Prognostic Value of Serum MICA Levels as a Marker of Severity in COVID-19 Patients

Faramarz Farzad
Mashhad University of Medical Sciences https://orcid.org/0000-0002-9870-1416
Neda Yaghoubi
Mashhad University of Medical Sciences
Farahzad Jabbari Azad
Mashhad University of Medical Sciences
Mahmoud Mahmoudi
Mashhad University of Medical Sciences
Mojgan Mohammadi (MohammadiMZH@mums.ac.ir)
Mashhad University of Medical Sciences https://orcid.org/0000-0002-0088-5916

Research Article

Keywords: COVID-19, sMICA, NKG2D, MHC class I related chain A

Posted Date: April 29th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-458280/v1

Abstract

Purpose: The global pandemic of COVID-19 and high mortality rates, necessitate the development of diagnostic and prognostic tools and expansion of testing capacity. The existing approaches to identification and characterization of SARS-COV-II infection are usually based on viral genome detection or measuring COVID-19-specific antibody levels. Despite being valuable, these methods are unable to anticipate disease outcomes in patients. Given the point that the innate immune cells, particularly NK cells, contribute significantly to anti-viral defense, this research aimed to determine the prognostic value of serum secretory MHC class I polypeptide–related sequence A (sMICA) levels as an important ligand for NKG2D receptor, the master regulator of NK cell development and responsiveness.

Methods: Serum levels of MICA were measured by ELISA assay. Sera (n = 60) were collected from SARS-CoV-2 positive patients and disease severity was assessed by clinical criteria. The patient group consisted of 30 cases with mild disease and 30 severely ill patients examined versus 30 controls.

Results: Our data revealed that serum levels of MICA were considerably higher in patients compared with controls, particularly in cases with severe complications ($P<0.0001$).

Conclusion: Higher serum levels of MICA may be associated with respiratory failure in COVID-19 and can serve as a marker of clinical severity for patients with SARS-CoV-2 infection in particular whenever the clinical manifestations are not sufficient to make a confident prediction.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative pathogen of COVID-19 disease, continues to spread and place great burdens on public health system [1]. To date, numerous studies have attempted to introduce novel biomarker testing of COVID-19 into clinical practices so that enable the globe to fight against the life-threatening complications of COVID-19 infection [2-4]. A vast majority of investigations into marker development have focused on the pathogenesis of SARS-COV-II infection, although, many features of the viral pathogenicity are yet to be understood [5]. In general, the molecular mechanisms of COVID-19 disease include suppression of anti-viral immune responses, oxidative stress, and inflammatory processes due to excessive cytokine secretion which leads to acute lung disease, tissue fibrosis, coagulopathy, and pneumonia [6]. Given the nature of mentioned pathologic events, immune dysregulation may largely be associated with COVID-19 progression and worse disease outcomes [7]. The close relationship between the immune system and different clinical manifestations of the COVID-19 disease also illustrates the point that COVID-19 could be an immune-related disease with viral origin and pathogenicity [8].

Normally, the virus-infected cells are first recognized by innate immune cells; indeed, early intervention of natural immunity by type I interferon (IFN-1) and natural killer (NK) cells ensures a quick, but unspecific defense against cytopathic viruses [9]. In response to tissue damage and pathogen invasion, the innate immune cells produce several cytokines, including IL-1, IL-6, TNF-α, IL-2, GM-CSF, and IFN-γ[10].

Overproduction of these pro-inflammatory cytokines as double-edged swords causes the immune cells such as neutrophils, macrophages, and T cells to migrate from the blood circulation to the infected tissues and trigger damage causing cellular death [10]. In the same way, the shifting between natural and adaptive immune responses is of great value for predicting disease severity of SARS-CoV-2 infections; early CD4+ and CD8+ T cell responses as well as sufficient T-cell activation play protective roles, by contrast, dysregulated and exacerbated responses may fail to clear viruses [11]. Similarly, immune responses developed by plasma cells and cytotoxic T lymphocyte (CTL) -mediated immunity contribute to anti-viral responses by generating neutralizing antibodies which in turn produce cytokines and effector molecules to destroy virus-infected cells [11]. In general it seems that maintaining the balance between protective and destructive immune responses may affect the ultimate outcome of the viral infection [12]. In this regard, NK cells have drawn researchers' attention as important modulators of immune responses [12].

