AMERICAN JOURNAL OF BIOLOGY AND NATURAL SCIENCES
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Article

Estimation of KLRK1 (NKG2D) and 4-1 BB Gene expression as a biomarker for prognosis and diagnosis of Severe COVID-19

Rawaq Haider Al-Mihanya[1], Ihsan Raisan Ibrahim[*2], Hayder Saud Mayih[3]
1,2. Department of Clinical Laboratory Sciences, College of Pharmacy, University of Al-Qadisiyah, Diwaniyah, Iraq
3. Ministry of Education,Open educational college, Diwaniyah, Iraq
*Correspondence: ihsan.raisan@qu.edu.iq

Citation: Al-Mihanya, R. H., Ibrahim, I. R., Mayih, H. S. Estimation of KLRK1 (NKG2D) and 4-1 BB Gene expression as a biomarker for prognosis and diagnosis of Severe COVID-19. American Journal of Biology and Natural Sciences 2026, 3(9), 7-16.

Received: 18th Jul 2026
Revised: 05th Aug 2026
Accepted: 25th Aug 2026
Published: 04th Sept 2026

Abstract: The complications of the respiratory system, that demining from pneumonia to acute respiratory distress syndrome, are the marker to disease severity of COVID-19. Natural killer (NK) is innate immune cells that contribute in body defense against viral infections; a decrease in these cells affects the development and severity of COVID-19. The study consists of 34 cases diagnosed as severe COVID-19, 19 females and 15 males aged (23-75) years, who attended Al- Diwaniyah teaching hospital during the period (October 2023- July 2024). The healthy group included 20 samples, 13 males and 7 females aged (27-61) years. After diagnosis of COVID-19 -2 by the positive result of RT-PCR from nasopharyngeal specimens and chest CT scan, RNA was extracted from the blood specimens for measuring mRNA KLRK1 and 4-1BB via RT- qPCR technique. The result in the research revealed lower KLRK1 mRNA in patients with COVID-19 (0.51 ±0.25) compared to the healthy group (1.22 ±0.56), while 4-1BB was elevated in patients with COVID-19 (2.07±0.94) compared to the healthy group (1.21±0.58) at a significant level ($P \leq 0.01$). This study indicated that the deceased mRNA KLER1 and elevated 4-1BB in COVID-19 patients might be an independent biomarker for prognosis and diagnosis of the severity of COVID-19 infection.

Keywords: KLRK1, NKG2D, 4-1BB, COVID-19, qPCR, ROC

Introduction

The pandemic of ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) represents a profound challenge facing the health system worldwide. Coronavirus disease (COVID-19) is induced by SARS-CoV-2, that are belongs to the Beta-coronavirus genus,genomic (+ssRNA) viruses, which noticed under electron microscope as spherical entities with an envelope with club-shaped spikes. SARS-CoV-2 consist of 4 structurally proteins like the envelope (E), spike (S), the nucleocapsid (N) and protein membrane (M), the S protein is related to the virus pathogenesis because of its associated domain to the expressed of receptor in the host cells, this glycoprotein is high affinity to (ACE2) receptors which present on in the upper and lower respiratory tract and the tissue of lung , therefor SARS-CoV-2 can infect respiratory tract leading to pneumonia with dangerous complications and high rate of death [1].The clinical manifestations of COVID-19 is multiple and about 80% of infected individual are asymptomatic, or moderate symptomatic, the remaining individuals about 20% suffer from the complication of severe infection [2,3]. The clinical

forms of severe infection are observed in the cases included dys-regulations of complex immune and an increased in the released of inflammatory cytokines which lead to pulmonary edema, severe pneumonia, acute respiratory distress syndrome (ARDS) and dysfunction in multi-organ or eventual death [4].Natural killer (NK) cells are important lymphocytes of innate immune that have the ability to destroy virus through the expression of Natural Killer group 2D (NKG2D) encoded by a gene KLRK1 (Killer Cell Lectin-Like Receptor K1). NKG2D is a receptor have an essential role in activation and regulation the cytotoxicity of NK cells and T cell subsets that interact with NKG2DLs which structurally homolog to MHC-I molecules and consist of (MICA /B) and the UL16-binding proteins (ULBP1-6) that might be induced with different many types of cellular stress such as viral infection and tumor cells [5,6,7]. The change in the activity of NK associated with severity of COVID-19 progression [8].The maturation of NK cells constitute about 10-15 % from circulating lymphocytes. NK cells are present in bone marrow, spleen, liver and intestinal mucosa, and have important role against several bacterial and viral lung infections like SARS-CoV-1, Influenza, and COVID-19 and MERS-CoV infections [9], 4-1BB (CD137, TNFRSF9) is a powerful co-stimulatory glycoprotein receptor necessary for robust immune responses, is highly expression following the activation of many leukocyte populations ncluding CD4+ and CD8+ T cells, regulatory T (Treg) cells, natural killer (NK) cells, Upon relate to high-affinity receptors called 4-1BB ligand (4-1BBL), which is expressed on the surface of several professional APCs, such as B cells, dendritic cells (DCs), and macrophages to induce cell survival, proliferation, and effector functions[10,11].This study aimed to investigate the role of KLRK1 and 4-1 BB gene expression as prognostic and diagnostic factor of patients with severe COVID-19.

