Identification and Analysis the Polymorphism in FGA Gene among Covid-19 Patients in Babylon Province/Iraq

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Abstract
SARS-COV-2, a beta coronavirus, was discovered to be the virus that caused the coronavirus disease pandemic in 2019 (Covid-19). It's been proven that abnormal coagulation function has a role in COVID-19 illness progression. However, there is no apparent link between D-dimer levels and COVID-19 severity. The goal of the study was to see if there was a link between D-dimer levels and the severity of COVID-19 investigation of SNPs in FGA gene. Interactors have been found in a variety of proteins, and fibrinogen chains (FGA) are a type of fibrinogen, which is an anti-infective organ, the liver produces this glycoprotein. And serves as an important coagulation factor and an acute phase reactant; for its versatile role in coagulation, inflammation, blood viscosity, and the implications in the management of (COVID 19). Fibrinogen's role in acute COVID 19 patients and clot formation has been studied been investigated by researchers. There have been no research on the direct association of Fibrinogen alpha chain (FGA) with SARS-Cov-2 so far. The current study proved that FGA did not affect the vaccinated people, but effect of sars-cov2 infected patients. Sequencing study of the investigated section revealed 392 bp of the gene FGA. The presence of an insertion mutation in the fifth axon results in a shift in amino acid sequence from Histidine CAU (GTA) to Threonine ACA (TGT), resulting a change in the amino acid sequence.
Keywords: SARS-CoV-2, FGA Gene, Iraqi Human, PCR, Nucleotide Sequences.

1. Introduction

The corona virus disease (2019) COVID-19 is to blame for SARS-Cov2 driven infection produced by the corona virus2 of acute respiratory syndrome numerous coagulopathies that can culminate in either thrombosis or embolism (Perico et al., 2021; Gupta et al., 2020). FGA gene is protein encoded and is the alpha component of fibrinogen, a blood-borne glycoprotein composed of three pairs of non - identical polypeptide chains (Ro, *et al.*, 2013).
FGA, FGG, & FGB fibrinogen chains COVID-19 mortality is connected to top hub proteins, while APOA2, ORM2, ORM1, and CFP are neighbor proteins linked to fibrinogen chains. Considering the coagulation process' molecular pathways, which comprise fibrinogen chains, may provide a window. Into illness management. During COVID-19 patients' acute inflammatory phase hospitalization, the liver overreacts and secretes a variety of reactants including C reactive protein (CRP), ferritin, and a number of cytokines are all examples of fibrinogen (Guan et al.,2019; Wang et al., 2019).
Furthermore, most SARS patient's exhibit thrombocytopenia and increased D-dimers', indicating that the coagulation and fibrin polymerization pathways' are dysregulated (Lee *et al.*, 2003 ; Peiris *et al.*, 2003; Wong *et al.*, 2003; Wu, *et al.*, 2003).In labs', up regulation of SARS-COV-infected peripheral blood mononuclear cells also had fibrinogen mRNA' (Ng, *et al.*, 2004).
Fibrinolysis and coagulation factors keep the balance between thrombosis and damage in check. The link between fibrinolysis and COVID19 has received a lot of attention. According to a paper, COVID19 causes fibrinolysis impairment" and "hypercoagulability," as well as "venous thromboembolic events," "stroke," and renal failure." COVID-19 patients had a high level of D-dimers, which could indicate a clogged fibrinolytic system (Yuan Yee Lee et al; 2022).

Materials and procedures

Taking blood samples

Blood samples' (10) were collected from a selection made at random from Iraqi human coronavirus patients and vaccinated subjects selected from the hospital in Babylon province. About 3 ml of blood samples by jugular vein puncture using disposable needle for the period from the first of October 2021 to February 2022

2. DNA Extraction

DNA was extraction from peripheral blood using genomic DNA extraction kit (Favor gen - Taiwan). A Nano drop (Bio Drop LITE, Bio drop, UK) was used to evaluate the concentration and purity of DNA after it was extracted. The likelihood of DNA degradation was then determined using a conventional 0.8 percent (w/v) agarose gel electro-phoresis with ethidium bromide pre-staining (1 µg/mL) in TBE buffer.