Natural killer cells may function as regulators of adaptive immune responses regardless of serving as the first-line defense against infections [12]. Their function is regulated by a balanced cooperation between activatory receptors like natural killer group 2 member D (NKG2D), CD244, NKp30, NKp46- and inhibitory receptors such as killer cell immunoglobulin-like receptors (KIRs), and the lectin-like CD94-NKG2A heterodimers [12]. Upon cellular stress, infection or cancer, the activatory receptors overcome the inhibitory ones resulting in NK cell activation and killing target cells by various mechanisms such as direct lysis, antibody-dependent cell-mediated cytotoxicity (ADCC), interaction with Toll-like receptor (TLR) ligands, and generating IFN-γ [12]. One of the most important activatory and co-stimulatory receptors that viruses usually try to evade is NKG2D (killer cell lectin-like receptor K1 or KLRK1, CD314) [13]. This molecular sensor of cellular stress is expressed on a variety of immune cells including NK, γδT cells, and several subsets of CD4+and CD8+T cells [13]. These effector cells recruit NKG2D receptors in the surveillance of inflammation [14] through binding to several stress-induced molecules such as the MHC class-I-related chain A (MICA)/MICB and the UL-16 binding proteins 1–6 [15].

MICA/B were the first identified ligands for NKG2D whose genes have been mapped to the MHC class I region, in the proximity of HLA-B locus [13]. These highly glycosylated proteins are present in membrane-bound and soluble isoforms and bear complete polymorphism with more than 100 alleles [13, 16, 17]. Previous research has established that prolonged interactions between NKG2D and MIC ligands could impair NK and CD8+ T-cell immune responses by down-regulation of NKG2D [18]. Moreover, virus-infected cells have developed a variety of mechanisms to block the engagement of NKG2D/NKG2D ligands such as intracellular storage of molecules, shedding them in soluble forms, and in particular modulating MICA expression in respiratory epithelial cells [18, 19]. Given the key role of NK cells in acute viral infections, high prevalence of NK cells in lung tissues, and master regulation of NK cell development and activation by NKG2D receptor, this study sought to analyze serum levels of MICA and its probable link to COVID-19 exacerbation.

Materials And Methods

Study design and participants

This research project was a cross-sectional comparative study carried out in one of the centers dedicated to COVID-19 patients in Mashhad, Northeastern Iran, (Mashhad University of Medical Sciences). The study population consisted of 60 patients diagnosed with COVID-19 and 30 control subjects without any respiratory viral infection. Infected individuals were categorized into two different groups: 30 patients with mild disease, without any respiratory failure or radiological findings; and 30 hospitalized severely ill patients with extreme clinical manifestations of acute respiratory distress syndrome. All positive cases for COVID-19 were confirmed by the Real-time PCR test. The exclusion criteria for all groups comprise having any comorbidity, autoimmunity, immunodeficiency, cancer, taking immune suppressive or booster medicines and receiving anti-viral therapy.

Assays to measure serum MICA levels

Commercial ELISA kits from Invitrogen (Thermo Fisher Scientific, USA) were used to analyze serum levels of MICA. All samples were examined according to the manufactures' instructions. In brief, a MICA-specific antibody has been coated in the wells of the supplied microplate. Pre-diluted samples, standards, and controls were then added into these wells and bound to the immobilized antibody. The sandwich ELISA was formed by adding the second antibody (biotin conjugate). The next stage involved one-hour incubation at room temperature with gentle shaking. Afterwards, a prepared streptavidin-HRP solution was added to the mixture and the plate was incubated again for 45 minutes. Finally, the substrate solution was integrated with the enzyme-antibody-MICA complex to produce a blue color which then changed to yellow by adding the stop solution. The intensity of the color was proportional to the concentration of MICA in the serum samples.