Materials and Methods

Study samples

Five milliliters blood was collected from 34 patients with severe COVID-19 (19 females and 15 males) aged (23-75) year, COVID-19 was determined by RT-PCR for nasopharyngeal specimens which collected from Al- Diwaniyah teaching hospital during period (October 2023- July 2024), healthy group included 20 samples (13 males and 7 females) aged (27-61) years.

Real- time reverse transcription quantitative PCR (RT-qPCR) technique

RNA total was isolated from the blood sample by utilizing TRIZOL reagents, according to the procedure of (PreAnalytiX; Qiagen Ltd), and RNA was converted to cDNA based on (AccuPower® RT PreMix Kits) , which consists of blank-dried components necessary for the synthesis of cDNA, like reaction buffer, M-MLV Reverse Transcriptase, and RNase inhibitor. With 1μM random hexamer primer in the tubes, the protocol was conducted following the instructions of the AccuPower RT PreMix (BIONEER / Korea). Measuring the gene expression profiles of KLRK1 and 4-1BB using the SYBR Green RT-PCR kit (Promega Company). Specific sequences KLRK1 were designed in this study through the design of Primer three online based on NCBI-Gene Bank database(Macrogen,Korea),as follows: 4-1 BB F 5-TGCTTGTGAATGGG ACGAAG-3 R 5-ACGTCAGCGCAAGAAGAAG-3 [12], GAPDH F 5- ATGGGGAAGGTGAAGGTCG-3 R 5- GGGTCATTGATGGCAACAATATC-3 [13]. The data were expressed as copy numbers relative to (GAPDH) as a housekeeping gene The total reaction size consists of (5 μl cDNA, 2 μl primer, and 13 μl master (water /mix). PCR amplifications were carried out with initial denaturation for 3 minutes at 95˚C, followed by 44 cycles with a two-step (denaturation 10 sec at 95˚C, annealing 30 sec at 58˚C) melting curve, with a 5-second hold at 65˚C and 0.5 at 95˚C, as in (Figure 1). The calculation of relative expression levels of KLRK1 and 4-1BB was performed based on the reference method as follows:

ΔCT (normalization)= CT (target)-CT (reference)

ΔΔCT(calibration) = ΔCT (experiential sample) - ΔCT (control sample)

Relative expression $=2^{(-\Delta\Delta CT)}$ [14].

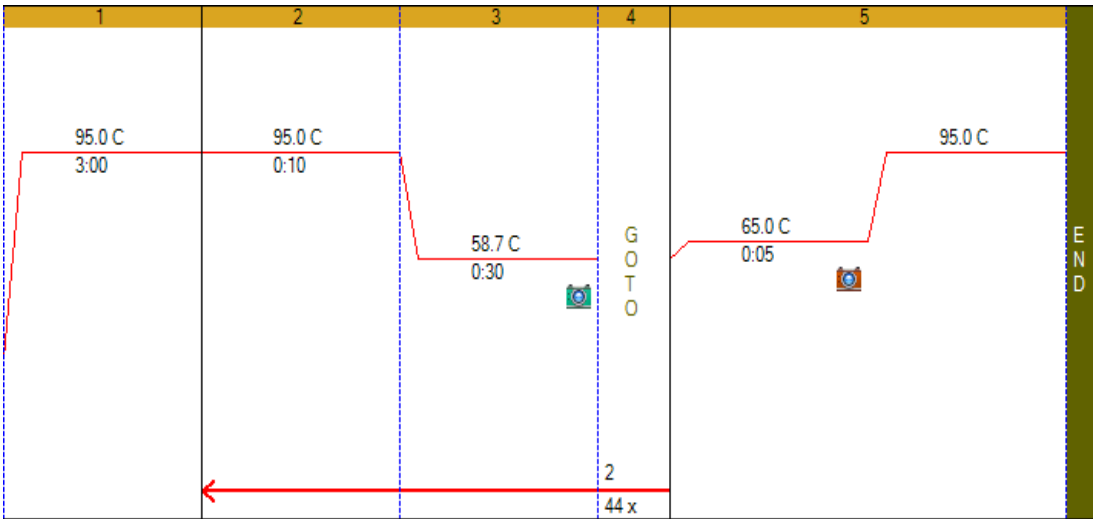

Figure 1. Annealing curve of genes study.

Statistical analysis

The data of this study was underwent for statistical analysis based on used SPSS VR. 24, T-test was performed to compare the means of patients with healthy group at significant level (P≤ 0.01), Receiver Operating Characteristic (ROC) to analyze the diagnostic precision of KLRK1 and 4-1BB [15, 16].

Results

The demographic characteristics of study groups are revealed in Table 1. The patients aged (60-75)years was higher (41.17 %) , while the percentage of low age (40-59) years was (23.52%). Percentage of females was increased (55.88%) compared to males (44.11%).

Table 1. Demographic characteristics of study groups.

