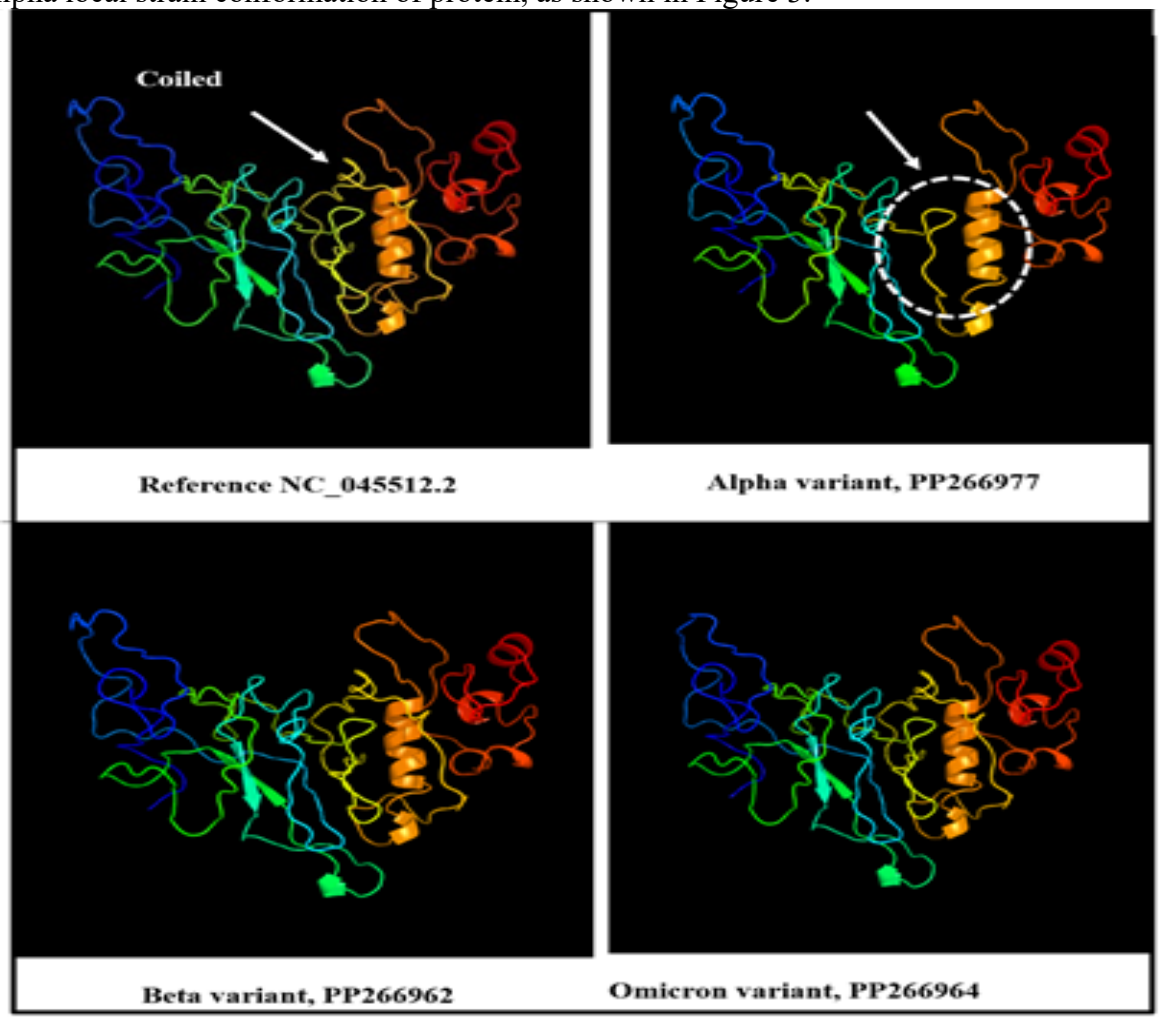

Figure 3: Protein prediction of partial N gene SARS-CoV-2 variants comparing with reference strain by RaptorX tool.