Data Analysis and Statistics:

The whole data was analyzed employing Statistical Package for the Social Sciences (SPSS) version 16.0 and Graph Pad Prism 6. Variables across different groups were compared with ANOVA and Independent Sample T-Tests. The possible associations between variables were analyzed by Pearson correlation test. A p-value less than 0·05 was assigned as statistically significant.

Ethical Considerations

The research project was carried out based on the ethical standards and codes of the institutional and national research committee and in compliance with the Helsinki Declaration on human research. The study was reviewed and approved by the research ethics committee of Mashhad University of Medical Sciences (reference number: IR.MUMS.MEDICAL.REC.1399.771).

Results

As shown in Table 1, the study population consisted of 52 males (58 %) and 38 females (42%) aged between 25 and 68 years. The patient group contained 60 individuals, 35 male (58.3%) and 25 female

(41.7%) with a mean ± SD age: 38·7±6·9 and 47·3±10·3 years for patients with mild disease and severe COVID-19, respectively. Similarly, control subjects consisted of 17 male (57 %) and 13 female (43 %) with a mean ± SD age: 39·5±8·8 years. As it can be noted from the data, over half of the infected patients were middle-aged men older than 30 years.

Assessing serum sMICA levels across the study groups

A significant increasing trend in sMICA levels was seen in patients with severe form of COVID-19 compared with patients who suffered from the mild symptoms (Mean ± SD sMICA: 1874±427·8 and 1072±187·2 pg/ml for severe and mild cases of COVID-19, respectively) (P-value: 0·0001). Moreover, a statistically significant increase in MICA levels in patients with both mild and severe form of disease was detected as compared with controls (Mean ± SD sMICA for healthy controls: 434·6±188·9 pg/ml) (P-value: 0·0001) (Fig. 1).

We also assessed the correlation among the three variables; sMICA levels, age, and gender. Our findings revealed that there was a positive significant correlation between MICA serum concentrations and age (r: 0·34, p-value: 0·04). This close relationship was particularly of great importance in the case of participants aged between 40 and 50 years as the most at risk group of patients. However, there was no statistically significant association between sMICA levels and gender (p-value: 0·43).

Discussion

COVID-19 pandemic has caught the world by surprise and fiercely condemned many infected individuals to death. Clinical manifestations of COVID-19 patients are highly diverse ranging from exhaustion and slight fever to acute respiratory distress syndrome (ARDS), septic shock, and multi-organ failure [20]. This broad range of symptoms mainly stems from host factors such as age, gender, genetic background, and in particular regulated or dysregulated immunologic responses [20]. However, the identification of infected individuals developing severe cases of the disease is of great value to provide effective treatments for patients most in need and reduce mortality rates [21]. Thus, it is necessary to define easily measured biomarkers that can represent abnormality and predict disease outcomes for patients with COVID-19 infection. The present study was designed to explore the possible relationship between sMICA levels as effective molecules in natural immunity and COVID-19 exacerbation. The most valuable finding to emerge from this research is that elevated sMICA concentrations may contribute to severe respiratory failure during COVID-19 infection.

Several lines of evidence suggest that severe respiratory failure in COVID-19 is strongly linked to immunologic disturbances including CD4 cell and NK Cell cytopenia, decreased expression of the activatory receptor NKG2D and -IFN-γ, suppressing the cytotoxic activity of NK cells and CD8 T lymphocytes, secreting excessive amounts of pro-inflammatory cytokines by monocytes, and deficiency in B cell function [22-24]. Indeed, impairments of both innate and adaptive immunity during SARS-COV-II infection lead to an immunologic imbalance that may be associated with COVID-19 severity.

Despite the growing number of investigations into the anti-viral immune responses against the SARS-COV-II virus, the enigma of the host immunity of COVID-19 patients with severe disease is still unsolved. In this regard, numerous studies have focused on NK cells as the first-line responders to human viral pathogens as well as regulators of immunologic homeostasis by releasing activating or inhibitory cytokines [25]. It is now well established from a variety of studies that NK cells, macrophages, and plasmacytoid dendritic cells (pDCs) can migrate into the lungs at the early stages of SARS-COV-II infection [25]. However, constitutive activation of NK cells within infected lungs may cause exhaustion, hyporesponsiveness, and hyper inflammatory immune responses due to the reduced number of NK cells [25]. Different variables have been found to be related to the altered number and function of NK cells such as NK cell redistribution in lungs, apoptosis, higher production of IL-6, IL-10, and TGF-β cytokines, and modulating the expression of NKG2D receptor [25].