Amplification via PCR

PCR was used to amplify the FGA gene using one set of particular primers. According to (Gen Bank acc.No.NC 000004.12), the Primers were desired. Macrogene was the

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source of the lyophilized primers (Korea). The order of events of Forward: 5'GTTGTTAGGCCTCGCGTTC3'andReverse: 5'ATGGAACCGGATCAGAGACG3'. PCR reaction was performed using promega. 1 U of Top DNA polymerase, 250 M of dNTPs, 10 mM Tris-HCl (pH 9.0), 30 mM KCl, and 1.5 mM MgCl2 were included in each 20 l of PCR premix. 10 pmol of each primer and 50 ng of genomic DNA were added to the reaction mixture.

The gradient PCR thermoscycler was used to start the program was started by the inaugural den-turation at 92°C. For 3 minutes, follow-d by 32 cycles of den-turation at 92°C. For 30 second, annealing at 57.5°C. For 3 second, and el-ngation at 72°C. For 3 second, and was concluded with a final extension at 72°C for 3 minutes'. In a gradient PCR thermos-cycler. Resolving the PCR amplified products on 1.5 percent agarose in conjunction with a 100 bp DNA ladder validated the PCR amplified products. Gel electrophoresis was carried out in 1X TBE buffer at a constant voltage of 100 V for 45 minutes. It was ensured that all PCR determined bands were distinct and only contained one band (392bp fragment each).

DNA sequencing

Ten samples' were sent to Micro gene Company for the purpose of genetic analysis to compare it with the standard sources in NCBI.

3. Results and Discussion

Ten samples' have been included in the locus, with 392bp amplicons of the FGA locus being designed. These amplicons are located on Chromosome4 gives advice on how to make a protein called fibrinogen one portion (subunit) of the fibrinogen protein is the alpha (A) chain.

All amplified FGA amplicons were evaluated for specific, and clean bands before being sent to sequencing assays. The amplified products' unmistakable identities were disclosed by the sequencing processes, through using NCBI blasts to determine their identity.

Using-Bio-Edit Sequence Alignment Editor Software Version. 7.1, the sequencing findings of various PCR products were edited, aligned, and assessed in comparison to the relevant sequences in the reference database (DNA STAR, Madison, WI, USA). Each sequenced sample's detected differences were numbered in PCR amplicons and their matching positions within the referring genome.. The PCR amplified sequence of FGA for sequence homology searches in public databases, the query gene was run through NCBI's nucleotide blast (http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi).In this study, FGA gene was studied for the Homo sapiens'. PCR was used to amplify a 392-bp segment of the FGA genes of the exons 5. (Fig. 1).

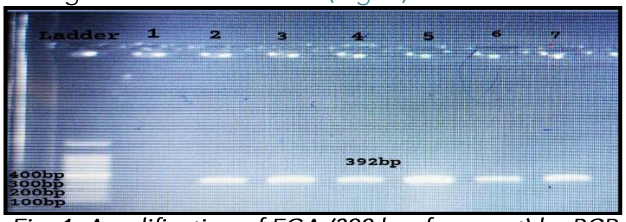


Fig. 1: Amplification of FGA (392 b p fragment) by PCR in 1.5% agarose gel, 100 b p DNA ladder.

The current study comprised twelve samples from this locus that had previously been demonstrated to amplify CD147 gene sequences in human chromosome 19. After running these PCR amplicons via NCBI BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the sequencing reactions revealed the exact identity. The NCBI BLAST engine found 99 percent sequence similarity between sequenced samples and target sequences for the alleged 392 bp amplicons. The By comparing the observed DNA sequences of these local samples with the returned DNA sequences, the approximate positions and other properties of the acquired PCR fragments were discovered (Gen Bank acc. NC_000004.12) (Fig. 2).

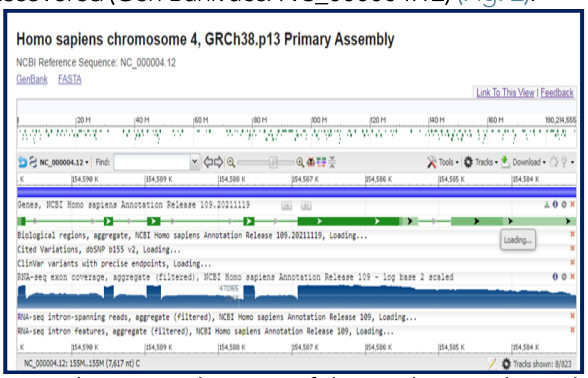


Fig. 2. The precise location of the 392 b p amplicon that partially encompassed a region of the genome that was A fragment of the FGA gene on chromosome 4 was covered (Gen Bank acc. no. NC-000004.12).

