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Title: The Pattern of Neuroinflammatory miRNAs, C-Reactive Protein, and Alanine Aminotransferase During Hospitalization In Recovered or Not-Recovered COVID-19 Patients

Running title: Neuroinflammatory Markers in COVID-19

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Abstract

Background: Our aim was to investigate the expression of miRNAs, C-reactive protein as a blood inflammation marker, and alanine aminotransferase as a tissue inflammation marker in recovered and not-recovered COVID-19 patients.

Methods: This cross-sectional project was done in three medical center of Iran from December to March, 2021. Generally, a total of 20 confirmed cases of COVID-19 with grade III and 20 healthy subjects were enrolled in the study. Then, the neuroinflammatory expression of miRNAs (miR-199, miR-203, and miR-181), C-reactive protein, and alanine aminotransferase were investigated during hospitalization from week 0 to week 2.

Results: In not-recovered COVID-19 subjects, the expression of miR-199, miR-203, and miR-181 were decreased and the levels of C-reactive protein and alanine aminotransferase were increased during hospitalization. Conversely, in recovered COVID-19 subjects, the relative expression of miR-199, miR-203, and miR-181 were increased and the levels of C-reactive protein and alanine aminotransferase were decreased during hospitalization.

Conclusions: The expression pattern of neuroinflammatory miRNAs is depends on whether the COVID-19 patient is recovering or deteriorating. Their expression is down-regulated in not-recovered COVID-19 patients and up-regulated in recovered COVID-19 patients.

Keywords: miRNAs, COVID-19, Neuroinflammatory, Hospitalization

Backgrounds

Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is from the Coronaviridae family and is similar to 2003 SARS outbreak (Wang et al., 2020). RNA viruses evolve rapidly with the help of mutations (Girardi, López, & Pfeffer, 2018). On the other hand, human host develops a variety of ways to fight viral infections, including innate immunity. Viral components also lead to produce different cytokines to limit the virus. In the case of SARS-CoV-2, different pathways associated with stress, apoptosis, autophagy, and innate immunity can be activated (Fung & Liu, 2019).

It has been found that the activation and regulation of immune response are modulated by miRNAs which are non-coding RNAs with 19-24 nucleotides (Nejad, Stunden, & Gantier, 2018). They regulate gene expression through binding to mRNA and this process can regulate cell differentiation, cell growth, apoptosis, and disease progression (Lou et al., 2019). During viral infection, miRNAs play an important role in various signaling pathways and interfere virus transmission and pathogenicity by regulating host genes and activation antiviral immune responses (Y. Li et al., 2012). Previously, the role of miRNAs in other viral diseases such as HIV, herpes, and Ebola has been determined (Bernier & Sagan, 2018). Recently, some miRNAs have been identified in COVID-19 using both experimental and bioinformatics tools (Ahmed et al., 2020; Khan, Sany, Us, Islam, & Islam, 2020). However, these reports are limited and there is insufficient information on neuroinflammatory miRNAs in COVID-19 patients during hospitalization.

Our aim was to investigate the expression of miRNAs, C-reactive protein as a blood inflammation marker, and alanine aminotransferase as a tissue inflammation marker in recovered and not-recovered COVID-19 patients during hospitalization.

Methods

Blood sample collection

This study was done in three medical center, including Yazd, Zahedan, and Tehran province of Iran. It was started from December and ended at March 2021. Here, 20 confirmed COVID-19 patients with grade III were enrolled, according to WHO guidance and diagnostic criteria (Sohrabi et al., 2020). Also, 20 healthy subjects were enrolled in the study. This study was under

ethics committee of Zahedan University of Medical Sciences, Zahedan, Iran. (Ethical code: IR.ZAUMS.REC.1399.316). Here, five mL of whole blood was obtained from each patient at week 0, week 1, and week 2 and kept at -80 °C.

The expression of miRNAs

Total RNAs were first isolated by mirPremier isolation kit (Sigma-Aldrich, USA) and confirmed by a NanoDrop ND-1000 UV-VIS spectrophotometer. In the next step, Mir-X miRNA First-Strand Synthesis kit (Takara Bio Inc, USA) was used to synthesize cDNA. Mir-X miRNA qPCR SYBR (Invitrogen, UK) was applied for Realtime-PCR. In this study, *RNU 48* was considered as internal reference.