As previously said, NKG2D as a molecular sensor of cellular stress and activator of the immune system plays major roles in the identification and clearance of infected cells by NK, CD8+, and γδ+ T cells [15]. Additionally, it may serve as a checkpoint to regulate CD4+ Th cell activation during inflammatory processes [15]. Therefore, reduced expression of NKG2D receptor may affect SARS-COV-II elimination and inflammatory responses simultaneously. On the other hand, previous research has established that RNA viruses such as respiratory syncytial virus (RSV) infection could induce the expression of different NKG2D ligands contributing to NK cell proliferation and IFN-γ production [26]. Indeed, RSV infection of lung epithelial cells increased the production of IL-15, expression of MICA and sMICA serum levels [19]. On the contrary, the human metapneumovirus (HMPV) as a causative pathogen of influenza-like illness in children has proved to downregulate MICA/B through viral protein M2.2 [27]. Our results showed that serum MICA levels increased in patients with COVID-19 infection particularly in severe cases. The increase in sMICA levels could be attributed to the body's protective reactions to immune evasion through viral proteins or altering the trafficking of MHC-I molecules on the infected cell surface during viral infection that helps the virus deceive immune cells [28]. Another possible explanation for this is that increased sMICA levels may affect suppressive signaling network related to immunologic tolerance due to the virus presence [29], however, more future research is needed to determine the exact mechanisms of interaction.

It is unfortunate that the study did not include more patient information to be assessed concerning sMICA levels such as nutrition status or medications. We excluded patients with unmet inclusion criteria such as comorbidities, autoimmunity, and cancer to limit the confounder factors. Notwithstanding the relatively limited sample, this work shows valuable insights into diagnostics for COVID-19 exacerbation that may be of great assistance to manage patients with SARS-COV-II infection.

Conclusion

The significant increase in sMICA levels in patients compared with uninfected controls could explain the pathogenesis of the infection to some extent and may have the propensity to be used for diagnostic, prognostic, and therapeutic objectives.

Declarations

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

Patients signed informed consent regarding publishing their data.

Funding

This study was supported by Mashhad University of Medical Sciences, ID 991307.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Ethics approval

The research project was carried out based on the ethical standards and codes of the institutional and national research committee and in compliance with the Helsinki Declaration on human research. The study was reviewed and approved by the research ethics committee of Mashhad University of Medical Sciences (reference number: IR.MUMS.MEDICAL.REC.1399.771).

Data and/or Code availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contribution statements

Writing - review and editing/ methodology/ Formal analysis and investigation: Faramarz Farzad

Conceptualization / Writing - original draft preparation: Neda Yaghoubi

Resources: Farahzad Jabbari Azad

Resources: Mahmoud Mahmoudi

Funding acquisition / Supervision: Mojgan Mohammadi

Acknowledgment: This work was supported by a grant from Mashhad University of Medical Sciences (grant no: 991307). We appreciate all patients and controls for participating in this study. We also thank all staff at the special covid-19 ward for their kind assistance.

References

[1]. Baud D,Qi X,Nielsen-Saines K,Musso D,Pomar L,Favre G. Real estimates of mortality following COVID-19 infection. Lancet Infect Dis. 2020; 20: 773.

[2]. Shen B,Yi X,Sun Y,Bi X,Du J,Zhang C, et al. Proteomic and Metabolomic Characterization of COVID-19 Patient Sera. Cell. 2020; 182: 59-72.e15.

[3]. Aceti A,Margarucci L M,Scaramucci E,Orsini M,Salerno G,Di Sante G, et al. Serum S100B protein as a marker of severity in Covid-19 patients. Sci Rep. 2020; 10: 18665.

[4]. Ren H,Yang Y,Wang F,Yan Y,Shi X,Dong K, et al. Association of the insulin resistance marker TyG index with the severity and mortality of COVID-19. Cardiovasc Diabetol. 2020; 19: 58.

