Genotyping of Corona virus infections in Baghdad city

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Abstract
Background: The discovery of genotypes linked to geographic and temporal infectious clusters suggests that genotyping analysis can be used to track and monitor the transmission of corona virus. Objective: To explore the clinical value of causative agent for corona virus infection (CoVI) by using different genes (SARS-HCoV2 ORF1ab JINZA1 and JINZA2 gene and HCoV NL63, HCoV OC43 and HCoV 229E in the diagnosis of causative agent for corona virus disease and severity of infection to know speed transmission this pandemic and control of disease. Patients and methods: Different types from human samples included nasal swabs, throat swabs and blood samples(plasma) from patients with CoVI and pneumonia. To diagnosis SARS-HCoV2 ORF1ab JINZA1 and JINZA2 gene, and HCoV NL63 gene, HCoV OC43 gene and HCoV 229E gene. The positive ratio of SARS-HCoV2 ORF1ab gene in the diagnosis of COVID-19 pneumonia confirmed by conventional PCR then gene sequencing by sanger method by using PCR product were sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation Korea. The results were received by email then analyzed using geneious software. Results: Assay for CoV the results shown P = 0.001 Highly sign. (P<0.01) within NL63 gene from nasal and throat swab positive n = 2 (10.53%) while negative n =17 (89.47 %) and P = 0.00 Highly sign. (P<0.01) within CT for NL63 gene positive n = 2 (4 %) while negative n = 48 (96 %). In addition to CoV result by PCR were P = 0.033 Sign. (P<0.05), positive n =17 (34%) and negative n =33 (66 %) from total n =50, and P = 0.019 Sign. (P<0.05) within SARSHCOV2 ORF1ab gene from nasal swab by PCR positive n =4(21.05%), negative n =15(78.95%) from total n =19 and P = 0.648 Non sign. (P>0.05) 229E gene from nasal and throat swab positive n =11(57.9%), negative n =8 (42.1%) from total n=19 (100%). While undetectable from OC43HCOV gene by real time PCR and by conventional PCR that indicated all results were negative for blood samples and from nasal and throat swab: Conclusion: Genotyping very important to know type of gene caused corona virus infection by using PCR real time PCR and conventional PCR indicated the study on the present other types of corona virus were HCOV 229E and NL63 HCOV and PCR product confirmed by Sanger sequencing using ABI3730XL, automated DNA sequences, the results concluded discovery two new isolates called SARSHCOV2ORF1ab JINZA1 gene and SARSHCOV2ORF1ab JINZA2 gene in Baghdad/Iraq patients and submitted in National Center for Biotechnology Information. SARSHCOV2ORF1ab JINZA1 OK486620 gene and JINZA2 OK586822 gene. The names of both genes according to name of PhD student Jnan Jafar Baksh, Supervisal Prof. Dr. Nazar Shiyaa Mohammed and Assist prof. Dr. Ahmed Saadi Hassan. BLAST results indicated because transmission by travel between Iraq and USA. Both of two patient's loss of their life due to severity of infection for JINZA1 and JINZA2 and were critical class for this pandemic.Recommendation: 1) Chosen specific primers for specific gene to avoid coinfection with other viruses and using confirmed tests include real time PCR or conventional PCR and gene sequencing for genotyping for corona virus to know speed viral transmission and control of disease: 2) Nasal swab and throat swab for detection from corona virus mostly greatest than blood samples because viral load higher and development molecular techniques and instruments for detection from virus when very low viral load.

Keywords: Baghdad city; Corona virus infections, Genotyping.

1. Introduction

Coronaviruses contain a positive sense, single stranded RNA genome, the genome size for coronaviruses ranges from 26 to 32 kilobases. The genome size is one of the largest among RNA viruses. The genome has a 5′ methylated cap and a 3′ polyadenylated tail (Fehr and Perlman, 2015).
Fehr and Perlman in 2015 revealed the genome organization for a coronavirus is 5′-leader-UTR-replicase (ORF1ab)-spike (S)-envelope (E)-membrane (M)-nucleocapsid (N)-3′UTR-poly (A) tail. The open reading frames 1a and 1b, which occupy the first two-thirds of the genome, encode the replicase polyprotein (pp1ab), also the replicase polyprotein self cleaves to form 16 nonstructural proteins (nsp1–nsp16). The later reading frames encode the four major structural proteins: spike, envelope, membrane, and nucleocapsid, spread between these reading frames are the reading frames for the accessory proteins, the number of accessory proteins and their function is unique depending on the specific coronavirus (Fehr and Perlman, 2015).