The Phylogenetic trees of SARS-CoV-2 isolates for N genes

As we can see in the phylogenetic tree below (Figure 4), there are three variants in the phylogenetic tree below Omicron, Beta, and Alpha. The similarity between these three isolates and references with a few variants can be observed.

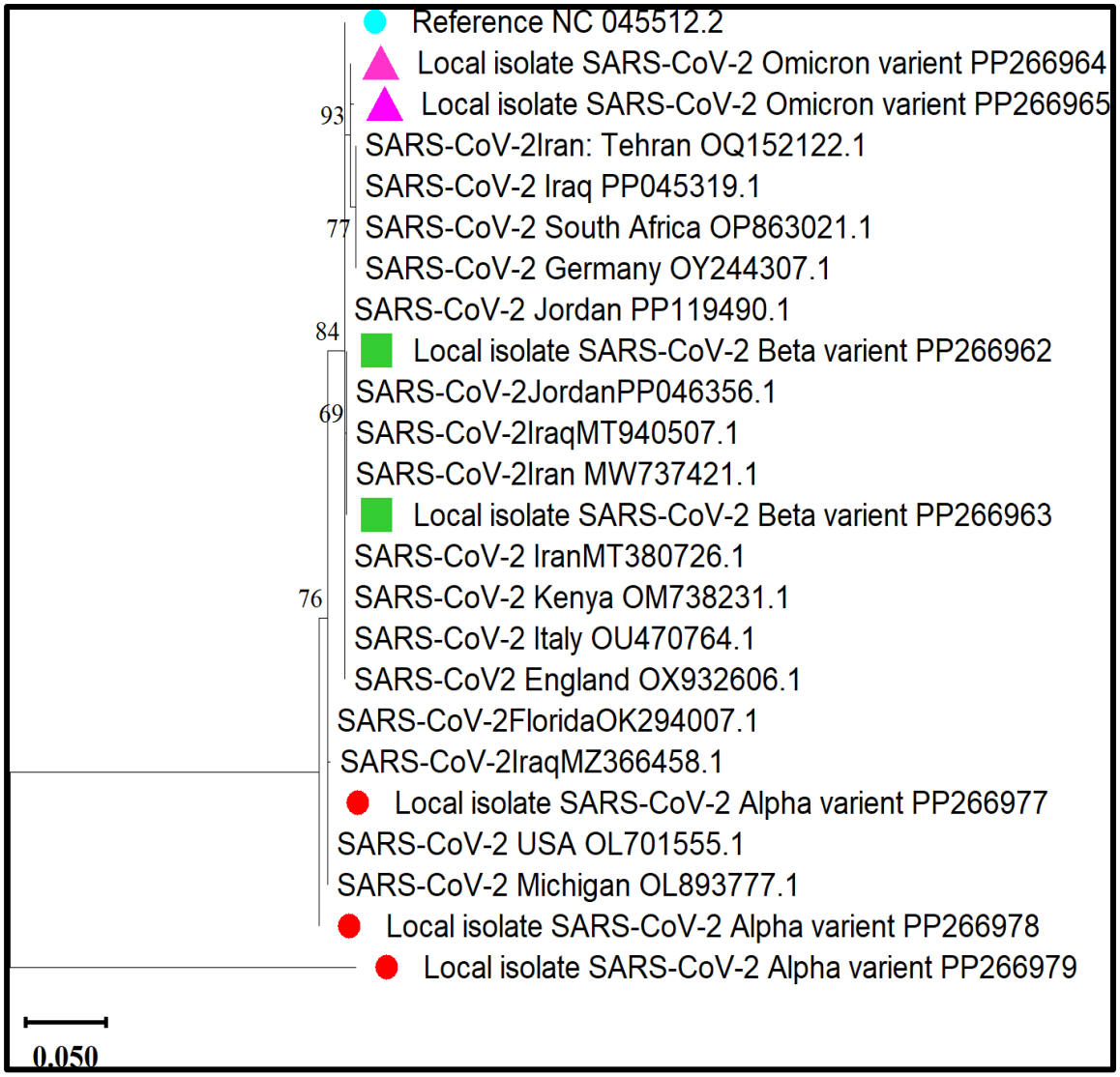

Figure 4: Evolutionary relationships of local isolates of SARS-CoV-2 as demonstrated as a phylogenetic tree. The Neighbor-Joining method was used to infer the evolutionary history. MEGA6 was used to conduct the evolutionary analyses.

