Expression of HCOV NL63 and 229E genes in Corona virus infections with elevation of serum Zinc levels

Jnan J. Baksh[1], Nazar Sh. Mohammed[2], Ahmed S. Hassan[3]
[1,2,3]Department of Medical Laboratories, Medical Technical College / Middle Technical University, Baghdad/ Iraq (MTU)
Corresponding Author: jnanjafar999@gmail.com

Abstract
A total of 100 serum samples were collected from patients with 20 nasal and throat swab samples of Corona virus and 100 samples as a control group from patients were recumbent in al-Yarmook and Al- Numan Hospitals from period 2st January 2021 to 1st September 2021. The mean of patient's ages were high compared to control in Corona virus infection (NS), the IgG of patients were high compared to healthy persons HS., the IgM mean was more than healthy persons HS. While the mean of serum zinc levels of patents a high compared to control HS. The mean of patient's ages with mild was HS, sever was (HS), and critical infection was (Sign), the IgG mild, sever was NS, and the critical also was NS. The IgM mean the mild was NS, the sever was HS, and the critical was HS. The mean of serum zinc levels with mild infection, sever and critical infection were NS, with the validity tests, serum zinc sensitivity 89%, Specificity94.1% positive predictive value (PPV) 93.4% and accuracy 91.6% P=0.00 (HS). Ct positive of genotyping was (24%) are low and the high positive 3(%) in patents have corona virus infections. The positive assay of NL63 gene (10.53%) isolated from nasal and throat swab patients have corona virus infections, the positive CT for NL63 gene (4%), also 229E gene (56.9%) isolated from nasal and throat swab. The NL63 and E229 genes expression was investigated in patients who were infected with Corona virus in comparison with the healthy controls by using real-time PCR. The results showed that there was high Ct value for patients and controls with high Ct value of templates, preoperational to the gene concentration.
Keywords: Expression, HCOV NL63 gene 229E gene, Corona virus, serum Zinc

1. Introduction

Coronaviruses are a group of related RNA viruses that cause diseases in humans, they cause respiratory tract infections that can range from mild to severe (Fan et al., 2019). Human coronavirus 229E continued to be studied in subsequent decades (Geller et al., 2012; Myint,1995). Other human coronaviruses have since been identified, including NL63- HCoV in 2003 (Zhu et al., 2020). Zn is involved in several cellular processes and has a variability of direct and indirect antiviral properties. It was confirmed that Zn deficiency is associated with low production of antibody in addition to affect function of the innate immune system such as low natural killer cell activity, decreased production of cytokine by monocytes, and the chemotaxis and oxidative burst of neutrophil granulocytes which demonstrated by Ibs et al., in (2003). It also results in thymic atrophy, altered thymic hormones production, lymphopenia, and defective cellular and antibody mediated responses that lead to increased degrees and period of infection (Saha et al.,1998).

2. Material and Methods

The primers for HCoV (229E and NL63) genes detection by real time PCR were supplied by Macrogen Company in a lyophilized form as in table 2.1

Table1: Primers used

Primer name	Froward primer (5' - 3')	Reverse primer (5' - 3')
HCoV 229E gene	CAGTCAAATGGGCTGATGCA	AAAGGGCTATAAAGAGAATAAGGTATTCT
HCoV NL63 gene	ACGTACTTCTATTATGAAGCATGATATTAA	AGCAGATCTAATGTTATACTTAAAACTACG

Table 2: Cycling: NL63

Target	NL63 → NL63
Normalisation	Dynamic
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	0.998 starting at cycle 1

Table 3: Cycling: E229

Target	E229 → E229
Normalisation	Dynamic
Ignore	First 21 cycles
Exclusion	Extensive with fluorescence cutoff of 5%
Threshold	0.100 starting at cycle 1

3. Statistical Analysis

Data were expressed as mean (±) SEM (standard error of mean), and the p-value <0.05 was regarded significant.