Quantification of C-Reactive Protein and Alanine Aminotransferase

According to the article of Aceti et al, quantification of C-Reactive Protein and Alanine Aminotransferase in serum was done, according to standard laboratory protocol (Aceti et al., 2020).

Severity of illness

According to WHO guidelines (Aceti et al., 2020), COVID-19 patients are divided into 5 grades, according to the severity of the disease, including Grade I or Asymptomatic Infection, Grade II or Mild Illness, Grade III or Moderate Illness, Grade IV or Severe Illness, and Grade V or Critical Illness.

Statistical analysis

Mean $\pm$ SD was used to show data and one-way ANOVA was used to show significant differences at *P*-value less than 0.05.

Results

The analysis of microRNA expression in recovered and not-recovered COVID-19 subjects

In not-recovered COVID-19 subjects, the expression of miR-199 (**Figure 1a**), miR-203 (**Figure 1c**), and miR-181(**Figure 1e**) were significantly decreased ($P<0.0001$). Conversely, in recovered COVID-19 subjects, the expression of miR-199 (**Figure 1b**), miR-203 (**Figure 1d**), and miR-181(**Figure 1f**) were significantly increased ($P<0.0001$).

The analysis of inflammation markers in recovered and not-recovered COVID-19 subjects

In not-recovered COVID-19 subjects, the normalized level of C-reactive protein (**Figure 2a**) and alanine aminotransferase (**Figure 2c**) was significantly increased ($P<0.0001$). Conversely, in recovered COVID-19 subjects, the normalized quantity of C-reactive protein (**Figure 2b**) and alanine aminotransferase (**Figure 2d**) was significantly decreased ($P<0.0001$).

The analysis of grade of illness in recovered and not-recovered COVID-19 subjects

In not-recovered COVID-19 subjects, the grade of illness was significantly increased ($P<0.0001$) (**Figure 3a**) and conversely, in recovered COVID-19 subjects (**Figure 3b**), the grade of illness was significantly decreased ($P<0.0001$).

Discussion

Although SARS-CoV-2 attacks the lungs, the virus can spread to other tissues, such as brain, and it causes neurological complications, especially in the elderly (Huang et al., 2020; Wolfel, Corman, & Guggemos, 2019; Wu & McGoogan, 2020). Previous analysis shows that up to 20-30% of COVID-19 patients have brain complications (Chen et al., 2020; Varatharaj et al., 2020). An important question is how does SARS-CoV-2 damage the CNS? Although SARS-CoV-2 enter brain by several routes, the virus can directly infect nerve cells (Song et al., 2021). The binding of the virus to ACE2, which is present on the surface of cells (Zeisel et al., 2015), neurons, and neuroglial cells (Nemoto et al., 2020) is critical. Moreover, endosomal proton pump

and NAADP-sensitive intracellular two-pore channel 2 are important parts for endocytosis (Petersen, Gerasimenko, & Gerasimenko, 2020). Unfortunately, the virus enters the brainstem through the olfactory bulb after entering the nose and eyes and then spreads to all parts of the brain (K. Li et al., 2016). The problem is exacerbated when the virus crosses the BBB barrier and spreads to the entire human brain or body, at which point a cytokine storm can happen, leading to death (Coperchini, Chiovato, Croce, Magri, & Rotondi, 2020). As the virus enters the brain, peripheral immune cells enter, followed by microglial cells and astrocytes. This is not under normal circumstances (Engelhardt, Vajkoczy, & Weller, 2017). One research has shown that astrocytic calcium-binding protein S100b, tissue and blood inflammatory markers are increased in COVID-19 patients (Aceti et al., 2020). In response to viral infections, microglia and astrocytes are activated and secrete proinflammatory cytokines (Shabab, Khanabdali, Moghadamtousi, Kadir, & Mohan, 2017). These events are generally called neuroinflammation and protect the brain against pathogens (Kempuraj et al., 2016).