Discussion

Research of genetic sequencing has found numerous mutations, mainly consisting of insertion/deletions and single nucleotide polymorphisms (SNPs), that are typically benign or slightly damaging. Yet, a minor portion of mutations has the potential to alter the virus' fitness and assist in its acclimation [11]. These removals or alterations may impact the polarity of the peptides, resulting in modifications to the function and structure of viral proteins associated with infectivity, pathogenicity, antigenicity, and transmissibility.

The sequences testing positive for coronavirus in the study showed different mutations in the variable regions of N gene [12]. Various mutations, such as transversion, transition, and deletion, were observed in local isolates and are listed in Table 1. There were three occurrences of transversion mutations and seven occurrences of transition mutations in total

isolates. Furthermore, ten missense mutations were induced by both transition and transversion, along with three deletions.

A transition mutation involves swapping similar types of nucleotides, while a transversion mutation swaps different classes of nucleotides. In terms of protein synthesis, a missense mutation results in a single amino acid substitution in the resulting protein, while a nonsense mutation causes a premature stop in the protein-building process, leading to a potentially non-functional protein [13]. Transitions include changing two-ring purines (A, G) or one-ring pyrimidines (C, T), while transversions involve substituting purine with pyrimidine bases, leading to the exchange of one-ring and two-ring structures [13]. Transition mutations occur more often than transversions because of molecular processes ("wobble"), leading to a lower chance of amino acid changes and the potential to remain as "silent substitutions" in populations as single nucleotide polymorphisms. Additionally, transitions are less prone to elimination by natural selection since they frequently result in synonymous substitutions that encode the same amino acid sequence as the original DNA [14]. Three Alpha strains, two Beta, and the other Omicron have been discovered locally. The Alpha isolate exhibited 3 transversions (missense mutations) and 3 transitional mutations, whereas the Beta isolate displayed 1 transition (missense mutations), and the other isolate, Omicron, exhibited 3 transitions (missense mutation) and 3 deletions (Table 1). During genome replication, the RNA viruses have minor fidelity for RNA-dependent RNA polymerase, and the activity of proofreading was lacking; therefore, the presence of new variants (genotypes or serotypes) was missed through a particular set of probes or oligonucleotide primers [15]. There are high mutation rates in RNA viruses because of the vicinal 2' hydroxyl group of the sugar moiety. Also, when the virus enters the cell, RNA genomes face numerous cytoplasmic and enzymes of nucleoplasmic that may splice, degrade, and modify them, and in the absence of proofreading activity, RNA viruses could produce new variants that might go undetected. Nonetheless, the coronavirus family of viruses is recognized for possessing a proofreading function linked to the exoribonuclease (ExoN) domain found in nsp14 [16].

The variant of Alpha (B.1.1.7) is related to a 55% (95% CI 39-72%) larger danger of death compared to the original SARS-CoV2 virus; the Alpha variant had major virulence and transmittance, the studies show that the Alpha variant had resistance to neutralization by monoclonal antibodies aiming the NTD supersite.

In replication dynamics in cultured human airway epithelial cells. The Beta shows reduced fitness compared to the Alpha variant. The ability of replicative for Beta was reduced compared to that of the Alpha or progenitor variant when observed in hamster models; as a result, it is important to make key differences, as the variants Alpha and Beta might possess the improved ability to bind to the ACE2 receptor, though the Alpha may replicate more than Beta after the cell infection. Data comparing SARS-CoV-2 WT with the Delta, Beta Alpha, and Omicron variants in a transgenic mouse model showed a trend of attenuation in the emerging

variants, with Omicron being the least severe. This same investigation showed that the Omicron display reduced virus replication in animal cells and humans when compared with the WT viral strain. Infections with Omicron in humans might lead to fewer severe lung symptoms compared to infections with other variants, though it is significant to highlight that studies in cell lines and animals may not accurately reflect the condition of human lungs [17].

