RESEARCH ARTICLE

Discovering of JINZA1 and JINZA2 Genes of SARS COV2 by using ABI3730XL, Sequencer in Iraqi Patients

Jnan J. Baksh, Nazar Sh. Mohammed, Ahmed S. Hassan

Department of Medical Laboratories, Medical Technical College, Middle Technical University, Baghdad, Iraq

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ABSTRACT

A total of (200) whole blood, nasal and throat swab samples were collected from patients infected with the Coronavirus who were recumbent in Al-Nouman and Al-yarmook hospitals from 1st January to 1st November 2021. Demographic results showed that the highest infection rate was among the age group (20–40) years followed by (41–60) years, with no significant difference ($p > 0.05$). While the distribution of infection according to gender revealed that the females were higher 58 (58%) than males 42 (42%). The distribution of the severity of infection among patients showed that the mild infection was among the age group (20–40) years 38(66.7%), the moderate infection was among the age group (41–60) years 20(55%), but the severe infection was among the age group (61–80) years 16(44.4%). While the females were shown to be more infected among patients with mild and moderate infections with Coronavirus. The mild symptomatic infections 38(76%) were highest among other cases. Two mutated strains, JINZA1 and JINZA2, belonging to Iraqi patients who died after contracting Coronavirus, were identified and registered in NCBI at the Gene Bank USA

Keywords: Genes ABI3730XL, Genotyping, JINZA1, JINZA2.

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INTRODUCTION

Coronaviruses cause respiratory tract infections that can range from mild to severe. Mild human diseases involve certain common cold cases that may cause severe acute respiratory syndrome (SARS), middle east respiratory syndrome (MERS), as well as coronavirus disease (COVID-19).[1] The size of coronavirus genome ranges between (26–32) Kilo base. The genomes possess 5′ methylated caps and 3′ polyadenylated tails.[2] Fehr and Perlman in 2015 revealed that the organization of the genome for Coronavirus is 5′-leader-UTR-replicase (ORF1ab.)-spike (S)-envelop (E)-membrane (M)-nuclocapsid (N)-3′UTR-Poly (A) tail. Replicase polyprotein (pp1ab) is encoded by open readings frames 1a and 1b, that occupies the 1st 2/3rd of the genome, also replicase polyprotein self is cleaved to form 16 nonstructural proteins (nsp-1,nsp-16). The four main structural proteins spike, membrane, envelope and nuclocapsid are encoded by the later readings frames, and the reading frames for accessory proteins spread between those reading frames. Based on specific coronavirus, the number and functions of accessory proteins is unique.[2] In the subsequent years, 229E and OC43 continued to be studied.[3] The strain B814 of coronavirus has been lost, it is still not known which is the current human coronavirus.[4] Since then, other human coronaviruses have been recognized, such as SARS-HCOV, 2003, NL63- HCOV, 2003, HKU1- HCOV, 2004, MERS-HCOV, 2013 as well as SARS-HCoV-2 , 2019.[5] Genotyping of COV, this pandemic resulted in a significant health emergencies and economic stresses worldwide. Thus, understanding viruses' nature and finding out methods to control its spread in the pandemic are essential steps in controlling the disease, there were different molecular aspects of COV relevant to this pandemic. The discovery of genotypes related to temporal and geographic infection clusters illustrates the ability of using the genome sequence signature to monitor and track COV transmission.[6]

MATERIALS AND METHODS

A total of 200 whole blood samples, nasal and throat swab samples were collected from patients infected with Coronavirus who were recumbent in Al-Nouman and Al-yarmook hospitals during the period from 1st January to 1st November 2021. This study involved two groups, patients and controls for isolation of Coronavirus from 732 samples, only 200 samples were identified according to pneumonia and clinical signs and symptoms of COVI) in symptomatic and asymptomatic patients. The isolates were confirmed by the molecular