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229E and OC43 continued to be studied in subsequent decades (Geller et al., 2012 ; Myint,1995).

The coronavirus strain B814 was lost, it is not known which present human coronavirus it was (Corman et al., 2014). Other human coronaviruses have since been identified, including SARS-HCoV in 2003, NL63- HCoV in 2003, HKU1- HCoV in 2004, MERS-HCoV in 2013, and SARS-HCoV-2 in 2019 (Zhu et al., 2020).

Genotyping of CoV, this pandemic has caused a substantial health emergency and economic stress in the world. Therefore, understanding the nature of this virus and deriving methods to monitor the spread of virus in the pandemic are critical in disease control, there were several molecular facets of the CoV pertinent to this pandemic. The discovery of genotypes linked to geographic and temporal infectious clusters suggests that genome sequence signatures can be used to track and monitor the transmission of CoV (Changchuan Yin, 2020).

2. Method

This study involved two groups, patient and control groups for isolation of corona virus from total of 732 samples, according to pneumonia and clinical signs and symptoms of CoVI) in symptomatic and asymptomatic patients. The isolates were characterized by confirmed tests molecular identification (real time PCR and conventional PCR) by used primers as in table 3.1 (Hamid et al., 2020) and gene sequencing by sanger method, sequencing using ABI3730XL, automated DNA sequences.

2.1 Kits

Table 2.1 Maxwell® 16 Viral Total Nucleic Acid Purification Kit	
Kits	Company/ Origin
Maxwell® 16 Viral Total Nucleic Acid Purification Kit, GoTaq® 1-Step RT-qPCR System, MgCL2, Nuclease Free Water.	Promega, USA (Xiang et al., 2015; Bharti et al., 2016); (Hamid et al., 2020).
Primers	Macrogen, Korea

2.2 Primer.

Table 2.2 Primers used in this study for real time PCR and conventional PCR then confirmed by gene sequencing by sanger method (Hammit et al., 2011).		
Gene name	Froward primer (5′ - 3′)	Reverse primer (5′ - 3′)
SARS HCoV ORF1ab gene	CTAGGACCTCTTTCTGCTCA	ACACTCTCCTAGCACCATCA
HCoV 229E gene	CAGTCAAATGGGCTGATGCA	AAAGGGCTATAAAGAGAATAAGGTATTCT
HCoV OC43 gene	CGATGAGGCTATTCCGACTAGGT	CCTTCCTGAGCCTTCAATATAGTAACC
HCoV NL63 gene	ACGTACTTCTATTATGAAGCATGATATTAA	AGCAGATCTAATGTTATACTTAAAACTACG

2.3 Real time PCR for HCoV (229E, OC43 and NL63) genes detection.

Table 2.3 Primer preparation		
Primer Name	Vol. of nuclease free water (µl)	Concentration (pmol/µl)
HCoV 229E-F	300	100
HCoV 229E-R	300	100
HCoV OC43-F	300	100
HCoV OC43-R	300	100
HCoV NL63-F	300	100
HCoV NL63-R	300	100

Table2.4 Reaction setup and thermal cycling protocol PCR component calculation				
No. of Reaction		rxn	Annealing temperature of primers	55
Reaction Volume /run	10	µl	No. of primers	3
		%	No. of PCR Cycles	40

Table 2.5 Master mix components					
Master mix components	Stock	Unit	Final	Unit	Volume
qPCR Master Mix	2	X	1	X	5
RT mix	50	x	1	x	0.25
MgCl2					0.25
Forward primer	10	µM	1	µM	0.5
Reverse primer	10	µM	1	µM	0.5
Nuclease Free Water					
RNA		ng/µl		ng/µl	3.5
Total volume					10
Aliquot per single rxn	6.5µl of Master mix per tube and add 3.5 µl of Template				

Table 2.6. Real Time PCR Program			
Steps	°C	m: s	Cycle
Initial Denaturation	95	05:00	
Denaturation	95	00:20	40
Annealing	55	00:20 acquiring on Green	
Extension	72	00:20	

These primers were supplied by Macrogen Company in a lyophilized form, lyophilized primers were dissolved in a nuclease free water to give a final concentration of 100pmol/µl as a stock solution. Working solution of these primers was prepared by adding 10µl of primer stock solution (stored at freezer -20 C) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl.as in table 2.3

A working solution of these primers was prepared by adding 10µl of primer stock solution (stored at freezer -20 C) to 90µl of nuclease free water to obtain a working primer solution of 10pmol/µl (Hamid et al., 2020; Akta et al.,1989; Clinical Laboratory Standards Institute 2007).