4. Result

The mean of patient's ages (40.71 ±1.62) compared to control (41.32±14.95) in Corona virus infection

doi.org/10.31838/hiv23.01.150 Received: 14.09.22, Revised: 28.10.22, Accepted: 25.12.22

(NS), the IgG of patients were (14.49±6.61) compared to healthy persons (0.056± 0.09) was HS., the IgM mean was (1.93±1.04) in compassion to healthy persons (0.072±0.13) HS. While the mean of serum zinc levels of patents (22.41±2.24) compared to control (90.37±0.95) HS., shown in table 4.

Table 4: The mean of parameters compared to control group

Parameters		N	Mean	Std. Deviation	Std. Error	Range		P – value
						Mini.	Maxi.	
Age/Year	Control	100	41.32	14.95	1.49	17	78	P = 0.779 Non sign. (P>0.05)
	Patient	100	40.71	16.19	1.62	6	78	
IgG	Control	100	0.056	0.09	0.009	0.01	0.66	P = 0.00 Highly sign. (P<0.01)
	Patient	100	14.49	6.61	0.66	1.5	33.3	
IgM	Control	100	0.072	0.13	0.013	0.01	0.66	P = 0.00 Highly sign. (P<0.01)
	Patient	100	1.93	1.04	0.104	1	6.5	
Zinc	Control	100	90.37	9.51	0.95	76.76	111.4	P = 0.00 Highly sign. (P<0.01)
	Patient	100	47.44	22.41	2.24	12.6	112.6	

The mean of patient's ages with mild (34.12±1.862) HS, sever (46.94±14.485), (HS), and critical infection (Sign), the IgG mild (14.17±6.531), sever (14.58± 6.525) NS, and the critical (15.37± 8.469) NS. While the IgM mean the mild was (14.17±1.0884) NS, the sever (1.79±0.818) HS, and the critical (3.16±1.276) HS. The mean of serum zinc levels with mild infection (49.62±23.352), sever (45.98±22.035) and critical infection (37.19±13.401) NS, as shows in table 5.

Table 5: correlation between studied parameters and severity for corona virus infection

Parameters		N	Mean	Std. Deviation	Std. Error	Range		ANOVA P-value	LSD P-value
						Mini.	Maxi.		
Age / Year	Mild	57	34.12	14.06	1.862	14	70	P = 0.00 Highly sign. (P<0.01)	P^1=0.00 HS
	Sever	36	46.94	14.485	2.414	6	78		P^2=0.00 HS
	Critical	7	62.14	8.668	3.276	50	75		P^3=0.011 S
	Total	100							
IgG	Mild	57	14.17	6.531	1.0884	1.5	27	P=0.896	P^1=0.768 NS
	Sever	36	14.58	6.625	0.86422	4.1	33.3		P^2=0.769 NS
	Critical	7	15.37	8.469	3.2012	1.5	28.2		P^3=0.662 NS
	Total	100							
IgM	Mild	57	14.17	6.531	1.0884	1.5	27	P = 0.004 Highly sign. (P<0.01)	P^1=0.694 NS
	Sever	36	1.79	0.818	0.10838	1.01	4.6		P^2=0.001HS
	Critical	7	3.16	1.276	0.48223	1.69	4.9		P^3=0.002 HS
	Total	100							
Zinc	Mild	57	49.62	23.352	3.09299	12.6	109.9	P=0.343	P^1=0.446 NS
	Sever	36	45.98	22.035	3.67243	16.4	112.6		P^2=0.169 NS
	Critical	7	37.19	13.401	5.06500	22.1	60.6		P^3=0.345 NS
	Total	100							

Table 6 and figure 1 showed the validity tests, serum zinc sensitivity 89%, Specificity94.1% positive predictive value (PPV) 93.4% and accuracy 91.6% P=0.00 (HS).