Although neuroinflammation is present in many disorders, the related molecular processes are not often clear (DiSabato, Quan, & Godbout, 2016). The major cells that cause neuroinflammation are microglia, a static macrophage made from myeloid progenitor cells in early embryonic life (Wieghofer & Prinz, 2016). Under normal circumstances, microglia are resting and they constantly scan the environment, phagocyte, and release neurotrophic agents (Kabba et al., 2018). Microglia have molecular recognition receptors, cytokine receptors, and a variety of neural receptors, and can respond to a variety of unfamiliar and dangerous molecules (Harry, 2013). Activation of microglia induces morphological changes and they take the form of amoeba (Kabba et al., 2018). Microglia have two function shapes, including M1 (pro-inflammatory) and M2 (anti-inflammatory). M1 releases inflammatory mediators, such as TNF α , IL-6, and IL-1 β (Tang & Le, 2016) and M2 releases anti-inflammatory cytokines, such as IL-10, TGF- β , IL-4, and IL-13 [9]. Astrocytes are another type of glial cell in the brain and cause an inflammatory response in CNS (Cekanaviciute & Buckwalter, 2016). In a healthy and normal brain, astrocytes induce synaptogenesis, deplete neurotransmitters, and regulate nervous signaling to help neural homeostasis (Vasile, Dossi, & Rouach, 2017). Astrocytes also play an important role in responding to pathogens (Dossi, Vasile, & Rouach, 2018).

In this study, we found that in not-recovered COVID-19 subjects, the expression of miR-199, miR-203, and miR-181 were significantly decreased from week 0 to week 2. Conversely, in recovered COVID-19 patients, their relative expression were significantly increased from week 0 to week 2. Also, we found that in not-recovered COVID-19 patients, the normalized level of C-reactive protein, alanine aminotransferase, and COVID-19 grade was significantly increased and conversely, in recovered COVID-19 patients, these parameters was significantly decreased.

Host-directed therapies, such as miRNAs, are a new therapeutic approach that targets the factors influencing the host cell. In the case of SARS-CoV, scientists are now finding specific molecules to block important pathways. Some cytokines can activate the antiviral defense during viral infections. Interestingly, there are different miRNAs to control the antiviral genes. For example, Sardar et al found 2197 human miRNAs that could target host genes (Sardar, Satish, & Gupta, 2020). Satyam et al introduced six putative miRNAs in SARS-CoV-2. They reported that hsa-miR-214-3p could be increased by SARS-CoV 2 (Satyam et al., 2020). Brogaard et al showed late regulation of hsa-miR-223-5p in circulating leukocytes of pig model of influenza A (Brogaard et al., 2016). Moffett et al illustrated that miR-31 could inhibit CD8+ T cell function and is as an important regulator in chronic infection (Moffett et al., 2017). Nersisyan et al introduced six miRNAs, including miR-21-3p, miR-195-5p, miR-16-5p, miR-3065-5p, miR-424-5p and miR-421, in coronavirus-host (Nersisyan et al., 2020). Jafarinejad et al showed that miR-29 family had high affinity on the SARS-CoV-2 genome (Jafarinejad-Farsangi, Jazi, Rostamzadeh, & Hadizadeh, 2020).

Conclusion

Taken together, this study showed that in not-recovered COVID-19 subjects, the expression of miR-199, miR-203, and miR-181 were significantly decreased from week 0 to week 2. Conversely, in recovered COVID-19 patients, their relative expression was significantly increased from week 0 to week 2. Also, we found that in not-recovered COVID-19 patients, the normalized quantity of C-reactive protein, alanine aminotransferase, and COVID-19 grade was significantly increased and conversely, in recovered COVID-19 patients, these parameters was significantly decreased.

Abbreviations:

SARS-CoV-2: Acute Respiratory Syndrome Coronavirus 2

COVID-19: Coronavirus disease-2019

miRNAs: micro Ribonucleic acid

snRNA: Small nuclear Ribonucleic acid

CNS: central nervous system

ACE2: angiotensin converting enzyme 2

NAADP: Nicotinic acid adenine dinucleotide phosphate

BBB : blood–brain barrier

TNF: Tumor necrosis factor

IL: Interleukin

Ethics approval and consent to participate

All experiments were under the guidelines of the National Institute of Health, the provisions of the Declaration of Helsinki, and the ethics committee of Zahedan University of Medical Sciences, Zahedan, Iran. (Ethical code: IR.ZAUMS.REC.1399.316).