Following the placement of the 392 b p the sequences of amplicons on chromosome 4, the sequences of the forward primer of the 392 b p amplified amplicons were highlighted in detail (0 1).

Table 1: Shows the locations and lengths of 392 bp PCR amplicons used to amplify a segment of the FGA gene on chromosome 4. (Gen Bank acc. no. NC-000004.12). The forward and reverse primer positions were represented by the grey colored sequences'.

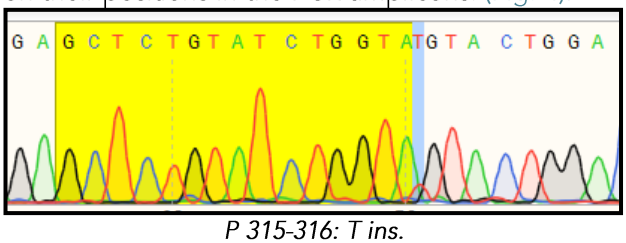
Amplicon'	Sequences of the referring locus (5' - 3')	Length'
Internal DNA sequences of the FGA gene	*ATGGAACCGGATCAGAGACGGAA AGCCCCAGGAACCCTAGCAGTGCT GGAAGCTGGAACTCTGGGAGCTCT GGACCTGGAAGTACTGGAAACCG AAACCCTGGGAGCTCTGGGACTG GAGGGACTGCAACCTGGAAACCT GGGAGCTCTGGACCTGGAAGTACT GGAAGCTGGAACTCTGGGAGCTCT GGAACTGGAAGTACTGGAAACCAA AACCCTGGGAGCCCTAGACCTGGT AGTACCGGAACCTGGAATCCTGGC AGCTCTGAACGCGGAAGTGCTGG GCACTGGACCTCTGAGAGCTCTGT ATCTGGTAGTACTGGACAATGGCA CTCTGAATCTGGAAGTTTTAGGCCA GATAGCCCAGGCTCTGGGAACGC GAGGCCTAACAAC**	392 bp

* refers to the sequences of forward primers.
** refers to the sequences of reverse primer.

In contrast to their corresponding reference DNA sequences, The 392 bp samples' alignment findings confirmed the presence of little SNPs that were variously distributed in the analyzed human samples'. (Fig. 3).

Fig. 3. DNA sequences alignment of ten genetic groups of human with the 392 bp amplicons of the FGA genetic DNA sequences' corresponding reference sequences the position of each substitution and insertion mutation in the PCR products was marked. The sign "ref" stands for the NCBI referencing sequence, while "S1-S10" stands for samples 1 through 10.

The detected substitution and insertion SNPs sequencing chromatograms, The chromatogram details of the observations, as well as their thorough comments, were noted SNPs were displayed based on their positions in the PCR amplicons. (Fig. 4).



P 315-316: T ins.

Fig. 4. The pattern of insertion mutations found in the desired target's DNA chromatogram 392 bp amplicons with-n the human FGA gene'. The sites of the detected insertion mutations were they've been highlighted based on where they appear in the PCR products. S1 – S10 refer to the studied no. 1 to no. 10 samples. The phrase "ins" refers to insertion mutation.

To summarize all of the findings from the 392 bp fragments that were sequenced, in the NCBI reference sequences, the specific sites of the detected changes were given. (Table 2).

Table 2. In comparison to their matching NCBI reference sequences, the pattern of detected SNPs in the 392 bp amplicons of Homo sapiens (Human) (GenBank acc. no. NC- 000004.12). The letter "S" stands for sample number.

Sample No.	Native Allele	Position in the PCR fragment
S5, S6	T ins.	315-316

The detected differences in the targeted 392 bp amplicons exhibited one different distribution in the investigated samples. The insertion mutation at position 315-316 causes a change in the amino acid sequence, which causes the next nucleotid bases to change as a result of the genetic difference in the fifth axon. This mutation was detected in only a few samples of sars-cov2 infected patients and did not occur in people who had been vaccinated.

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