[5]. Zheng J,Wong L R,Li K,Verma A K,Ortiz M E,Wohlford-Lenane C, et al. COVID-19 treatments and pathogenesis including anosmia in K18-hACE2 mice. Nature. 2021; 589: 603-607.

[6]. Mrityunjaya M,Pavithra V,Neelam R,Janhavi P,Halami P M,Ravindra P V. Immune-Boosting, Antioxidant and Anti-inflammatory Food Supplements Targeting Pathogenesis of COVID-19. Front Immunol. 2020; 11: 570122.

[7]. Fathi F,Sami R,Mozafarpoor S,Hafezi H,Motedayyen H,Arefnezhad R, et al. Immune system changes during COVID-19 recovery play key role in determining disease severity. Int J Immunopathol Pharmacol. 2020; 34: 2058738420966497.

[8]. Paces J,Strizova Z,Smrz D,Cerny J. COVID-19 and the immune system. Physiol Res. 2020; 69: 379-388.

[9]. Maggi E,Canonica G W,Moretta L. COVID-19: Unanswered questions on immune response and pathogenesis. J Allergy Clin Immunol. 2020; 146: 18-22.

[10]. Hosseini A,Hashemi V,Shomali N,Asghari F,Gharibi T,Akbari M, et al. Innate and adaptive immune responses against coronavirus. Biomed Pharmacother. 2020; 132: 110859.

[11]. Toor S M,Saleh R,Sasidharan Nair V,Taha R Z,Elkord E. T-cell responses and therapies against SARS-CoV-2 infection. Immunology. 2021; 162: 30-43.

[12]. van Eeden C,Khan L,Osman M S,Cohen Tervaert J W. Natural Killer Cell Dysfunction and Its Role in COVID-19. Int J Mol Sci. 2020; 21.

[13]. Burgess S J,Maasho K,Masilamani M,Narayanan S,Borrego F,Coligan J E. The NKG2D receptor: immunobiology and clinical implications. Immunol Res. 2008; 40: 18-34.

[14]. Liu H,Osterburg A R,Flury J,Huang S,McCormack F X,Cormier S A, et al. NKG2D Regulation of Lung Pathology and Dendritic Cell Function Following Respiratory Syncytial Virus Infection. J Infect Dis. 2018;

218: 1822-1832.

[15]. Babic M,Dimitropoulos C,Hammer Q,Stehle C,Heinrich F,Sarsenbayeva A, et al. NK cell receptor NKG2D enforces proinflammatory features and pathogenicity of Th1 and Th17 cells. J Exp Med. 2020; 217.

[16]. Groh V,Wu J,Yee C,Spies T. Tumour-derived soluble MIC ligands impair expression of NKG2D and T-cell activation. Nature. 2002; 419: 734-738.

[17]. Luo Q,Guo X,Peng S,Luo W,Tian F,Yu P, et al. The association between MICA/MICB polymorphism and respiratory syncytial virus infection in children. Int J Immunogenet. 2017; 44: 328-336.

[18]. Zdrenghea M T,Mallia P,Johnston S L. Immunological pathways in virus-induced COPD exacerbations: a role for IL-15. Eur J Clin Invest. 2012; 42: 1010-1015.

[19]. Zdrenghea M T,Telcian A G,Laza-Stanca V,Bellettato C M,Edwards M R,Nikonova A, et al. RSV infection modulates IL-15 production and MICA levels in respiratory epithelial cells. Eur Respir J. 2012; 39: 712-720.

[20]. Zhang X,Tan Y,Ling Y,Lu G,Liu F,Yi Z, et al. Viral and host factors related to the clinical outcome of COVID-19. Nature. 2020; 583: 437-440.

[21]. Perlman S. COVID-19 poses a riddle for the immune system. Nature. 2020; 584: 345-346.

[22]. Giamarellos-Bourboulis E J,Netea M G,Rovina N,Akinosoglou K,Antoniadou A,Antonakos N, et al. Complex Immune Dysregulation in COVID-19 Patients with Severe Respiratory Failure. Cell Host Microbe. 2020; 27: 992-1000.e1003.