In both South Africa and the US, the case fatality rate for the Omicron was half compared with other variants. A mathematical model produced to measure the case fatality rate and

transmissibility of the Omicrn in South Africa showed that the transmissibility rate was higher, and the infection fatality was reduced by 78.7% compared to other variants [18].
Additionally, Gribble *et al.* have demonstrated through experiments that nsp14-ExoN could play a crucial role in RNA recombination events during viral replication, leading to the creation of genetic variants. We investigate important mutations seen in viral proteins that are relevant for diagnostics, treatment, and vaccine advancement [8].

N Protein Nucleocapsid mutation (D3L, G204R, T205I, E31del ,P13L, S33del, R32del,G204R, R203K)

The outcomes of the study by Wu *et al*. displayed that in NS3a Q57H and G251V, S194L and R203K/G204R in N made alterations in the protein structure and also had an effect on the binding affinity of intraviral protein-protein interactions during release of virus and assembly. So, these alterations might be related to the evolution of the virus and advantageous for the virus and its virulence [19].

In-silico study showed that the N protein mutations, especially in the N-terminal domain, changed the flexibility and structure of the protein, while the substitutions in the C-termini are thought to impact the dimerization potential also identified mutations within several domains of the N protein that include common ones such as G204R and R203K.However, the mutations of N-protein on infectiousness and transmission rate remain unclear [20,21]. Variants like the variant of European 20A.EU1 with N-mutation A220V or S-mutation A222V became dominant during the summer of 2020, and in the N gene of the Omicron variant, there is a high number of deletions, affecting diagnostics [22,23]. These mutations and effects on the pathogenicity of the virus require further research.

The structure of proteins

To predict the structure of the protein (coils and helixes), RaptorX software was used (http:reptox.uchicago.eDu/predict) and for detection of the changes in protein conformational for the references and three different samples taken from NCBI. The model structure analysis showed changes in the protein structure for local strains compared to the references in terms of secondary structure folding. The reference had significant dissimilarities to one of the local strain (alpha). However, Beta and Omicron strains of the local isolates had similarities to the references, as shown in Figure (3).

Thus, variations of the amino acid sequence and the appearance of new regions with binding sites affected the functions of the proteins. As for the alpha variant, it was very apparent that there were changes in the structural features of the protein, such as the coiling of the protein. The structures and properties of many proteins of SARS-CoV-2 are still unknown, even though these proteins are involved in the life cycle of the virus. Of these proteins, the N protein is quite paramount in the packaging and assembly of the RNA genotype of the virus into virus particles [24,25,26]. The N protein is an integral of the SARS COV 2 virus [27]. Its primary role entails identifying and enveloping the virus' RNA in a symmetrical helical form and was a highly multifaceted factor in the coronavirus life cycle [28]. Furthermore, N protein is responsible for mRNA transcription and replication of viruses, cytoskeletal tissue homeostasis, and immune control. N protein has also been demonstrated to suppress host anti-virus response and may also induce an immune response; therefore, its detection could be considered important in diagnostics as well as a target for vaccine development [29]. These differences affect the structure of proteins, which in turn affect the function of the N gene.

Phylogenetic trees of SARS-CoV-2 isolates For N gene

The phylogenetic tree shows three isolates Beta, Alpha, and Omicron. There is a clear similarity between the reference and the three isolates. The Bootstrap value for the local isolates compared to GenBank sequences was considerably high, and each isolate is grouped with similar isolates. Omicron and Beta show a high degree of similarity with isolates from various countries, including previous local isolates in Iraq, as well as those from Iran, Jordan, South Africa, and Germany. On the other hand, Alpha is the least similar to Omicron and Beta due to its distinct variants and mutations. These findings further support the notion that the genotype of local isolates is present globally, and the prevalence may significantly differ depending on location and genotype.

The study contains some limitations. First, the small sample size of the patients and controls. Second, a lack of information sources about the variants, mutation, and vaccine in COVID-19, as well as the reliance on specific databases.

Conclusion:

The *N gene* mutations showed specific associations with each variant of SARS-CoV-2, which is essential for severity containment and maintaining the sensitivity and specificity of diagnostic tools. There are three variants, Alpha ,Beta, and Omicron, which have the highest infection percentages. Despite ongoing efforts, the virus persists and spreads, prompting the need to closely monitor any genetic variations and continue research efforts to control the disease.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors declare that there were no conflicts of interest.