Characteristic	Category	Patient N=34 (%)	Healthy N=20(%)
Age	23-39	12 (35.29)	9(45)
	40-59	8(23.52)	7(35)
	60-75	14 (41.17)	4(20)
Sex	Male	15(44.11)	13(65)
	female	19(55.88)	7(35)
Diagnosis virus	Result of real time PCR	Positive 34(100)	0(0)
		Negative 0(0)	20(100)
	Chest CT scan as viral pneumonia	Positive 34(100)	0(0)
		Negative 0(0)	20(100)
characteristic	Category	Patient N=34 (%)	Healthy N=20(%)
Age	23-39	12 (35.29)	9(45)
	40-59	8(23.52)	7(35)
	60-75	14 (41.17)	4(20)
Sex	Male	15(44.11)	13(65)
	Female	19(55.88)	7(35)
Diagnosis virus	Result of real time PCR	Positive 34(100)	0(0)
		Negative 0(0)	20(100)

	Chest CT scan as viral pneumonia	Positive 34(100) Negative 0(0)	0(0) 20(100)

CT = computed tomography, PCR= Polymerase Chain Reaction

Estimation of KLRK1 and 4-1BB gene expression by real-time quantitative PCR

To estimate the transcriptional profiles of the target genes, RT-qPCR data were calculated based on the cycle threshold (CT) ratios of KLRK1 and 4-1BB relative to the GAPDH housekeeping gene in the patient and healthy groups. The amplification diagram revealed distinct differences in the (CT) values for both target genes in patients compared to healthy controls. KLRK1 was decreased in patients at CT range (32-34) compared to the healthy group at CT range (30-33) as shown in Fig 2. 4-1 BB increased in patient with CT value (27-30) compared to healthy (29-31) (Fig 3), while there was no difference in the CT data of GAPDH in the range (24- 26) between study groups as in as seen in (Figure 4).

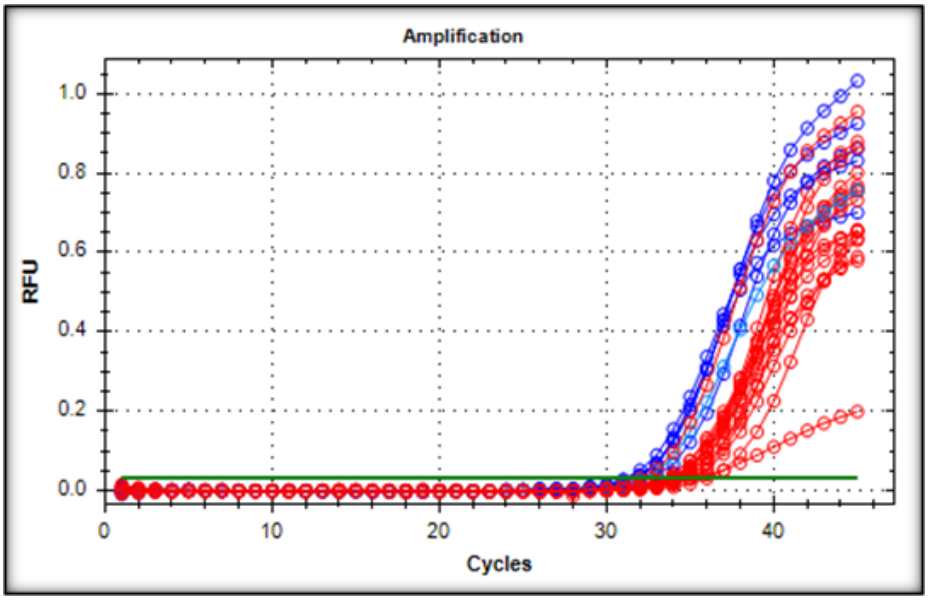

Figure 2. Quantitative PCR plot of KLRK1 gene in study group.

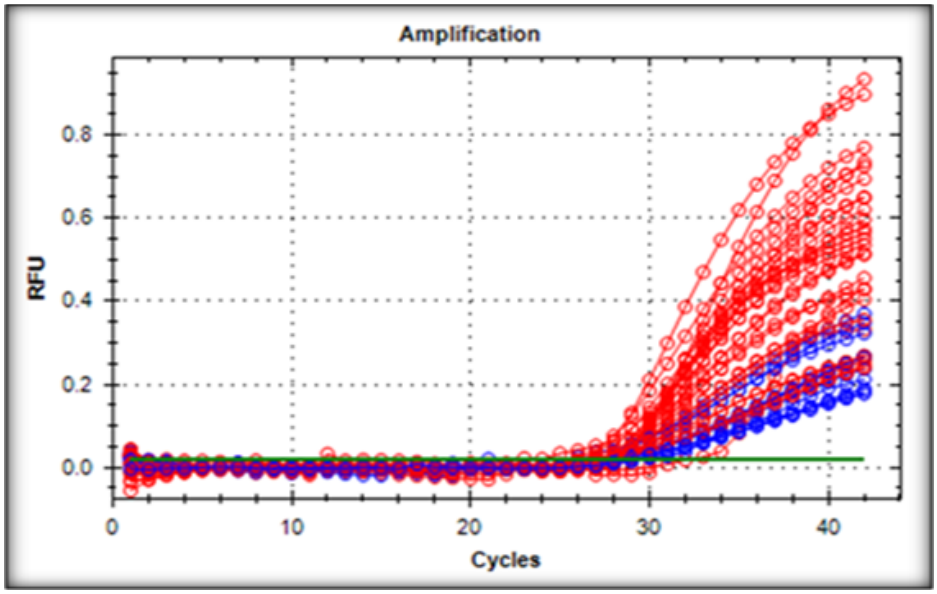

Figure 3. Quantitative PCR plot of 4-1BB gene in study group.