[23]. Varchetta S,Mele D,Oliviero B,Mantovani S,Ludovisi S,Cerino A, et al. Unique immunological profile in patients with COVID-19. Cell Mol Immunol. 2021; 18: 604-612.

[24]. Yao C,Bora S A,Parimon T,Zaman T,Friedman O A,Palatinus J A, et al. Cell-Type-Specific Immune Dysregulation in Severely Ill COVID-19 Patients. Cell Rep. 2021; 34: 108590.

[25]. Khadke S,Ahmed N,Ahmed N,Ratts R,Raju S,Gallogly M, et al. Harnessing the immune system to overcome cytokine storm and reduce viral load in COVID-19: a review of the phases of illness and therapeutic agents. Virol J. 2020; 17: 154.

[26]. Ebihara T,Masuda H,Akazawa T,Shingai M,Kikuta H,Ariga T, et al. Induction of NKG2D ligands on human dendritic cells by TLR ligand stimulation and RNA virus infection. International Immunology. 2007; 19: 1145-1155.

[27]. Diab M,Schmiedel D,Seidel E,Bacharach E,Mandelboim O. Human Metapneumovirus Escapes NK Cell Recognition through the Downregulation of Stress-Induced Ligands for NKG2D. Viruses. 2020; 12.

[28]. Fink A,Mikuličić S,Blaum F,Reddehase M J,Florin L,Lemmermann N A W. Function of the cargo sorting dileucine motif in a cytomegalovirus immune evasion protein. Medical Microbiology and Immunology. 2019; 208: 531-542.

[29]. Goodnow C C. COVID-19, varying genetic resistance to viral disease and immune tolerance checkpoints. Immunol Cell Biol. 2021; 99: 177-191.

Tables

Table 1 is not available with this version.

Figures

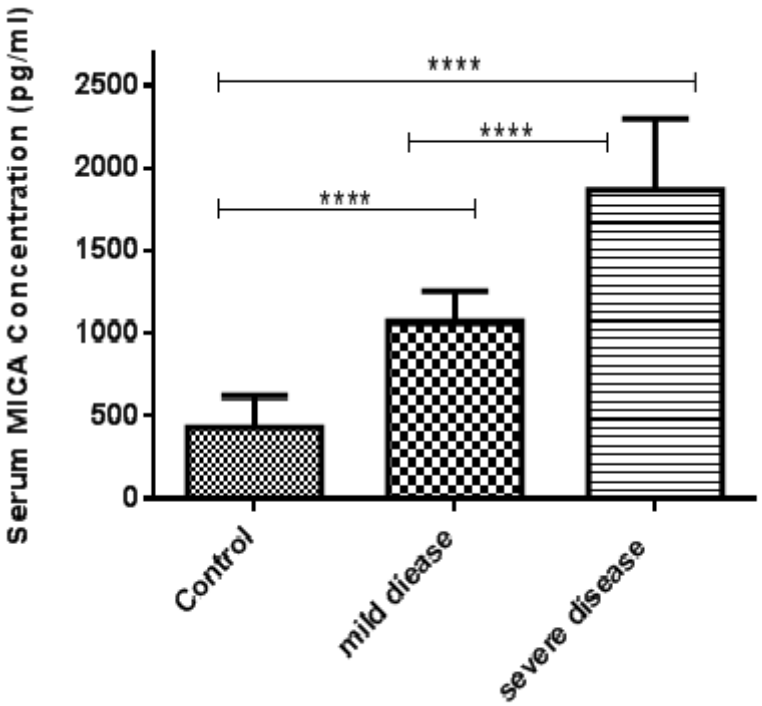

Figure 1

Serum levels of MICA across studied groups. ANOVA test was used to determine the differences, indicated by asterisks. The values show significant differences between control (N=30) and severely-ill patients (N=30), (****), (p = 0·0001), control and asymptomatic patients (N=30)(****), (p = 0·0001), and between two groups of COVID positive patients(****), (p = 0·0001). The graph pad prism version 6 was used to create figure