Acknowledgments

The authors thank the medical staff at Al-Shifa Medical Center at Baghdad Medical City, as well as the Central Public Health Laboratory (CPHL) / Molecular Biology Department in Baghdad for their kind cooperation.

References:

[1] C. Huang . "Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China", *The Lancet*, vol. 395, no. 10223, pp. 497-506, 2020. Available: 10.1016/s0140-6736(20)30183-5.

[2] P.Yadav,V.Potdar,M.Choudhary,D.Nyayanit,M.Agrawal,S.Jadhav,T.Majumdar,A.Shete-Aich,and A.Basu,P.Abraham. "Full-Genome Sequences of the First Two SARS-CoV-2 Viruses from India". *Indian Journal of Medical Research,* vol.151, no.200. PP 423-429.2020

[3] A.Wu,Y.Peng,B.Huang,X.Ding,X.Wang,P.Niu,J.Meng,Z.Zhu,Z.Zhang,andJ.Wang."Genom e Composition and Divergence of the Novel Coronavirus (2019-NCoV) Originating in China". *Cell Host Microbe*. vol.*27*,no.70.pp 325–328. 2020.

[4] R.Lu,X.Zhao,J.Li,P.Niu,B.Yang,H.Wu,W.Wang,H.Song,B.HuangandN.Zhu,"Genomic Characterisation and Epidemiology of 2019 Novel Coronavirus: Implications for Virus Origins and Receptor Binding". *Lancet*.vol. 395,no.70.pp 10224. 2020.

[5] A. Rahimi, A. Mirzazadeh,A.Tavakolpour and S. Genetics." Genomics of SARS-CoV-2: A Review of the Literature with the Special Focus on Genetic Diversity and SARS-CoV-2 Genome Detection". *Genomics*,vol.14,no.20,pp 113. 2020

[6] C. Roy,S.M.Mandal,S.K.Mondol,S.Mukherjee,W.Ghosh and R.Chakraborty."Trends of Mutation Accumulation across Global SARS-CoV-2 Genomes: Implications for the Ecology and Evolution of the Novel Coronavirus".*Genomics*.vol,25,n0.13,pp123 .2020.

[7] D.Mercatelli and F.M.Giorgi."Geographic and Genomic Distribution of SARS-CoV-2 Mutations". *Front. Microbiol*.vol.11,no.22,pp132-131.2020,

[8] J. Ghazzi, R. Maeh, H. Fadhil, M. Jabir, K. AL-Azawi. The impact of TLR10 on the resolution of influenza A and B infections during the winter season. Microbial Biosystems.10(4)(2025)2025.416673.

[9] Maeh R K, AL-Tameemi A I, Mahmood Z S, Fadhil H Y, AL-azawi K. Co-Analysis of TLR7 and CCL2 as Predictive Biomarkers forH1N1 Influenza Infection Severity. Iran J Med Microbiol. 2025; 19(5):341-53.

[10] N. Wolter, J. Waasila and W. Sibongile. "Early assessment of the clinical severity of the SARS-CoV-2 omicron variant in South Africa: a data linkage study". *Lancet*, vol.399, pp.437–46, 2022.

[11] R. Sanjuán, MR. Nebot, N. Chirico, LM. Mansky and R. Belshaw. "Viral mutation rates". *Journal of Virology*. vol.89,no. 84,pp.9733–48. 2010.

[12] F. Robson, KS. Khan, TK. Le, C. Paris, S. Demirbag and P. Barfuss. "Coronavirus RNA. proofreading: molecular basis and therapeutic targeting". *Molecular Cell*. vol. 79,no.56,pp.710–27.

[13] J. Gribble, LJ. Stevens, ML. Agostini , J. Anderson-Daniels, JD. Chappell and X. Lu. "The coronavirus proofreading exoribonuclease mediates extensive viral recombination". *PLoS Pathogens*. vol.17,no.23,pp226 .2021.