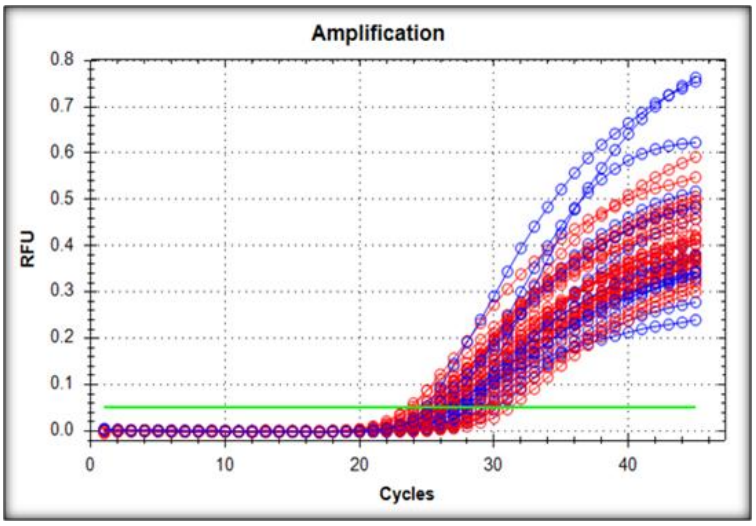

Figure 4. Quantitative PCR plot of GAPDH gene in study group.

Evaluation of KLRK1 and 4-1BB gene expression in study groups

The data of KLRK1 gene as in (Figure 5) showed that the mean of KLRK1gene was significantly decreased at rate (0.51±0.25) in COVID-19 patients compared with the mean of healthy group (1.22±0.56) at significant level ($P \leq 0.01$), while the mean of 4-1 BB elevated in COVID-19 patients (2.07±0.94) compared healthy (1.21±0.58) ($P \leq 0.01$) as seen in (Figure 6).

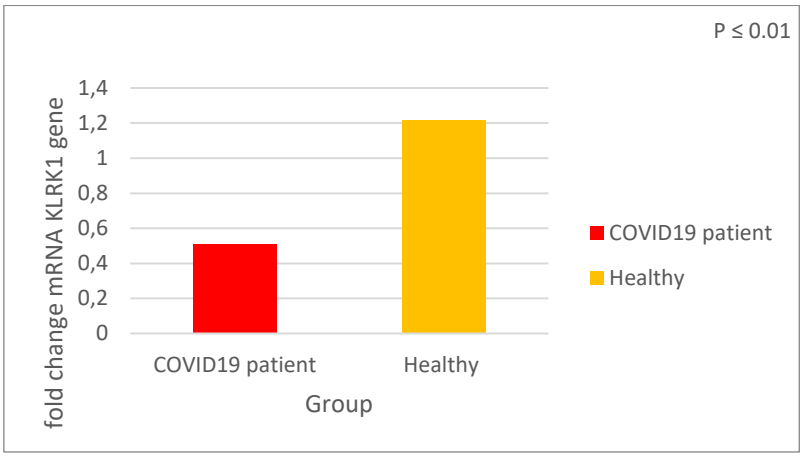

Figure 5. KLRK1 gene expression in COVID-19 patients and healthy group.

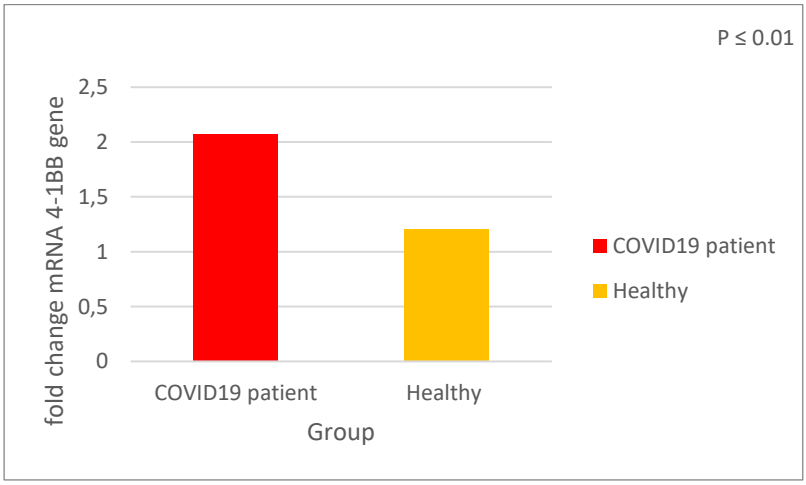

Figure 6. 4-1BB gene expression in COVID-19 patients and healthy group.

Area Under the ROC Curve (AUC-ROC) analysis

To assess the diagnostic efficacy of KLRK1 and 4-1BB, the ROC curve was analyzed for serum KLRK1 and 4-1BB in COVID-19 patients (n=34) vs. the control group (n=20). The area under the curve AUC values =0.81, CI=95%=0.65-0.96, sensitivity =76%, specificity =83%, cut off=0.76 was related to the level of KLRK1 in COVID-19 patient and healthy control at p-value =0.02 (Figure 7), while AUC value= 0.79,CI=95%=0.66-0.91, sensitivity =71%, specificity =85%, cut off=1.64 was correlated with 4-1BB level COVID-19 patient and healthy control at $P \leq 0.01$ (Figure 8).