To examine the optimum annealing temperature of primer, the DNA template was amplified with the same primer pair, (Forward) (Reverse), at annealing temperatures of 55, 58, 60, 63 and 65°C. PCR amplifications were performed with 20µl volumes containing 10µl GoTaq Green Master Mix (2X); 1µl for each primer (10pmol); 0.5µl RT Mix; 0.5µl MgCl2; 4µl nuclease free water and 3µl of template DNA.

PCR cycling was performed with PCR Express (Thermal Cycler, Thermo Fisher Scientific, USA) with the following temperature program: Hold at 37oC for 15 min; denatured at 94oC for 4 min followed by 40 cycles of denaturation at 94°C for 30 sec; annealing at 55, 58, 60, 63 or 65°C for 30 sec; and extension at 72°C for 30 sec. A final extension incubation of 7 min at 72oC was included, followed by a 10 min incubation at 4oC to stop the reactions (Hamid et al., 2020; Clinical Laboratory Standards Institute 2007).

2.4 Conventional PCR

Table 2.7 Primer preparation		
Primer Name	Vol. of nuclease free water (µl)	Concentration (pmol/µl)
SARSHCOV2ORF1ab gene F	300	100
SARSHCOV2 ORF1ab gene R	300	100

Table 2.8 Reaction Setup and Thermal Cycling Protocol PCR Component Calculation				
No. of Reaction		rxn	Annealing temperature of primers	58
Reaction Volume /run	25	µl	Length of PCR product (bp)	588
		%	No. of PCR Cycles	40

Table 2. 5 Master mix components					
Master mix components	Stock	Unit	Final	Unit	Volume
					1 Sample
qPCR Master Mix	2	X	1	X	12.5
RT mix	50	x	1	x	0.5
$MgCl_2$					0.5
Forward primer	10	µM	1	µM	1
Reverse primer	10	µM	1	µM	1
Nuclease Free Water					6.5
RNA		ng/µl		ng/µl	3
Total volume					25
Aliquot per single rxn	22µl of Master mix per tube and add 3µl of Template				

Table 2.9 Conventional PCR Program			
Steps	°C	m: s	Cycle
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	

For conventional PCR must agarose gel electrophoresis, preparation of solutions, preparation of agarose, casting of the horizontal agarose gel and DNA loading.

2.5 Standard Sequencing

PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequences, by Macrogen Corporation – Korea.

The results were received by email then analyzed using geneious software (Hamid et al., 2020).

2.6 Statistical analysis

Statistical analysis of the presented study was performed using SPSS statistical package for social sciences (version 21.0 for windows, SPSS).

Data are presented as mean ± SD for quantitative variables, and number and percentage for qualitative variables.

Relations were studied using Chi-square test and Student's t-test was used to test differences between groups. P-value < 0.05 was measured to show statistical significance.

3. Results

Table 3.1 Types of samples for all participants in the study from symptomatic and asymptomatic patients for CoVI.	
Number	Type of sample
713	Blood sample
12	Nasal swab
7	Throat swab
732	Total

Table 3.2 Courses of pneumonia in symptomatic and asymptomatic patients			
Courses of pneumonia in CoVI patient	N	%	P – value
Mild pneumonia in mild CoVI patient	57	57	P = 0.00 Highly sign. (P<0.01)
Sever pneumonia in sever CoVI patient	36	36	
Very sever pneumonia in critical CoVI patient	7	7	
Total	100	100	

Table 3.3 Demographics age groups and gender					
Demographics			Studied groups		P – value
			Control N = 100	Patient N= 100	
Age Groups / Year	< 20	N	4	6	P = 0.691Non 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 3.4 Assay for genotyping				
Corona virus result by PCR	Positive	17	34	P = 0.033 Sign. (P<0.05)
	Negative	33	66	
	Total	50	100	
SARSCOV2ORF1ab gene from nasal swab by PCR	Positive	4	21.05	P = 0.019 Sign. (P<0.05)
	Negative	15	78.95	
	Total	19	100	
NL63 gene from nasal and throat swab	Positive	2	10.53	P = 0.001 Highly sign. (P<0.01)
	Negative	17	89.47	
	Total	19	100	
CT for NL63 gene	Positive	2	4	P = 0.00 Highly sign. (P<0.01)
	Negative	48	96	
	Total	50	100	
229E gene from nasal and throat swab	Positive	11	57.9	P = 0.648 Non sign. (P>0.05)
	Negative	8	42.1	
	Total	19	100	