**Author for Correspondence: jnanjafar999@gmail.com*

identification test (RT-PCR) using primers as in Tables 1 and 2, gene sequencing by Sanger method, sequencing using ABI3730XL, and automated DNA sequencer. The primers are provided by Macrogen Company in a lyophilized form. Lyophilized primers are dissolved in nuclease-free water for giving final concentrations of 100 pmol/ µL as stock solutions. The preparation of the working solutions of such primers was done when 10 µL of primer stock solution was added to 90 µL of nuclease free water for obtaining working primer solutions of 10 pmol/µL.[7]

Primer Optimization

For the purpose of examining the primer's optimum annealing temperatures, template of DNA is amplified by the pair of same primer, (Forward, Reverse), at annealing temperature of 55°C, 58°C, 60°C, 63°C and 65°C. The amplification of PCR was carried out using 20 µL volume that contains (10 µL) GoTaq Green Master Mix (2X); (1 µL) to each primer (10) pmol; 0.5 µL RT Mix; 0.5 µL $MgCl_2$; 4 µL nuclease-free water with 3 µL of template DNA. The cycling of PCR was done using PCR express (Thermal cyclers, Thermo Fisher Scientific, USA) with the following temperatures programs: Hold at 37°C for 15 minute; denatured at 94°C for four minutes, then by 40 denaturation cycles at 94°C for 30 seconds; annealing at 55, 58, 60, 63 and 65°C for 30 seconds; and extensions at 72°C for 30 seconds. The ultimate incubation of extension for 7 minutes at 72°C was involved, then incubated for (10) minutes at 4°C to stop the reaction (Table 3).[7]

Agarose Gel Electrophoresis

After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplifications. PCR completely depended upon the criteria of extracted DNA.[7]

Solutions

1-X TAE buffer, loading dyes, DNA ladder markers and (10 mg/mL) Ethidium bromide.[7]

Agarose preparation

In a flask, 100 mL of 1X TAE was placed. To the buffer, 1.5 gm of the 1.5% agarose was added. The microwave boiled the solution until dissolving of all particles of the gel. To the agarose, 1-µL Ethidium bromide was added. The agarose was thoroughly stirred to obtain a good mixture and avoid bubble formation, then the solution was cooled to 500–60°C.[7]

Table 1: Primers used in the study

Primer name	*Sequence*	*Annealing temperature (°C)*	*Product size (bp)*
ORF1ab-F	5`-CTAGGACCT CTTTCTGCTCA-3`	58	588 (Hamid *et al.*, 2020).
ORF1ab-R	5`-ACACTCTCC TAGCACCATCA-3`		

Table 2: Primer preparation

Primer name	*Vol. of nuclease free water (µL)*	*Concentration (pmol/µL)*
SARSHCOV2-F	300	100
SARSHCOV2-R	300	100

Table 3: PCR program

Steps	*Temperature (°C)*	*m: s*	*Cycles*
Initial denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	58	00:30	
Extension	72	00:30	
Final extension	72	07:00	1
Hold	10	10:00	

Casting of the Horizontal Agarose Gel

To the gel tray, agarose solution was poured after sealing of both edges with cellophane tape and leaving the agarose to solidify for 30 minutes. at room temperature. Then, the comb was carefully removed, followed by placing the gel in the gel tray. The tray is filled with 1X TAE electrophoresis buffer until it reached (3–5) mm above the gel surface.[7]

DNA Loading

There was a direct loading of PCR products, when 5 µL was loaded immediately to the well. The electrical power is turned on for 60 minutes at 100 v/mAmps. DNA moved from cathode to anode pole. The gel imaging system was used to visualize the stained Ethidium bromide bands.

Standard Sequencing

The products of PCR were sent to Sanger sequencing by the use of ABI3730XL, automated DNA sequencers, from the Korean Macrogen Corporation. The result of sequencing was obtained via E-mail and analyzed by genius software.[7]

Statistical Analysis

The SPSS program version-21 was used for the statistical analysis of data in the study. Data are presented as mean ± SD for quantitative variable and number and percentage for a qualitative variable. Relations were studied using the Chi-square test and the Student t-test was applied to test variations among groups. The *p*-value (<0.05) is measured to show statistical significance.