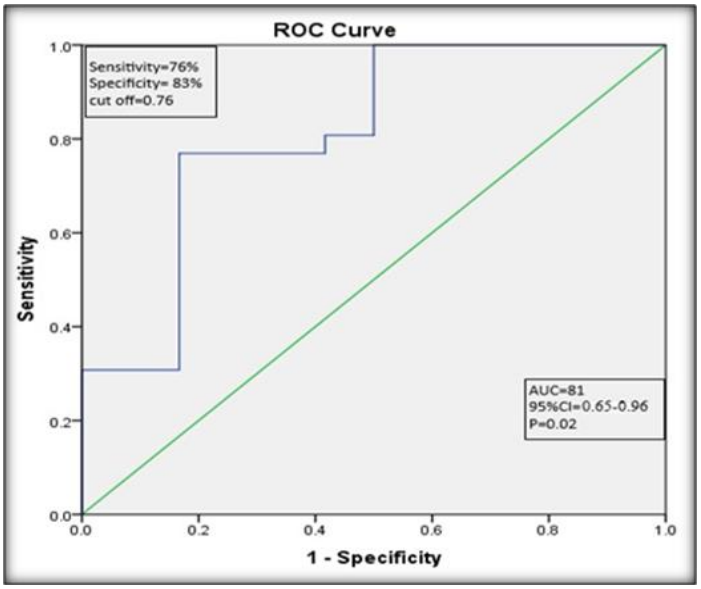

Figure 7. ROC curve of KLRK1 in study group

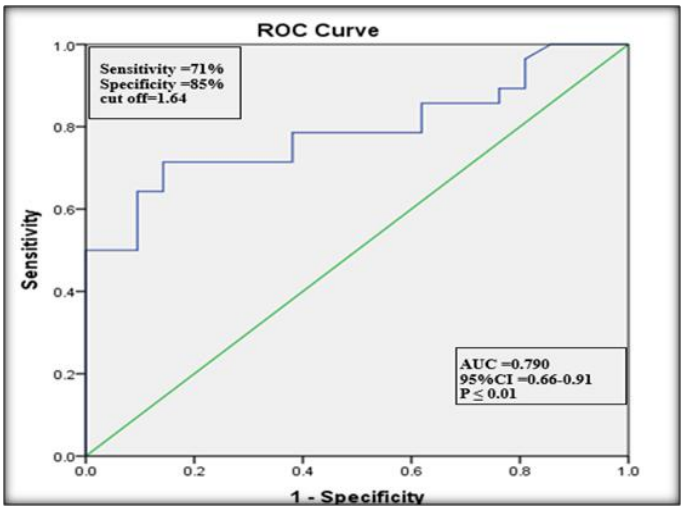

Figure 8. ROC curve of 4-1BB in study group

Discussion

COVID-19 affects females and males, without sparing any age group. The results in this study indicated that the female is high infected compared to males, and this result is consistent with a recent study of Flor *et al* that was carried out during the COVID-19 pandemic and showed that females were more infected than males [17]. The prevalence of virus in females may be return to several reason like psychological factor, females are more afraid of contracting the virus and their movement have been restricted, that lead to decrease in hospitalizations, also increase of visit to outpatient and emergency during the pandemic of virus [18], as well as, the evidence indicated that female have been severely affected by the consequences of the COVID-19 pandemic, females are vulnerable to economic and social factors [19]. The data in this study showed that patients aged 60-75 years are more likely to be infected with COVID-19. This result agrees with a recent study of Starke *et al* suggested that old age

has been recorded to be a factor related to the severity of COVID-19. The individual aged 60 years is considered more effectively infected with the virus [20]. COVID-19 might infect people of any age and cause serious illness, hospitalization, and death. In general viruse, can spread among younger individuals and focus primarily on older people aged more than 55 years [21]. Old age is a potent and independent risk factor to hospitalization and ICU admission [22].

The imaging of computed tomography (CT) has an essential role in the diagnosis and management of patients with COVID-19, while real-time PCR provides a test for COVID-19 diagnosis with high specificity. Several studies have recorded the important role of CT in the management of COVID-19 patients. The imaging used as an indicator for COVID-19 diagnosis has been established in some countries [23,24]. the result of the current study as seen in Table 1 revealed that the diagnosis of COVID-19 by chest CT an RT PCR was positive at rate (100%), this result agreed with study by Ai *et al* that carried out for 1014 patients with COVID-19 and referred that the sensitivity of chest CT in COVID-19 patients was at rate (97%) depending on the result of RT-PCR [25]. A study carried out by Böger *et al* showed that the chest CT isn't as sensitive as compared to laboratory test for the diagnosis of COVID-19, like RT-PCR, thus utilizing CT alone doesn't have clinical benefit in COVID-19 diagnosis [26], Other studies explained that chest CT might be confined to evaluate COVID-19 pneumonia complications and severity, such as ARDS, when an alternative diagnosis of disease is present [27,28].