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

Not applicable

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Authors' contributions

(I) Conception and design: R.K, S.M.H.S and A.J, (II) Administrative support: R.K and A.J, (III) Provision of study materials or patients: R.K, (IV) Collection and assembly of data: A.J, (V) Data analysis and interpretation: R.K and A.J, (VI) Manuscript writing: All authors, (VII) Final approval of manuscript: All authors

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References

- Aceti, Antonio, Margarucci, Lory Marika, Scaramucci, Elena, Orsini, Massimiliano, Salerno, Gerardo, Di Sante, Gabriele, . . . Ria, Francesco. (2020). Serum S100B protein as a marker of severity in Covid-19 patients. *Scientific reports*, 10(1), 1-8.
- Ahmed, Shiek SSJ, Paramasivam, Prabu, Raj, Kamal, Kumar, Vishal, Murugesan, Ram, & Ramakrishnan, V. (2020). Regulatory cross talk between SARS-CoV-2 receptor binding and replication machinery in the human host. *Frontiers in Physiology*, 11, 802.
- Bernier, Annie, & Sagan, Selenia M. (2018). The diverse roles of microRNAs at the host–virus interface. *Viruses*, 10(8), 440.
- Brogaard, Louise, Heegaard, Peter MH, Larsen, Lars E, Mortensen, Shila, Schlegel, Michael, Dürrwald, Ralf, & Skovgaard, Kerstin. (2016). Late regulation of immune genes and microRNAs in circulating leukocytes in a pig model of influenza A (H1N2) infection. *Scientific reports*, 6(1), 1-11.
- Cekanaviciute, Egle, & Buckwalter, Marion S. (2016). Astrocytes: integrative regulators of neuroinflammation in stroke and other neurological diseases. *Neurotherapeutics*, 13(4), 685-701.
- Chen, Tao, Wu, DI, Chen, Huilong, Yan, Weiming, Yang, Danlei, Chen, Guang, . . . Wang, Hongwu. (2020). Clinical characteristics of 113 deceased patients with coronavirus disease 2019: retrospective study. *bmj*, 368.
- Coperchini, Francesca, Chiovato, Luca, Croce, Laura, Magri, Flavia, & Rotondi, Mario. (2020). The cytokine storm in COVID-19: An overview of the involvement of the chemokine/chemokine-receptor system. *Cytokine & growth factor reviews*, 53, 25-32.
- DiSabato, Damon J, Quan, Ning, & Godbout, Jonathan P. (2016). Neuroinflammation: the devil is in the details. *Journal of neurochemistry*, 139, 136-153.
- Dossi, Elena, Vasile, Flora, & Rouach, Nathalie. (2018). Human astrocytes in the diseased brain. *Brain research bulletin*, 136, 139-156.
- Engelhardt, Britta, Vajkoczy, Peter, & Weller, Roy O. (2017). The movers and shapers in immune privilege of the CNS. *Nature immunology*, 18(2), 123.
- Fung, To Sing, & Liu, Ding Xiang. (2019). Human coronavirus: host-pathogen interaction. *Annual review of microbiology*, 73, 529-557.