Results

Demographics picture showed that the age group (20–40) years was more than other ages followed by (41–60) years, $p > 0.05$) NS. While the distribution of infection according to gender revealed that the females 58(58%), were highly infected than males 42 (42%) with no significant difference, as shown in Table 4.

Table 5 demonstrates the distribution the severity rate of infection among different age groups. The mild infection was the highest 38(66.7%) among the ages group (20–40) years, the modern infection was high 20(55%) among (41–60) years, but the severe infection was high 16(44.4%) among the age group

Table 4: Demographic pcture of age groups and gender

Demographics			*Studied groups*		*p-value*
			Control N = 100	*Patient N= 100*	
Age groups /Year	< 20	N	4	6	p = 0.691 Non sign. (p > 0.05)
		%	4%	6%	
	20–40	N	50	48	
		%	50%	48%	
	41–60	N	38	34	
		%	38%	34%	
	61–80	N	8	12	
		%	8%	12%	
Gender	Male	N	43	42	p = 0.886 Non sign. (p > 0.05)
		%	43%	42%	
	Female	N	57	58	
		%	57%	58%	

Table 5: Demographics picture of age group distributed according to the severity of infections

Patient (N = 100)						
Demographics mild Mmoderate			*Classes Sever*			*p - value*
Age groups /Year	< 20	N	5	1	0	p = 0.00 Highly sign. (p < 0.01)
		%	8.8%	2.8%	0%	
	20–40	N	38	10	0	
		%	66.7%	27.8%	0%	
	41–60	N	11	20	3	
		%	19.3%	55.6%	42.9%	
	61–80	N	3	5	4	
		%	5.3%	13.9%	57.1%	
Gender	Male	N	22	16	4	p = 0.601 Non sign. (p > 0.05)
		%	38.6%	44.4%	57.1%	
	Female	N	35	20	3	
		%	61.4%	55.6%	42.9%	

Table 6: Distribution of the classes according to the severity depending on the symptoms

Classes	*N*	*%*	*Chi-square test (p-value)*
Mild asymptomatic	2	4	p = 0.00 Highly sign. (p<0.01)
Sever asymptomatic	3	6	
Mild symptomatic	38	76	
Critical symptomatic	7	14	
Total	50	100	

(61–80) years $p < 0.01$ (HS). While Females showed higher mild and moderate infections than males.

Table 6 shows the severity depending on symptoms. The mild symptomatic 38 (76%) was the highest case among others, p < 0.01, HS.

RT-PCR result was positive line at 588bp M.W in sample number 36 and sample number 39 from nasal swabs while

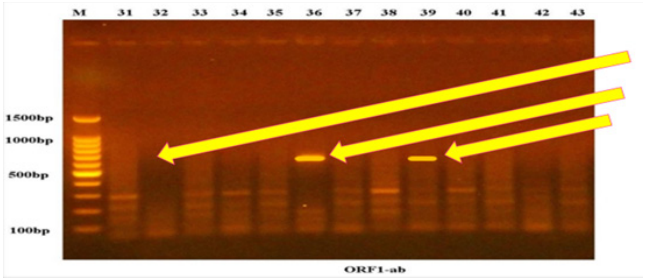

Figure 1: Amplification results of SARSHCOV2ORF1ab gene of virus specimens were fractionated on 1.5% agarose gel electrophoresis stained by ethidium bromide. M: 1000bp ladder marker. Lanes 31-43 resemble 588bp PCR products

negative in other types of samples for throat and whole blood.