NK cells have important in innate immune response and acts as defense barrier fight against the viruses of pulmonary during early phase of COVID-19,the amount of NK cells was elevated, thus NK cells can prevent fibrosis of lungs and allows the lungs to perform their normally function in some infected individuals, while in cases of severe COVID-19 the clearance of virus and progression of diseases correlated number of NK cells thus, the viral infection was a raised with dysfunction and decreased NK cells number, several studies have indicated that the decreased number and dysfunction of NK cells in the blood in patients with severe COVID-19, where these cells are lose ability to prevent the spread of illness and destruction the infected cells, that cause pulmonary inflammation progression in the pneumonia of COVID-19 and result in destruction of lung tissue [29, 30]. Also in study carried out Cao *et al* hypothesized reduction in the functions of NK cell, like impaired in abilities of anti-fibrosis and antiviral activity, that might be closely related to severe COVID-19 progression [31] The result of current study demonstrated that the decreased KLRK1 mRNA levels in severe COVID-19 patients might lead to reduce the active receptor NKG2D on surface of NK cells thus loss ability of these cells to effective immune response against to COVID-19 [32], this result is consistent with many finding indicated the exhaustion of NK cell and explained via the decreased of expression of NKG2D by NK cells [33,34].

The decreased of NK cells in peripheral blood might be due to several reason like the increment of NK cell apoptosis, the turnover change, and return to the inflamed/infected tissues [35].

Function of cytotoxic NK cells is inhibited in patients with COVID-19 by many mechanisms, one of these mechanism that cells with COVID-19 can evade from NK cell by induction the alteration and modulation in the response of NK cells to virus, where viral proteins Nsp1 and Nsp14 can down-regulation of ligands for the NK cell activating receptors NKG2D, thus in cells infected with virus the responses of NK cell less efficient against COVID-19 compared to the cells of uninfected,the COVID-19 can use another mechanisms to escaped from NK cell that mediated clearance of viral like, reduction of number of NK cells in the peripheral blood or induce NK cells exhaustion via increasing expression of the inhibitory receptor NKG2A and decreased of activating receptors like NKG2D, thus result in the severe destruction of lung tissue [36,37], Reduction of NK activity may occur via impairing NKG2D expression which blocked via soluble stress-stimulate molecules MICA/B. Thus, NKG2D was decreased in patients who died compared to survival patients [38], therefore, the down-regulation of NKG2D permits viral escape, high expression of pro-inflammatory cytokines, and increasing lung pathology [39]

The result of this study was agreed with several recent studies in patients with severe covid-19, NK cell might be exhausted due to decrease frequently of NKG2D receptor on surface of NK cells, thus activating receptor NKG2D expression by NK cells may be associated with severity of COVID-19 disease, the NKG2D expression was decreased in severe COVID-19 patients compared to negative

groups at (P≤ 0.01) [40,41],in addition the result indicated the higher significant increase of 4-1BBgene expression in COVID-19 patients compared to healthy control as seen in [Figure 6], These findings were recapitulated by Sanchez *et al* who explained the critical role 4-1BB co-stimulation on immune responses elicited by mRNA vaccine encoding the SARS-CoV-2 spike antigen in mice, and during the priming phase administering 4-1BB costimulatory antibodies did not stimulate immune responses, while the delay administering 4-1BB co-stimulatory antibodies after 96 hours, may lead to significant improvement in CD8 T cell responses was observed, providing greater protection against breakthrough infections [42], the result of this study also deal with another study that confirmed that s4-1BB were relegated to COVID-19 severity rate, and the expression of 4-1BB on surface $CD4^+$ and $CD8^+$ T cells is significantly increased severity cases compared to mild cases, reflecting systemic immune hyper-activation in severe cases[43]. The immune system aggressively fights off SARS-CoV-2 by activation of T cells, which up-regulate 4-1BB on their surface to promote clonal expansion and survival, also, several studies showed the direct role of 4-1BB in the hyper inflammatory response in severe COVID-19 patients, persistent stimulation of high levels of 4-1BB pathways is associated with exhausted T cells that lead to tissue damage and poor clinical outcomes [44,45].

Conclusion

This study concluded that ineffectiveness and exhaustion of NK-cell through down regulating of mRNA KLRK1 that encode to active receptor NKG2D are important factor related with severe COVID- 19 disease, while the elevated of 4-1BB gene expression indicated for exhausted of T cell, thus the better understand role of KLRK1 gene during the cases of severe COVID-19, can be carried out in more studies in the future about new strategies of the development of NK-cell-based treatment with elimination of virus and prevention the progression of COVID-19 disease by changes in the expression of receptors on surface of NK cell like NKG2D for improving NK cell function.

Acknowledgments

I thank all staff members at laboratory and UN in Teaching Hospital in Al- Diwaniyah, to their help in the collected data and specimens from a patients.

REFERENCES

[1] M. Ghasemzadeh, E. Hosseini, and A. Ghasemzadeh, "Exhausted NK cells and cytokine storms in COVID-19: Whether NK cell therapy could be a therapeutic choice," *Human Immunology*, vol. 82, no. 11, pp. 1–32, 2021, doi: 10.1016/j.humimm.2021.09.004.

[2] J. F.-W. Chan *et al*., "A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: A study of a family cluster," *The Lancet*, vol. 395, pp. 514–523, 2020, doi: 10.1016/S0140-6736(20)30154-9.

[3] N. Zhu *et al*., "A novel coronavirus from patients with pneumonia in China, 2019," *The New England Journal of Medicine*, vol. 382, no. 8, pp. 727–733, 2020, doi: 10.1056/NEJMoa2001017.

[4] N. Chen *et al*., "Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: A descriptive study," *The Lancet*, vol. 395, pp. 507–513, 2020, doi: 10.1016/S0140-6736(20)30211-7.