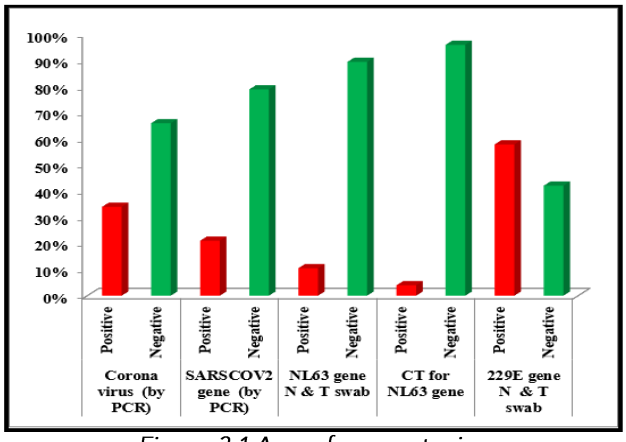


Figure 3.1 Assay for genotyping

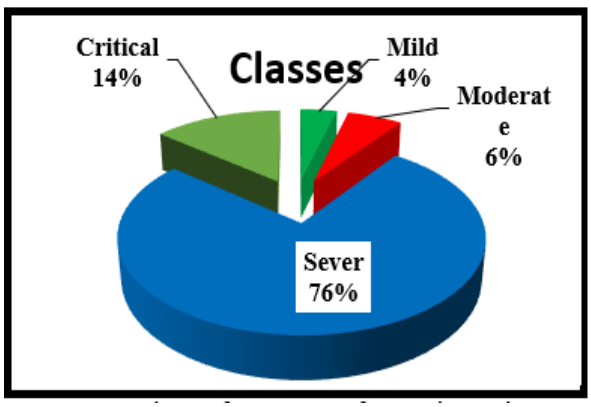


Figure 3.2 Classes for severity of CoVI depending on symptoms.

Table 3.5 Classes for severity of CoVI depending on 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	

Table 3.6 Genotype for CoVI			
Genotype	N	%	Chi-Square Test (P-value)
Positive (SARSCOV2)	4	8	P = 0.00 Highly sign. (P<0.01)
Positive (NL63)	2	4	
Positive (229E)	11	22	
Negative	33	66	
Total	50	100	

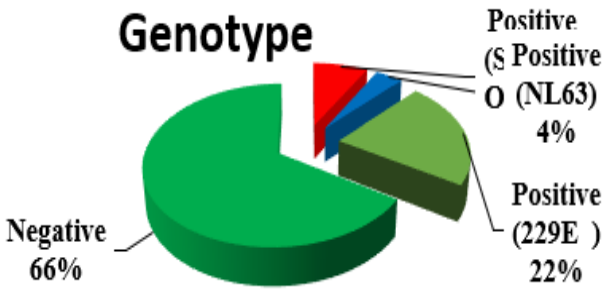


Figure 3.3 Genotype for CoVI

Table 3.7 CT or Cq for genotype			
CT or Cq for genotype	N	%	Chi-Square Test (P-value)
Negative	35	70	P = 0.00 Highly sign. (P<0.01)
Low	12	24	
High	3	6	
Total	50	100	

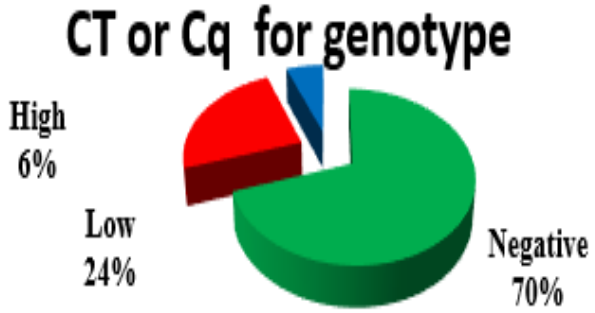


Figure 3.4 CT or Cq for genotype

Table 3.8 viral load or specimen's concentration			
viral load or specimen's concentration	N	%	Chi-Square Test (P-value)
Negative	35	70	P = 0.00 Highly sign. (P<0.01)
Low	12	24	
High	3	6	
Total	50	100	