For sequencing of JINZA1 gene from nasal swab sample

TCTAGGGACCTCTTTCTGCTCAAACTG-
GAATTGCCGTTTTAGATATGTGTGCTTCATT
AAAAGAATTACTGCAAAATGGTATGAATG-
GACGTACCATATTGGGTAGTGCTTTATT
AGAAGATGAATTTACACCTTTTGATGTTGTT-
AGACAATGCTCAGGTGTTACTTTCCAAAGTGCAGTGAAAA-
GAACAATCAAGGGTACACACCACTGGTTGTTACTCACAATTTTG
ACTTCACTTTTAGTTTTAGTCCAGAGTACTCAATG-
GTCTTTGTTCTTTTTTTTGTATGA

FASTA sequence Sample number 36 For sequencing of JINZA2 gene from nasal swab sample

AAATGCCTTTTTACCTTTTGCTATGGGTAT-
TATTGCTATGTCTGCTTTTGCAATGATGT
TTGTCAAACATAAGCATGCATTTCTCT-
GTTTGTTTTTGTTACCTTCTCTTGCCACT-
GTA GCTTATTTTAATATGGTCTATATGCCT-
GCTAGTTGGGTGATGCGTATTATGACATGGT
TGGATATGGTTGATACTAGTTTGTCTGGTTTTAA-
GCTAAAAGACTGTGTTATGTATGC ATCAGCTGTAGT-
GTTACTAATCCTTATGACAGCAAGAACTGTGTATGAT-
GATGGTGCT AGGA

FASTA Sequence Sample Number 39

DISCUSSION

The age group (20–40) years was more than other ages followed by (41–60) years, p > 0.05) NS. While the distribution of infection according to gender revealed that the females 58 (58%) were highly infected by coronavirus than males 42 (42%). These findings agreed with (Fleitas, P. E. *et al.*, 2021),[8] who reported that females were more susceptible to infection with coronavirus than males, and the reason may be the structure. Female physiology may be more affected by infection with coronavirus.[8] Verity, R. *et al.*, (2019) reported that the ages between 40 and 60 years are more susceptible to moderate symptoms when infected with the coronavirus, but the ages over sixty years are more susceptible to severe infection.[9] The mild symptomatic cases were the highest 38 (76%) among others with coronavirus infections. Oran, D. P. and Topol, E.

J. (2020),[10] demonstrated that the mild symptomatic patients were more than the moderate cases and the severely infected patients.[10] Two types of coronavirus genetic strains were recorded in NCBI, and these two strains were so deadly that the two infected people died in Iraq, Baghdad. These results matched with (Liu, D. X. *et al*., 2021),[11] who reported that seven Coronavirus (HCOVs) in humans were so far recognized, i.e., HCOV-229E, HCOV-OC43, HCOV-NL63, HCOV-HKU1, severe acute respiratory syndrome (SARS-COV), Middle East respiratory syndrome (MERS-COV) and novel Coronavirus (2019-nCOV, a.k.a. SARS-COV-2).[11]

CONCLUSION

Gene sequencing is very important to the discovery of new genes from SARSHCOV2 in patients suffering from COVID-19 pneumonia, which could be diagnosed by nucleic acid detection (RT PCR) required to be confirmed by Sanger sequencing using ABI3730XL, automated DNA sequencer,

The results showed the discovery of new isolates called SARSHCOV2ORF1ab JINZA1 gene and SARSHCOV2ORF1ab JINZA2 gene in Baghdad, Iraq, and submitted to NCBI SARSHCOV2ORF1ab JINZA1 OK486620 gene and JINZA2 OK586822 gene.

The names of both genes were according to Jnan J. Baksh, Nazar Sh. Mohammed and Ahmed S. Hassan. BLAST results were indicated because of transmission by travel between Iraq and USA.

JINZA: (JI) the first 2 letters of a name Jnan J. Baksh, (NZ): the first 2 letters for name Nazar Sh. Mohammed and (A): the first 1st letter of the name Dr. Ahmed S. Hassan.

Both patients lost their lives due to the severity of infection for JINZA1 and JINZA2 and were critical class for this pandemic.

RECOMMENDATION

Confirmed tests include RTPCR and gene sequencing to discover new genes for SARSHCOV2 in COVID-19 pneumonia patients to know speed viral transmission and control of disease.

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