[5] R. von Linsingen *et al*., "MICA and KLRK1 genes and their impact in cervical intraepithelial neoplasia development in the southern Brazilian population," *Human Immunology*, vol. 81, no. 5, pp. 249–253, 2020, doi: 10.1016/j.humimm.2020.02.007.

[6] C. Khairallah, J. Déchanet-Merville, and M. Capone, "γδ T cell-mediated immunity to cytomegalovirus infection," *Frontiers in Immunology*, vol. 8, Art. no. 105, pp. 1–13, 2017, doi: 10.3389/fimmu.2017.00105.

[7] E. R. Sturgill *et al*., "Natural killer cell evasion is essential for infection by rhesus cytomegalovirus," *PLOS Pathogens*, vol. 12, no. 8, pp. 1–25, 2016, doi: 10.1371/journal.ppat.1005868.

[8] H. Liu *et al*., "Role of NKG2D and its ligands in cancer immunotherapy," *American Journal of Cancer Research*, vol. 9, no. 10, pp. 2064–2078, 2019.

[9] M. Saresella *et al*., "NK cell subpopulations and receptor expression in recovering SARS-CoV-2 infection," *Molecular Neurobiology*, vol. 58, pp. 6111–6120, 2021, doi: 10.1007/s12035-021-02517-4.

[10] S. Salek-Ardakani, D. M. Zajonc, and M. Croft, "Agonism of 4-1BB for immune therapy: A perspective on possibilities and complications," *Frontiers in Immunology*, vol. 14, Art. no. 1228486, 2023, doi: 10.3389/fimmu.2023.1228486.

[11] Etxeberria *et al*., "New emerging targets in cancer immunotherapy: CD137/4-1BB costimulatory axis," *ESMO Open*, vol. 4, Suppl. 3, Art. no. e000733, 2020, doi: 10.1136/esmoopen-2020-000733.

[12] R. H. Al-Mihanya and I. R. Ibrahim, "The role of 4-1BB (TNFRSF9) gene expression during cytomegalovirus infection in patients with breast cancer," *MINAR International Journal of Applied Sciences and Technology*, vol. 271, no. 8234, pp. 156–166, 2024, doi: 10.47832/2717-8234.19.14.

[13] R. H. A. Al-Mihanya, "Evaluation of TIM-3 gene expression in patients with COVID-19," *AIP Conference Proceedings*, vol. 3092, Art. no. 020013, pp. 020013-1–020013-7, 2024, doi: 10.1063/5.0199729.

[14] K. J. Livak and T. D. Schmittgen, "Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method," *Methods*, vol. 25, no. 4, pp. 402–408, 2001, doi: 10.1006/meth.2001.1262.

[15] J. H. McDonald, *Handbook of Biological Statistics*, 2nd ed. Baltimore, MD, USA: Sparky House Publishing, 2009.

[16] N. S.-A. Saber and R. H. A. Al-Mihanya, "Global misuse of semaglutide for cosmetic weight loss in non-diabetic young population, an FDA public database and Google Trends data analysis," *AL-Kindy College Medical Journal*, vol. 21, no. 2, pp. 153–160, 2025, doi: 10.47723/qv85p752.

[17] L. S. Flor *et al*., "Quantifying the effects of the COVID-19 pandemic on gender equality on health, social, and economic indicators: A comprehensive review of data from March 2020 to September 2021," *The Lancet*, vol. 399, pp. 2381–2397, 2022, doi: 10.1016/S0140-6736(22)00008-3.

[18] S. De Rosa *et al*., "Reduction of hospitalizations for myocardial infarction in Italy in the COVID-19 era," *European Heart Journal*, vol. 41, no. 22, pp. 2083–2088, 2020, doi: 10.1093/eurheartj/ehaa409.

[19] P. Profeta, "Gender equality and public policy during COVID-19," *CESifo Economic Studies*, vol. 66, no. 4, pp. 365–375, 2020, doi: 10.1093/cesifo/ifaa018.

[20] R. K. Starke *et al*., "The isolated effect of age on the risk of COVID-19 severe outcomes: A systematic review with meta-analysis," *BMJ Global Health*, vol. 6, pp. 1–12, 2021, doi: 10.1136/bmjgh-2021-006434.

[21] D. A. Solomon, A. C. Sherman, and S. Kanjilal, "Influenza in the COVID-19 era," *Journal of the American Medical Association*, vol. 324, 2020, doi: 10.1001/jama.2020.14661.

[22] M. Clarfield and T. Dwolatzky, "Age and ageing during the COVID-19 pandemic: Challenges to public health and to the health of the public," *Frontiers in Public Health*, vol. 9, pp. 1–6, 2021, doi: 10.3389/fpubh.2021.655831.

[23] S. Salehi *et al*., "Coronavirus disease 2019 (COVID-19) imaging reporting and data system (COVID-RADS) and common lexicon: A proposal based on the imaging data of 37 studies," *European Radiology*, vol. 30, no. 9, pp. 4930–4942, 2020, doi: 10.1007/s00330-020-06863-0.

[24] E. P. Kanne *et al*., "Essentials for radiologists on COVID-19: An update—Radiology Scientific Expert Panel," *Radiology*, vol. 296, pp. 113–114, 2020, doi: 10.1148/radiol.2020200527.