[14] WT. Harvey, AM. Carabelli, B. Jackson, RK. Gupta, EC. Thomson and EM. Harrison. "SARS-CoV-2 variants, spike mutations and immune escape". *Nature Reviews Microbiology*. vol.19.no.20.pp.409–24. 2021.

[15] X. Xia."Domains and functions of spike protein in sars-cov-2 in the context of vaccine design". *Viruses*. vol. 13.no.12.pp.109. 2021.

[16] A. I. S. Al-Assaf , H. M. Ali and A. H. Ad'hiah. "Gene Expression of NLRP3 Inflammasome in Celiac Disease of Iraqi Children". *IHICPAS*.2645.2021.

[17] A.A.Mahmood and R.F.Abbas."Assessment of NLRP3 Gene Polymorphisms with Periodontitis as Compared with Healthy Periodontium in Iraqi Arabs Patients". *European Journal of Dental*,vol.17.pp.1338–1348.2023.

[18] B. Diao , K.Wen, J.Zhang, J. Chen, C. Han and Y.Chen . "Accuracy of a nucleocapsid protein antigen rapid test in the diagnosis of SARS-CoV-2 infection". *Clinical Microbiology and Infection*. vol.27,no.289.pp.281–284. 2021.

[19] WE.Matchett,V.Joag,JM.Stolley,FK.Shephard,CF.Quarnstrom and CK.Mickelson. "Nucleocapsid vaccine elicits spike-independent SARS-CoV-2 protective immunity". *bioRxiv*.vol.15,no.8,pp 166-173. 2021.

[20] T. Gao, Y. Gao, X. Liu, Z. Nie, H. Sun and K. Lin."Identification and functional analysis of the SARS-COV-2 nucleocapsid protein". *BMC Microbiol*. 21:58. 2021.

[21] NK. Dutta, K. Mazumdar and JT. Gordy."The nucleocapsid protein of SARS- CoV-2: a target for vaccine development". *Journal of Virology*. vol. 94.pp. 00647- 20. 2020.

[22] SB. Brosnahan, AH. Jonkman, MC. Kugler, JS. Munger and DA. Kaufman." COVID-19 and respiratory system disorders: current knowledge, future clinical and translational research questions". *Arteriosclerosis, Thrombosis, and Vascular Biologyl*.vol.40:2586–972020.

[23] Z. Jesus, M. Daniel, O. Josue, C. Angeles and C. Tecalco. "Implications of the Immune Polymorphisms of the Host and the Genetic Variability of SARS-CoV-2intheDevelopmentof COVID-19". *Viruse*s,vol.14:1-34, 2022.

[24] Z.A. Abdulla, S.M., Al-BashirAlzoubi, H. Al-Salih, N.S., Aldamen, A.A., and Abdulazeez, A.Z. "The Role of Immunity in the Pathogenesis of SARS-CoV-2 Infection and in the Protection Generated by COVID-19 Vaccines in Different Age Groups". *Pathogens*,vol.12.no.2.pp.329. 2023.

[25] CK.Chang, MH. Hou,CF.Chang, CD. Hsiao and TH. Huang. "The SARS coronavirus nucleocapsid protein–forms and functions". *Antiviral Research*.vol.103.no.145.pp.39–50. 2014.

[26] Y. Peng, N. Du, Y. Lei, S. Dorje, J. Qi and T. Luo. "Structures of the SARS- CoV-2 nucleocapsid and their perspectives for drug design". *EMBO Journal*.vol.39.no.45. e105938. 2020.

[27] YA. Malik."Properties of coronavirus and SARS-CoV-2". *Malaysian Journal of Pathology*.vol.42.no.67.pp.3–11. 2020.

[28] D. Zhao, W. Xu, X. Zhang, X. Wang, Y. Ge and E. Yuan. "Understanding the phase separation characteristics of nucleocapsid protein provides a new therapeutic opportunity against SARS-CoV-2". *Protein Cell*.vol.12.no.67.pp.734–40. 2021.

[29] Y. Cong, M Ulasli, H .Schepers, M. Mauthe, P. V'Kovski and F. Kriegenburg. "Nucleocapsid protein recruitment to replication-transcription complexes plays a crucial role in coronaviral life cycle". *Journal of Virology*.vol.94:e01925-e2019. 2020.