- Girardi, Erika, López, Paula, & Pfeffer, Sébastien. (2018). On the importance of host microRNAs during viral infection. *Frontiers in genetics*, 9, 439.
- Harry, G Jean. (2013). Microglia during development and aging. *Pharmacology & therapeutics*, 139(3), 313-326.
- Huang, Chaolin, Wang, Yeming, Li, Xingwang, Ren, Lili, Zhao, Jianping, Hu, Yi, . . . Gu, Xiaoying. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The lancet*, 395(10223), 497-506.
- Jafarinejad-Farsangi, Saeideh, Jazi, Maryam Moazzam, Rostamzadeh, Farzaneh, & Hadizadeh, Morteza. (2020). High affinity of host human microRNAs to SARS-CoV-2 genome: An in silico analysis. *Non-coding RNA Research*, 5(4), 222-231.
- Kabba, John Alimamy, Xu, Yazhou, Christian, Handson, Ruan, Wenchen, Chenai, Kitchen, Xiang, Yun, . . . Pang, Tao. (2018). Microglia: housekeeper of the central nervous system. *Cellular and molecular neurobiology*, 38(1), 53-71.
- Kempuraj, D, Thangavel, R, Natteru, PA, Selvakumar, GP, Saeed, D, Zahoor, H, . . . Zaheer, A. (2016). Neuroinflammation induces neurodegeneration. *Journal of neurology, neurosurgery and spine*, 1(1).
- Khan, Md, Sany, Md, Us, Rabi, Islam, Md, & Islam, Abul Bashir Mir Md. (2020). Epigenetic regulator miRNA pattern differences among SARS-CoV, SARS-CoV-2, and SARS-CoV-2 world-wide isolates delineated the mystery behind the epic pathogenicity and distinct clinical characteristics of pandemic COVID-19. *Frontiers in genetics*, 11, 765.
- Li, Kun, Wohlford-Lenane, Christine, Perlman, Stanley, Zhao, Jincun, Jewell, Alexander K, Reznikov, Leah R, . . . McCray Jr, Paul B. (2016). Middle East respiratory syndrome coronavirus causes multiple organ damage and lethal disease in mice transgenic for human dipeptidyl peptidase 4. *The Journal of infectious diseases*, 213(5), 712-722.
- Li, Yingke, Fan, Xiaohua, He, Xingying, Sun, Haijing, Zou, Zui, Yuan, Hongbin, . . . Shi, Xueyin. (2012). MicroRNA-466l inhibits antiviral innate immune response by targeting interferon-alpha. *Cellular & molecular immunology*, 9(6), 497-502.
- Lou, Weiyang, Liu, Jingxing, Ding, Bisha, Chen, Danni, Xu, Liang, Ding, Jun, . . . Fan, Weimin. (2019). Identification of potential miRNA-mRNA regulatory network contributing to pathogenesis of HBV-related HCC. *Journal of translational medicine*, 17(1), 1-14.

- Moffett, Howell F, Cartwright, Adam NR, Kim, Hye-Jung, Godec, Jernej, Pyrdol, Jason, Äijö, Tarmo, . . . Golub, Todd R. (2017). The microRNA miR-31 inhibits CD8+ T cell function in chronic viral infection. *Nature immunology*, 18(7), 791.
- Nejad, Charlotte, Stunden, H James, & Gantier, Michael P. (2018). A guide to miRNAs in inflammation and innate immune responses. *The FEBS journal*, 285(20), 3695-3716.
- Nemoto, Wataru, Yamagata, Ryota, Nakagawasai, Osamu, Nakagawa, Koharu, Hung, Wan-Yi, Fujita, Maho, . . . Tan-No, Koichi. (2020). Effect of spinal angiotensin-converting enzyme 2 activation on the formalin-induced nociceptive response in mice. *European journal of pharmacology*, 872, 172950.
- Nersisyan, Stepan, Engibaryan, Narek, Gorbonos, Aleksandra, Kirdey, Ksenia, Makhonin, Alexey, & Tonevitsky, Alexander. (2020). Potential role of cellular miRNAs in coronavirus-host interplay. *PeerJ*, 8, e9994.
- Petersen, Ole H, Gerasimenko, Oleg V, & Gerasimenko, Julia V. (2020). Endocytic uptake of SARS-CoV-2: the critical roles of pH, Ca²⁺, and NAADP. *Function*, 1(1), zqaa003.
- Sardar, Rahila, Satish, Deepshikha, & Gupta, Dinesh. (2020). Identification of novel SARS-CoV-2 drug targets by host microRNAs and transcription factors co-regulatory interaction network analysis. *Frontiers in genetics*, 11, 1105.
- Satyam, Rohit, Bhardwaj, Tulika, Goel, Sachin, Jha, Niraj Kumar, Jha, Saurabh Kumar, Nand, Parma, . . . Kesari, Kavindra Kumar. (2020). miRNAs in SARS-CoV 2: A Spoke in the Wheel of Pathogenesis. *Current pharmaceutical design*.
- Shabab, Tara, Khanabdali, Ramin, Moghadamtousi, Soheil Zorofchian, Kadir, Habsah Abdul, & Mohan, Gokula. (2017). Neuroinflammation pathways: a general review. *International Journal of Neuroscience*, 127(7), 624-633.
- Sohrabi, Catrin, Alsafi, Zaid, O'Neill, Niamh, Khan, Mehdi, Kerwan, Ahmed, Al-Jabir, Ahmed, . . . Agha, Riaz. (2020). World Health Organization declares global emergency: A review of the 2019 novel coronavirus (COVID-19). *International journal of surgery*, 76, 71-76.
- Song, Eric, Zhang, Ce, Israelow, Benjamin, Lu-Culligan, Alice, Prado, Alba Vieites, Skriabine, Sophie, . . . Dai, Yile. (2021). Neuroinvasion of SARS-CoV-2 in human and mouse brain. *Journal of Experimental Medicine*, 218(3).
- Tang, Yu, & Le, Weidong. (2016). Differential roles of M1 and M2 microglia in neurodegenerative diseases. *Molecular neurobiology*, 53(2), 1181-1194.