[25] T. Ai *et al*., "Correlation of chest CT and RT-PCR testing for coronavirus disease 2019 (COVID-19) in China: A report of 1014 cases," *Radiology*, vol. 296, no. 2, pp. 32–40, 2020, doi: 10.1148/radiol.2020200642.

[26] B. Böger *et al*., "Systematic review with meta-analysis of the accuracy of diagnostic tests for COVID-19," *American Journal of Infection Control*, vol. 49, pp. 21–29, 2021, doi: 10.1016/j.ajic.2020.07.011.

[27] G. D. Rubin *et al*., "The role of chest imaging in patient management during the COVID-19 pandemic: A multinational consensus statement from the Fleischner Society," *Radiology*, vol. 296, pp. 172–180, 2020, doi: 10.1148/radiol.2020201365.

[28] M. P. Revel *et al*., "COVID-19 patients and the radiology department—Advice from the European Society of Radiology (ESR) and the European Society of Thoracic Imaging (ESTI)," *European Radiology*, vol. 30, no. 9, pp. 4903–4909, 2020, doi: 10.1007/s00330-020-06865-y.

[29] C. Eeden *et al*., "Natural killer cell dysfunction and its role in COVID-19," *International Journal of Molecular Sciences*, vol. 21, Art. no. 6351, pp. 1–18, 2020, doi: 10.3390/ijms21176351.

[30] M. Jeyaraman *et al*., "Bracing NK cell based therapy to relegate pulmonary inflammation in COVID-19," *Heliyon*, vol. 7, no. 7, pp. 1–9, 2021, doi: 10.1016/j.heliyon.2021.e07635.

[31] W. J. Cao, F. S. Wang, and J. W. Song, "Characteristics and potential roles of natural killer cells during SARS-CoV-2 infection," *Infectious Disease Immunology*, vol. 3, no. 1, pp. 29–35, 2023, doi: 10.1097/ID9.0000000000000075.

[32] M. J. Lee and C. A. Blish, "Defining the role of natural killer cells in COVID-19," *Nature Immunology*, vol. 24, pp. 1628–1638, 2023, doi: 10.1038/s41590-023-01560-8.

[33] S. Varchetta *et al*., "Unique immunological profile in patients with COVID-19," *Cellular & Molecular Immunology*, vol. 18, no. 3, pp. 604–612, 2021, doi: 10.1038/s41423-020-00557-9.

[34] M. Li *et al*., "Elevated exhaustion levels of NK and CD8+ T cells as indicators for progression and prognosis of COVID-19 disease," *Frontiers in Immunology*, vol. 11, pp. 1–10, 2020, doi: 10.3389/fimmu.2020.580237.

[35] B. Krämer *et al*., "Early IFN-α signatures and persistent dysfunction are distinguishing features of NK cells in severe COVID-19," *Immunity*, vol. 54, no. 11, pp. 2650–2669, 2021, doi: 10.1016/j.immuni.2021.09.002.

[36] N. Vaninov, "In the eye of the COVID-19 cytokine storm," *Nature Reviews Immunology*, vol. 20, no. 5, pp. 277–277, 2020, doi: 10.1038/s41577-020-0305-6.

[37] H. Vietzen *et al*., "Deletion of the NKG2C receptor encoding KLRC2 gene and HLA-E variants are risk factors for severe COVID-19," *Genetics in Medicine*, vol. 23, no. 5, pp. 963–967, 2021, doi: 10.1038/s41436-020-01077-7.

[38] P. K. Brlić and I. Brizić, "Taking on SARS-CoV-2," *eLife*, vol. 11, pp. 1–3, 2022, doi: 10.7554/eLife.80552.

[39] S. Toro-Arreola *et al*., "NK cells at the crossroads in COVID-19: A question of timing," *Ciencia, Tecnología y Salud*, vol. 7, no. 3, pp. 309–324, 2020, doi: 10.36829/63CTS.v7i3.975.

[40] H. Liu *et al*., "NKG2D regulation of lung pathology and dendritic cell function following respiratory syncytial virus infection," *Journal of Infectious Diseases*, vol. 218, pp. 1822–1832, 2018, doi: 10.1093/infdis/jiy151.

[41] Zafarani *et al*., "Natural killer cells in COVID-19: From infection, to vaccination and therapy," *Future Virology*, vol. 18, no. 3, pp. 177–191, 2023, doi: 10.2217/fvl-2022-0040.

[42] S. Sanchez, "Time-dependent enhancement of mRNA vaccines by 4-1BB costimulation," *bioRxiv*, Art. no. 582992, 2024, doi: 10.1101/2024.03.01.582992.

[43] T. G. Paranga *et al*., "Distinct soluble immune checkpoint profiles characterize COVID-19 severity, mortality and SARS-CoV-2 variant infections," *Frontiers in Immunology*, vol. 15, Art. no. 1464480, 2024, doi: 10.3389/fimmu.2024.1464480.

[44] L. Yang *et al*., "COVID-19: Immunopathogenesis and immunotherapeutics," *Signal Transduction and Targeted Therapy*, vol. 5, no. 1, Art. no. 128, 2020, doi: 10.1038/s41392-020-00243-2.

[45] Kusnadi *et al*., "Severely ill COVID-19 patients display impaired exhaustion features in SARS-CoV-2-reactive CD8+ T cells," *Science Immunology*, vol. 6, no. 55, 2021.