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RESEARCH ARTICLE

Genetic Detection and Phylogenetic Tree Study for SARS COV-2 (N Gene) In Iraq

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ABSTRACT

Aim of the current work to discovered and evaluation the effect of SARS-CoV-2 in positive samples in terms of N-gene Material and method, techniques for covid-19 were created with the partial sequencing of the genome (N gene) in real-time reverse transcriptase-polymerase (RT-PCR) chain reaction. After SARS-CoV2 isolation, an RT-PCR diagnostic test was developed for nose and pharyngeal swabs. The FAM sequence's RNA-dependent RNA polymerase gene, as well as the SARS-E CoV-2's and N genes, were used in RT-PCR experiments (diagnostic kit). RT-PCR assays targeting positive samples. Result, the N-gene is further studied and amplified by PCR before being sequenced and recorded in gene bank for 10 samples (MW820281, MW820282, MW820283, MW820284, MW820285, MW820286, MW820287, MW820288, MW820289, MW820290). Conclusion, according to the results of this research, identifying the N-gene alone would mask more than 95% of positive cases going to follow viral nucleic acid screening of COVID-19 patients. As a result, tracking repositive COVID-19 patients can become more easy, reliable, and cost-effective. This conclusion must be confirmed in a larger society.

KEYWORDS:
Covid-19, N gene,
Phylogenetic, SARS-CoV2

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INTRODUCTION

SARS-CoV-2, a new coronavirus that appeared in Wuhan, China, and rapidly spread across the world, was discovered in early December 2019 (Munster, Koopmans, van Doremalen, van Riel, & de Wit, 2020). COVID-19 has posed a serious danger to public health around the world, prompting the World Health Organization to call it a pandemic(Joensen et al., 2020; Zhang, Li, Zhang, Chen, Lv, Xia, Sun, Shentu, Chen, & Li, 2020). The N protein, which is important for the transcription and replication of viral RNA, interactions with the cell cycle of host cells, and packaging of the encapsulated genome into virion, is the primary structural protein of SARS-CoV-2. Furthermore, coronaviruses include the N antigen, which has a high immunogenic potential and is widely distributed during viral infection. Potential proteins in sero-diagnosis are the(S-N)proteins as they are targeted of COVID-19, centered on S-N proteins, close to other diagnostic approaches that have been used to diagnose SARS disease (Kubina & Dziedzic, 2020; Suryadinata, Boengas, & Lorensia, 2021).

For improved disease management, the ability to treat patients more securely and reliably while minimizing the danger of infectious transmission is critical. The viral nucleic acid is used to diagnose COVID-9. Both mental symptoms and nucleic acid test findings are included when a COVID-19 patient has been cured. Current research, however, indicates that nucleic acid testing has a significant probability of false-negative results. (Wang et al., 2020). Even after the patient's clinical symptoms have faded, a positive viral check for certain COVID-19 patients might persist for a long period. (Zhou et al., 2020). After being released, some COVID-19 patients may have a repositive nucleic acid test or possibly

reactivation with symptoms. (An et al., 2020; Xing et al., 2020). There is presently no research into the features of nucleic acid test findings in people who test positive after a negative result. These data suggest that when it comes to nucleic acids, the N gene is still the most important gene to control. In SARS-CoV-2 positive samples, the current investigation found some new information regarding N genes. According to the findings of this study, detecting the N gene alone would hide more than 90% of positive instances in COVID-19 patients' subsequent viral nucleic acid test.

Aim of the study

Covid19 is a novel emergent warning that all researchers should be aware of, and investigating the N gene is crucial genetically to see whether it is identical to other countries or has mutated. Another thing to keep in mind is that confirmation of a favorable result is sufficient.

MATERIAL AND METHODS

Sample collection

On February 1, 2021, 44 COVID-19 patients were admitted to Morgan Hospital, and 44 nasopharyngeal swaps were retrieved by virus transport mediaVTM and sent to the general Health laboratory for analysis, where they were put in the Smarter apparatus for RNA extraction for 38 minutes. After that, RNA was loaded into a PCR tube with PCR blend (Dna Rna extraction identification Kit).

RNA Extraction and reverse transcription

Instructed by the manufacturer, RNA was extracted from Nasopharyngeal swabs using a nucleic acid purification kit based on the magnetic bead technique (Cat No. DA0630, DAAN Gene, China). The purity and quantity of viral RNA is determined using a Promega (fluorometer, ng/ul). At a final reaction volume of 20ul, the next RT-PCR started with total RNA, a random hexamer of 100 pmol, and a final reaction volume of 2ul. The BioRad Three thermal cycler was used to do the thermocycling (Biorun,). The initial state was changed to 15 Co for 10 minutes, cDNA synthesis started at 32Co for 20 minutes, and heat inactivation was set to 95Co for 5 minutes.

real-time reverse transcription polymerase chain reaction (RT-PCR), as previously described. A positive test result was defined as a period threshold value (Ctvalue) of 37, while a negative test result was defined as a Ct-value of 40 or higher. These diagnostic criteria were established in line with the guidelines of China's National Institute for Viral Disease Control and Prevention (Zhang, Li, Zhang, Chen, Lv, Xia, Sun, Shentu, Chen, & Li, 2020).