Table 6: Validity test for IgG, IgM, Zinc

Validity tests	IgG	IgM	Zinc
Sensitivity	100%	100%	89%
Specificity	100%	100%	94.1%
Positive predictive value (PPV)	100%	100%	93.4%
Negative predictive value (NPV)	100%	100%	89.6%
Accuracy	100%	100%	91.6%
Area under curve (AUC)	1	1	0.94
P - value	0.00 HS	0.00 HS	0.00 HS

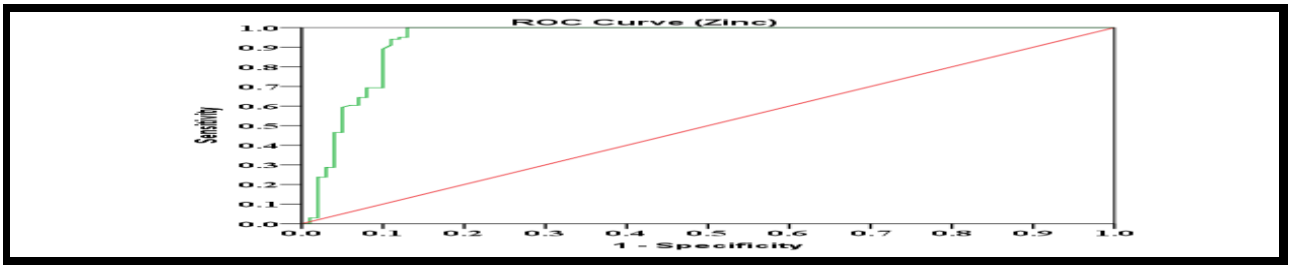

Figure 1: ROC curve Zinc

Ct positive of genotyping was 12(24%) are low and the high positive 3(%) in patents have corona virus infections, as shows in table 7.

Table 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	

The positive assay of NL63 gene 2(10.53%) isolated from nasal and throat swab patients have corona virus infections, the positive CT for NL63 gene 2(4%), also 229E gene 11(56.9%) isolated from nasal and throat swab, P<0.01), HS.

Table 8: Assays for NL63 gene genotyping CT for NL63 gene and229E gene				
Assays		N	%	Binomial Z Test (P-value)
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	

The NL63 gene expression was investigated in patients who were infected with Corona virus in comparison with the healthy controls by using real-time PCR. The results showed that there was high Ct value for patients and controls with high Ct value of templates, preoperational to the gene concentration, as shows in figure 2.

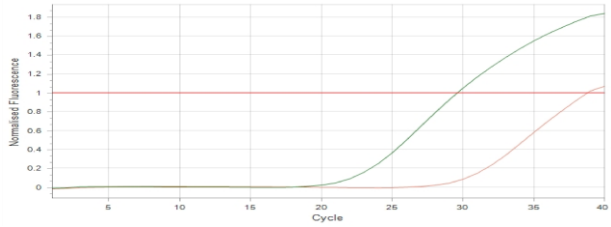

Figure 2: Expression of NL63 gene of Corona virus patients by using real-time PCR.

The 229E gene expression was investigated in patients who were infected with Corona virus in comparison with the healthy controls by using real-time PCR. The results showed that there was high Ct value for patients and controls with high Ct value of templates, preoperational to the gene concentration, as shows in figure 3.

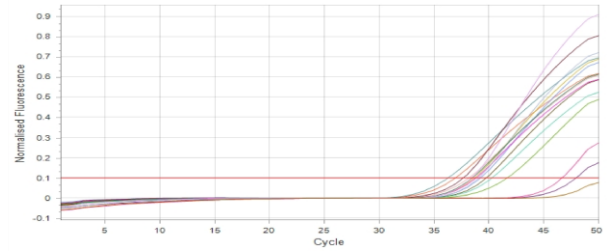

Figure 3: Expression of 229E gene of Corona virus patients by using real-time PCR.