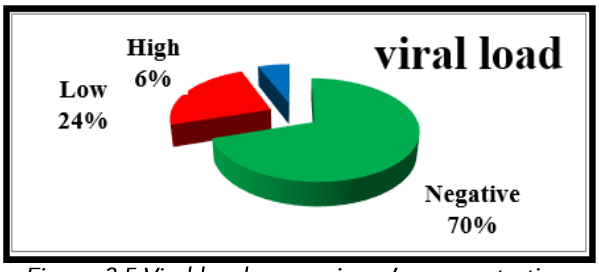


Figure 3.5 Viral load or specimen's concentration

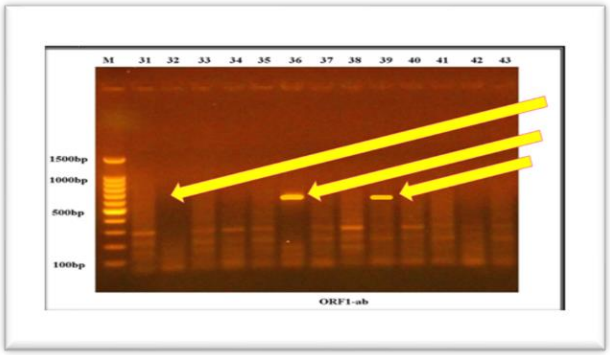


Figure 3.6 PCR Result

Results of the amplification of SARSHCOV2ORF1ab gene of virus samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 1000bp ladder marker. Lanes 31-43 resemble 588bp PCR products. The band present in sample36 and 39 and control in 31 well