Convert RNA to cDNA

Positive RNA for SARS-cov2 was obtained for genetic analysis, and the purity of the RNA was determined using a single tube-format protocol (promega) in which 0.5 mix was added to the tube and incubated for 5 mint before being analyzed using a Quantus™ Fluorometer (Use Blue Channel Ex 460nm, Em 515-575nm). cDNA amplification by polymer chains reaction (PCR).

For this analysis, the N gene primer was used because it is the key component of Covid19 diagnosis and discovering its genetic properties. Primer for the future 5'-GTCTTGGTTCACCGCTCTCA-3', 5'-ACGAGAAGAGGCTTGACTGC-3' (NC 045512.2), reverse primer 5'-ACGAGAAGAGGCTTGACTGC-3' (28274..29533) 406th product duration PCR substance tested by gel electrophoresis for bands indicating positive DNA. Annealing Tm57.2C 10l of master mix apply to the primer 0.5ul for each sample to the 3ul from DNA finishing volume to the 20 ul and inserted into thermo cycler. Sequencing of the PCR product was done by using the sanger dideoxy sequencing process, and the resulting Fasta file was analyzed using blast for the alignment, and 10 samples were entered into the Gene Bank data base with accession numbers (MW820281, MW820282, MW820283, MW820284, MW820285, MW820286, MW820287, MW820288, MW820289, MW820290). Phylogenetic analysis PhylogenyphyML was used for genetic research, and muscle aliment was performed for N Iraqi genes with ncbi genes in the construction of the tree, showing the closed strain and similarity.

RESULT

Both repositive samples and final positive findings had the SARS-CoV-2 N gene as a critical positive component. As illustrated in Figure 1, SARS-CoV-2 RNA is now identified utilizing real-time RT-PCR detection of three target genes: FAM, nucleocapsid protein (N), and envelope E gene. In this research, focus on the N gene as it the N gene had the largest proportion of positive effects . Before it can be utilized, RNA must be concerted and converted to cDNA in order to get an accurate result.

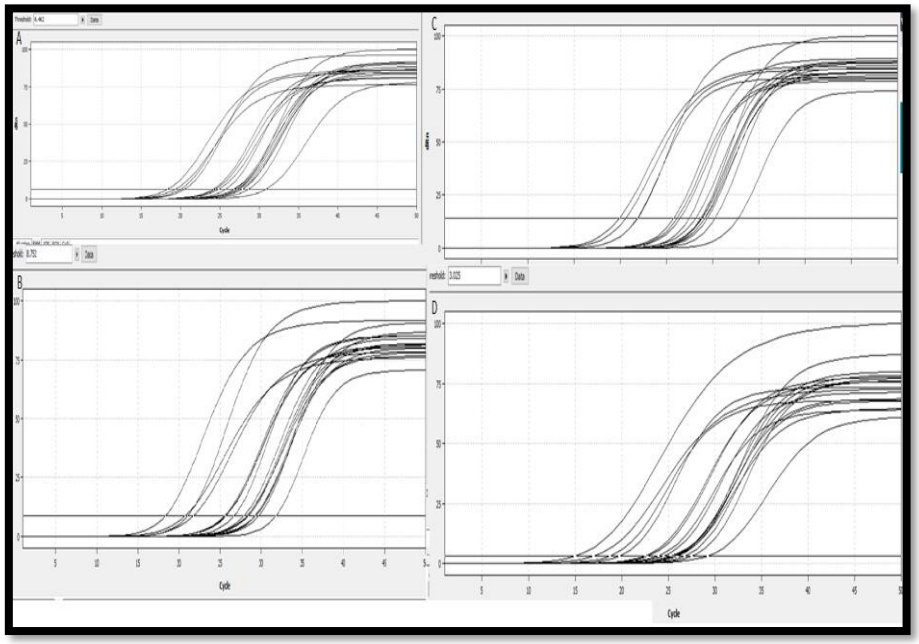

Fig.1: Rt(PCR) curve for a positive sample as A for Real time PCR FAM (COVID19) , B for E gene (Envelpe)C for the (internal control) and D PCR for the N gene(Nucleic acid)