5. Discussion

Corona virus is a risky microorganism may lead to death. The mean of patient's ages were high compared to control in Corona virus infection (NS), the IgG of patients were high compared to healthy persons HS., the IgM mean was more than healthy persons HS. Hou, et al, (2020) reported level of IgM was increased during the first week after SARS-CoV-2 infection and reached its peak level after 2 weeks, while IgG reached its peak in 3 weeks, which was maintained at a high level even over 48 days (Hou, et al, 2020). The mean of serum zinc levels of patents a high compared to control HS. The mean of patient's ages with mild was HS, sever was (HS), and critical infection was (Sign), the IgG mild, sever was NS, and the critical also was NS. The IgM mean the mild was NS, the sever was HS, and the critical was HS. These results agreed with (Krumm, et al, 2021) who illustrated that the sever and mild infections with corona virus was a high elevation of serum zinc level, high concentration of ant corona virus IgM antibodies and ant corona virus IgG antibodies (Krumm, et al, 2021). The serum zinc sensitivity 89%, Specificity94.1% positive predictive value (PPV) 93.4% and accuracy 91.6% P=0.00 (HS), (Skittrall, et al, 2020). The NL63 and E229 genes expression was investigated in patients who were infected with Corona virus in comparison with the healthy controls by using real-time PCR. Liu, et al, (2020) explained there is a large number of positive infections that were determined by the gene expression of both the NL63 and the E229 genes compared to the control group (Liu, et al, 2020).

References

Fan Y, Zhao K, Shi ZL, Zhou P (March 2019): Bat coronaviruses in China. 11 (3): 210.

Geller C, Varbanov M, Duval RE (November 2012): Human coronaviruses insights into environmental resistance and its influence on the development of new antiseptic strategies viruses. 4 (11): 3044–68.

Hamid Reza Mollaei, Abass Aghaei Afshar, Davood Kalantar-Neyestanaki, Mehdi Fazlalipour, and Behnaz Aflatoonian (2020 Jun): Comparison five primer sets from different genome region of COVID-19 for detection of virus infection by conventional RT-PCR ; 12(3): 185–193.
Myint SH (1995): Human coronavirus infections. The coronaviridae. The Viruses springer US. pp. 389–401.
Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. (February 2020): A novel coronavirus from patients with pneumonia in China, 2019. The New England Journal of Medicine. 382 (8): 727–733.
Ibs K.H., Rink L. Zinc-altered immune function. J Nutr. 2003;133(5 Suppl 1):1452S–1456S.2017 Dec;50(6):763-770.
Saha A.R., Hadden E.M., Hadden J.W. Zinc induces thymulin secretion from human thymic epithelial cells in vitro and augments splenocyte and thymocyte responses in vivo. Int J Immuno pharmacol. 1995; 17:729–733.
Huang C, Wang Y, Li X, et al. (2020): Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China [published correction appears in Lancet. 2020 Jan 30]. Lancet.; 395:497–506.
Novick S.G., Godfrey J.C., Pollack R.L., Wilder H.R. Zinc-induced suppression of inflammation in the respiratory tract, caused by infection with human rhinovirus and other irritants. Med Hypotheses. 1997;49:347–357.
Hou, H. Ting Wang, T. and Zhang, Bo. Detection of IgM and IgG antibodies in patients with coronavirus disease 2019, Clin Transl Immunology. 2020; 9(5): e1136. doi: 10.1002/cti2.1136.
Krumm, Z. A. Grace M. Lloyd, G. M. and Francis, C. P. Precision therapeutic targets for COVID-19, Krumm et al. Virol J (2021):18-66. doi.org/10.1186/s 12985-021-01526-y.
Skittrall, J. P. Wilson, M. and Smielewska, A. A. et al, Specificity and positive predictive value of SARS-CoV-2 nucleic acid amplification testing in a low-prevalence setting, Clin Microbiol Infect. 2021 Mar; 27(3): 469.e9–469.e15. 1 doi:0.1016/j.cmi.2020.10.003.
Liu, D. X. Jia Q. Liang, J. Q. and Fung. To. S. Human Coronavirus-229E, -OC43, -NL63, and -HKU1 (Coronaviridae)Encyclopedia of Virology. 2021: 428–440.
doi: 10.1016/B978-0-12-809633-8.21501-X.