4. Data Analysis

BLAST results

Sample 36

Sample 36

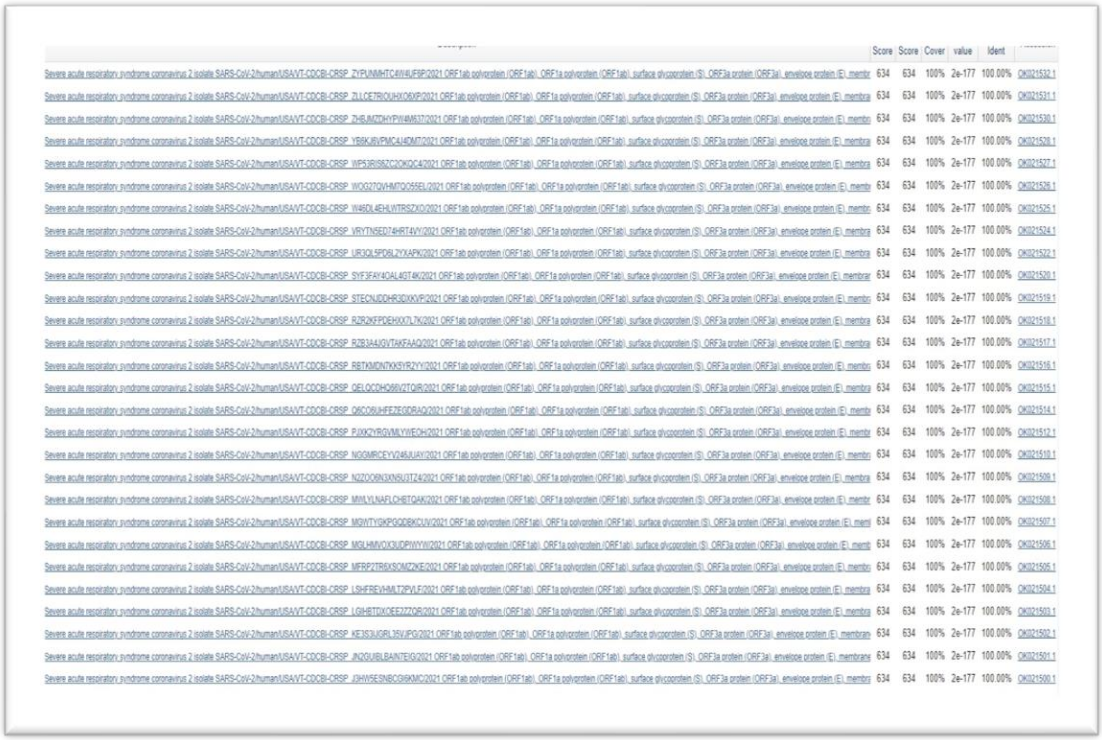
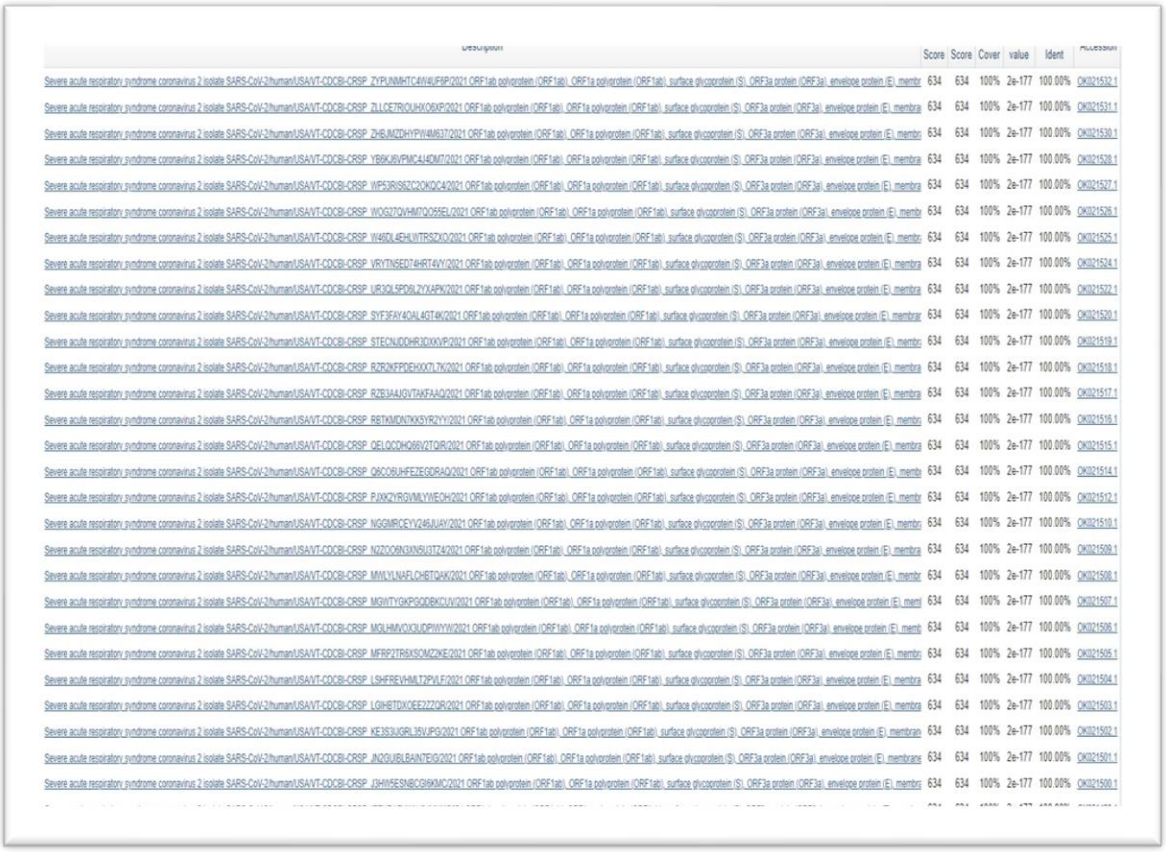