Fig.2: RNA volume in the samples

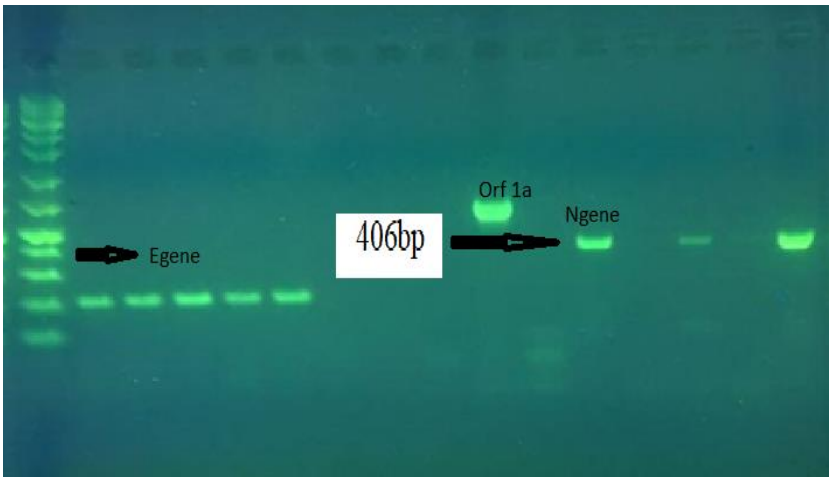

Fig.3: An agarose gel electrophoresis showing PCR product analysis for the N gene of Covid19 product size 406bp.

Fig.4: Multiply the sequence aliment (Muscle alignment) for N genes by (http://www.phylogeny.fr/) with number of NCBI genes that have been defined as having similarities using (blast database) accession number on phylogenetic tree. The amount of mutations and differences between isolates are shown by the alignment gap.

Fig.5: Phylogenetic tree construction using the Maximum Likelihood approach for 10 Ngene with NCBI genetic based genes from various countries using http://www.phylogeny.fr/ PhyloML (Dereeper et al., 2008) Yellow highlighted N genes are Iraqi and other from NCBI (blast) Genebank.

Phylogenetic analysis revealed that Iraqi strains, specifically the N gene, shared genetically from (90-100 percent) with the USA with some differences; however, Iraq strains for the N gene were separated by a number of mutations as four strains only had the same identity and the rest of the isolates were separated by countries but still had diagnosed N gene.

DISCUSSION

The numbers of people who were positive for the N gene were also the largest, led by those who were positive for both genes, more than 90% of persons tested positive for the N-gene . These findings show that the N -gene is still the most important when it comes to monitoring nucleic acids.(Zhang, Li, Zhang, Chen, Lv, Xia, Sun, Shentu, Chen, & Li, 2020). The current study revealed some novel information about SARS-CoV-2 positive samples for N- genes. The findings of this study indicate that in subsequent viral nucleic acid screening of COVID-19 patients, detecting the N gene alone will cover more than 90% of positive cases. As a result, it could be possible to track repositive COVID-19 patients in a more convenient, accurate, and cost-effective manner. This conclusion has to be validated in a broader community. The N gene is the most common positive fragment comparing it to the ORF1ab which was mentioned very limited. A positive rate comparable to that of detecting it by both genes can be obtained by detecting the N -gene alone(Sheikhzadeh, Eissa, Ismail, & Zourob, 2020). Since the N- gene has less nucleotide diversity than ORF1ab, its identification could be more stable than that of ORF1ab, which may explain why the N gene was identified more often in the positive than ORF1ab. Nucleotide variation was more common in 1ab than in N, and the hot spot nucleotide variation rate was higher in 1a than in N, according to a previous report(Goswami et al., 2020). Furthermore, nucleotide variations between reported primer-probe sequences and reference sequences were more prevalent in ORF1ab than in N;(Zhang, Li, Zhang, Chen, Lv, Xia, Sun, Shentu, Chen, Li, et al., 2020) nonetheless, despite being directed as N-gene, positive N genes showed substantial variance in our analysis. According to phylogenetic analysis, the N gene has particular relationships with ORF 1a, ORF 8, and ORF 10 when aligned by blast. Despite being isolated from patients who had the same residence and city, only four N genes clustered together with considerable distance on other N genes in the aliment.

CONCLUSION

Despite the fact that it was driven as N gene, had a high variance in our research. phylogenetic analysis revealed that the N gene has some similarities with ORF 1a, ORF 8 and 10. This variation in N genes was discovered in the aliment since only four N genes clustered together with a large gap on other N genes while being isolated from patients who shared the same house and city. These data suggest that when it comes to nucleic acids, the N gene is still the most important gene to control. In SARS-CoV-2 positive samples, the current investigation found some new information regarding N genes. According to the findings of this study, detecting the N gene alone would hide more than 95% of positive instances in COVID-19 patients' subsequent viral nucleic acid test.

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