- Varatharaj, Aravinthan, Thomas, Naomi, Ellul, Mark A, Davies, Nicholas WS, Pollak, Thomas A, Tenorio, Elizabeth L, . . . Zandi, Michael. (2020). Neurological and neuropsychiatric complications of COVID-19 in 153 patients: a UK-wide surveillance study. *The Lancet Psychiatry*, 7(10), 875-882.
- Vasile, Flora, Dossi, Elena, & Rouach, Nathalie. (2017). Human astrocytes: structure and functions in the healthy brain. *Brain Structure and Function*, 222(5).
- Wang, Huihui, Li, Xuemei, Li, Tao, Zhang, Shubing, Wang, Lianzi, Wu, Xian, & Liu, Jiaqing. (2020). The genetic sequence, origin, and diagnosis of SARS-CoV-2. *European Journal of Clinical Microbiology & Infectious Diseases*, 1-7.
- Wieghofer, Peter, & Prinz, Marco. (2016). Genetic manipulation of microglia during brain development and disease. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1862(3), 299-309.
- Wolfel, R, Corman, VM, & Guggemos, W. (2019). Virological assessment of hospitalized patients with COVID-19. *J. Mol. Bio*, 431, 1650-1670.
- Wu, Zunyou, & McGoogan, Jennifer M. (2020). Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *Jama*, 323(13), 1239-1242.
- Zeisel, Amit, Muñoz-Manchado, Ana B, Codeluppi, Simone, Lönnerberg, Peter, La Manno, Gioele, Juréus, Anna, . . . Betsholtz, Christer. (2015). Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science*, 347(6226), 1138-1142.

Tables and figures

Figure 1. The analysis of microRNA expression in recovered and not-recovered COVID-19 subjects. In not-recovered COVID-19 subjects, the expression of miR-199 (a), miR-203 (c), and miR-181(e) were significantly decreased. In recovered COVID-19 subjects, the expression of miR-199 (b), miR-203 (d), and miR-181(f) were significantly increased.

Figure 2. The analysis of inflammation markers in recovered and not-recovered COVID-19 subjects. In not-recovered COVID-19 subjects, the normalized level of C-reactive protein (a) and alanine aminotransferase (c) was increased significantly from week 0 to week 2. In recovered COVID-19 patients, the normalized quantity of C-reactive protein (b) and alanine aminotransferase (d) was significantly decreased.

Figure 3. The analysis of grade of illness in recovered and not-recovered COVID-19 subjects. In not-recovered COVID-19 subjects (a), the grade of illness was significantly increased ($P<0.0001$) and conversely, in recovered COVID-19 subjects (b), the grade of illness was significantly decreased ($P<0.0001$).