Figure 3.7 Data Analysis BLAST results

Data Analysis BLAST results Sample 39

Sample 39



Description	Score	Score	Cover	value	Ident	Accession
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_ZYPUNMHTC4W4UF9P/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021532.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_ZLLCE7RIOUHXO6XP/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021531.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_ZHBJMZDHYPW4M637/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021530.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_YB6KJ6VPMC4J4DM7/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021528.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_WP53RI9ZCCOKOC4/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021527.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_WOG27OVHM7QO55EL/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021526.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_W46DL4EHLWTRSZXO/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021525.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_VRYTN5ED74HRT4VY/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021524.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_UR3QL5PD6L2YXAPK/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021522.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_SYF3FAY4OALA4GT4K/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membrar	634	634	100%	2e-177	100.00%	OK021520.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_STECNUDDHR3DXKVP/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021519.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_RZRZKFPDEHXX7L7K/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021518.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_RZB3A4JGVTAKFAAQ/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021517.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_RBTKMDNTKKSYR2YY/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021516.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_QELQCDHQ66VZTQR/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021515.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_Q6CO6UHFEZEGDRAQ/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021514.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_PJXKQYRGVMLYWEOH/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021512.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_NGGMRCEYV246JUAY/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021510.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_NZZO0RN3XNSUSTZ4/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021509.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_MWLVLNAFLCHBTQAK/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021508.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_MGWTYGKPGGDBKCUV/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), mem	634	634	100%	2e-177	100.00%	OK021507.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_MGLHMVOXGUDPIWYY/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), mem	634	634	100%	2e-177	100.00%	OK021506.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_MFRP2TR6XSCMZ2KE/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021505.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_LSHFREVHMJT2PVLF/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021504.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_LGIHBTDXOEE2ZZOR/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membra	634	634	100%	2e-177	100.00%	OK021503.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_KE3S3UGRL35VJPG/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membran	634	634	100%	2e-177	100.00%	OK021502.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_JN2GUBLBAN7EIG/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membrane	634	634	100%	2e-177	100.00%	OK021501.1
Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/human/USA/VT-CDCBI-CRSP_J3HW5ESNBCGI6KMC/2021 ORF1ab polyprotein (ORF1ab), ORF1a polyprotein (ORF1ab), surface glycoprotein (S), ORF3a protein (ORF3a), envelope protein (E), membr	634	634	100%	2e-177	100.00%	OK021500.1

Figure 3.8 Data Analysis BLAST results

FASTA sequene Sample 36

FASTA sequencing Sample 39

AAATGCCTTTTTACCTTTTGCTATGGGTATTATT
GCTATGTCTGCTTTTGCAATGATGT

TTGTCAAACATAAGCATGCATTTCTCTGTTTGTT
TTTGTTACCTTCTCTTGCCACTGTA

GCTTATTTTAATATGGTCTATATGCCTGCTAGTT
GGGTGATGCGTATTATGACATGGT

TGGATATGGTTGATACTAGTTTGTCTGGTTTTAA
GCTAAAAGACTGTGTTATGTATGC

ATCAGCTGTAGTGTTACTAATCCTTATGACAGC
AAGAACTGTGTATGATGATGGTGCT AGGA

TCTAGGGACCTCTTTCTGCTCAA
ACTGGAATTGCCGTTTTAGATAT
GTGTGCTTCATT
AAAAGAATTACTGCAAAATGGT
ATGAATGGACGTACCATATTGGG
TAGTGCTTTATT
AGAAGATGAATTTACACCTTTTG
ATGTTGTTAGACAATGCTCAGGT
GTTACTTTCCAA
AGTGCAGTGAAAAGAACAATCA
AGGGTACACACCACTGGTTGTTA
CTCACAATTTTG
ACTTCACTTTTAGTTTTAGTCCA
GAGTACTCAATGGTCTTTGTTCT
TTTTTTTGTATGA

Figure 3.10 FASTA sequence Sample 39:

5. Discussion

Different types of samples for all participants in the study from symptomatic and asymptomatic patients for CoVI were blood sample (n = 713), nasal swab (n = 12), and throat swab (n = 7) from total samples (n = 732) as in Table 3.1. Patients were

4. Discussion

Different types of samples for all participants in the study from symptomatic and asymptomatic patients for CoVI were blood sample (n = 713), nasal swab (n = 12), and throat swab (n = 7) from total samples (n = 732) as in Table 3.1.

Fang et al., in (2020) represented the immune response to infection by pathogenic microorganisms is first expressed as an increase in the IgM antibody titer and then a rapid decrease until it disappears, while the IgG antibody titer normally increased in the middle and late stages of the infection, and it can be positive for a long time even after recovery. and agree with other study (Fang et al., 2020).

Demographics of the study revealed non-significant difference between age groups P = 0.691 Non sign. (P>0.05) and gender P = 0.886 Non sign. (P>0.05) for patient and control group as in table 3.3.

Demographics of Age groups / Year for control group < 20y (n = 4) (4%), (20 – 40) y (n = 50) (50%), (41 – 60) y (n = 38) (38%), and (61 – 80) y (n = 8) (8%).

Demographics of Age groups / Year for patient group < 20y (n = 6) (6%), (20 – 40) y (n = 48) (48%), (41 – 60) y (n = 34) (34%), and (61 – 80) y (n = 12) (12%).

Gender (Male) for control group (n = 43) (43%), female (n = 57) (57%), and gender (Male) for patient group (n = 42) (42%), female (n = 57) (57%), female (n = 58) (58%).

Courses of pneumonia in CoVI patient according to instruction of doctor in to three classes mild pneumonia in mild CoVI patient (n = 57) (57**%),** sever

pneumonia in sever CoVI patient (n =36) (36 %) and very sever pneumonia in critical CoVI patient (n =7) (7%) from total (n =100) P = 0.00 Highly sign. (P<0.01) according to as in table 3.2

Patients for molecular detection from genes were characterized by pneumonia as in Table 3.2 were divided to

two parts the first control group (n = 50) and the second were patient group (n = 50) the results indicated 10% asymptomatic patients and 90% symptomatic patients with P = 0.00 Highly sign. (P<0.01) as in table 3.5 and figure 3.2.

Table 3.6 and figure 3.3 represented genotype for CoVI was P = 0.00 Highly sign. (P<0.01) within positive SARSHCOV2 ORF1ab (n= 4), positive NL63(n=2) and 229E (n= 11), negative (n= 33) While OC43 gene negative results in all different samples from total (n= 50).

Table 3.4 and figure 3.1 Assay for CoV the results shown P = 0.019 Sign. (P<0.05) within SARSHCOV2 ORF1ab gene from nasal swab and throat swab by conventional PCR positive n =4(21.05%), negative n =15(78.95%) from total n =19.

Table 3.7 and figure 3.4 CT or Cq for genotype P = 0.00 Highly sign. (P<0.01) within (Low 12, High3 and negative 35) and shown the results undetectable from virus in blood sample due to very low viral low, in the contrast were nasal and throat swabs mostly positive due to viral load in nasal and throat swabs higher than blood samples.

Figure 3.6 shown the result of conventional PCR was positive line at 588bp M.W in sample number 36 and sample number 39 from nasal swabs while negative in other types of samples for throat and blood.

For sequencing only swab from nasal were positive were JINZA1 and JINZA2 as Figure 3.7 and Figure 3.8

The fate of two patients were suffer from COVID-19 pneumonia diagnosed by new type of this pandemic by PhD student Jnan Jafar Baksh, Supervisal Prof. Dr. Nazar Shiyaa Mohammed and Assist prof. Dr. Ahmed Saadi Hassan, also submitted in NCBI Both of two patient's loss of their life due to severity of infection for JINZA1 and JINZA2 new causative agents for COVID-19 pneumonia and were critical class for this pandemic.

Figure 3.9 Data Analysis BLAST results Sample 36 and figure 3.10 Data Analysis BLAST results Sample 39 shown both of two isolates from Baghdad/Iraq patients were SARSHCOV2 that indicated transmitted by travel between Iraq and USA

Figure 3.9 and figure 3.10 revealed and indicated both new isolates new causative agents for COVID-19 pneumonia with highly virulence for severity of this pandemic were critical class lead to loss of life both patients that indicated spread transmitted of this disease and prognosis of this disease by increased of complication by organ failure lead to death.

6. Conclusion

Detection of other COVID19 pneumonia as HCOV NL63, and 229E, also the study concluded that gene sequencing very important to discovery new genes from SARSHCOV2 in patients suffer from COVID-19 pneumonia could be diagnosed by nucleic acid detection (RT PCR) were required confirmed by Sanger sequencing using ABI3730XL, automated DNA sequences, the results concluded discovery new isolates called SARSHCOV2ORF1ab JINZA1 gene and SARSHCOV2ORF1ab JINZA2 gene in Baghdad/Iraq patients and submitted in NCBI SARSHCOV2ORF1ab JINZA1 OK486620 gene and JINZA2 OK586822 gene.

The names of both genes according to name of PhD student Jnan Jafar Baksh, Supervisal Prof. Dr. Nazar Shiyaa Mohammed and Assist prof. Dr. Ahmed Saadi Hassan. BLAST results indicated because transmission by travel between Iraq and USA.

JINZA: JI: PhD student Jnan Jafar Baksh, ZN: Proff. Dr. Nazar Shiyaa Mohammed and A: Assist. Proff. Dr. Ahmed Saadi Hassan.

Both of two patient's loss of their life due to severity of infection for JINZA1 and JINZA2 and were critical class for this pandemic.

7.Recommendation

Chosen specific primers for specific gene to avoid coinfection with other viruses and using confirmed tests include real time PCR or conventional PCR and gene sequencing for genotyping for corona virus to know speed viral transmission and control of disease. Nasal swab and throat swab must be used for detection from corona virus due to viral load higher than blood samples.

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