Opinion Paper

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Value of blood IFN-I levels in COVID-19 management

[COVID-19 yönetiminde kan IFN-I seviyelerinin önemi]

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Abstract: Coronavirus disease 2019 (COVID-19) which is a respiratory system disease has created inevitable pandemic. In the course of disease; the uncontrolled inflammatory response may cause tissue damage. 80–90% of patients undergo mild-to-moderate disease symptoms, the rest of the patients proceeds to critical or severe disease. Type I Interferons (IFN-I) are crucial immune mediators in order to host responses to viral infection likewise those are major components of the innate immune system moreover serve as critical antiviral molecules. IFN-I are within the first cytokines secreted during a viral infection. Until now, IFN-I response has been evaluated in patients with COVID-19 in a few publications and its contribution to the viral load control and inflammation is very little known. In those studies, the researchers have found that IFN-I deficiency which characterized by no IFN- β and low IFN- α production and activity is a hallmark of severe and critical COVID-19. Until recently, measurement of circulating serum levels of IFN- α have been limited owing to the limits of conventional immunoassay technology. Limit of detection problems of immunoassays has started to figure out thanks to SIMOA. On the top of that, generally the platforms that use SIMOA technology is 10–100 fold more sensitive than most conventional immunoassays. We should strive to do research that includes IFN-I with using platforms that include SIMOA technology, especially in severe COVID-19.

Keywords: biomarkers; COVID-19; interferons; method; SIMOA.

Öz: Bir solunum sistemi hastalığı olan Koronavirüs hastalığı 2019 (COVID-19) önlenemez bir pandemi yarattı. Hastalık seyrinde kontrolsüz inflamatuvar yanıt doku hasarına neden olabilir. Hastaların% 80–90’ı hafif-orta dereceli hastalık semptomları gösterirken, geri kalan hastalar kritik veya şiddetli hastalık geçirirler. Tip I İnterferonlar (IFN-I), viral enfeksiyonlara verilen cevapta çok önemli immün araçlardır. Aynı şekilde bunlar, doğal immün sisteminin ana bileşenleridir, ayrıca kritik antiviral moleküller olarak hizmet ederler. IFN-I, viral enfeksiyon sırasında salgılanan ilk sitokinler arasındadır. Şimdiye kadar, birkaç yayında COVID-19 hastalarında IFN-I yanıtı değerlendirildi ve bunun viral yük kontrolü ve inflamasyona katkısı çok az bilinmektedir. Bu çalışmalarda araştırmacılar düşük IFN- α üretimi ve aktivitesi ile karakterize olan IFN-I eksikliğinin şiddetli ve kritik COVID-19’da ayırt edici özelliği olduğunu bulmuşlardır. Yakın zamana kadar, IFN-a’nın dolaşımdaki serum seviyelerinin ölçümü, geleneksel immünoassay teknolojisinin sınırları nedeniyle sınırlıydı. SIMOA sayesinde immünolojik testlerin tespit sınırı problemleri çözülmeye başladı. Üstelik SIMOA teknolojisini kullanan platformlar, geleneksel immünolojik testlerin çoğundan 10–100 kat daha hassastır. Özellikle şiddetli COVID-19’da SIMOA teknolojisini içeren platformları kullanarak IFN-I içeren araştırmalar yapmaya çalışmaktayız.

Anahtar Kelimeler: Biyobelirteçler; COVID-19; İnterferonlar; Metot; SIMOA.

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Introduction

Coronavirus disease 2019 (COVID-19) which is a respiratory system disease has created inevitable pandemic. This disease has been generated by virus which is Severe Acute

Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). As SARS-CoV-2 enters the body firstly which activates innate and adaptive immune system. In the course of disease; the uncontrolled inflammatory response may cause tissue damage [1, 2].

80–90% of patients undergo mild-to-moderate disease symptoms, the rest of the patients proceed to critical or severe disease. In the majority of patients with severe COVID-19 reveal appreciably elevated serum levels of pro-inflammatory cytokines including IL-1 β and IL-6, on the top of that IL-2, IL-8, IL-17, G-CSF, GM-CSF, IP10, MCP1, CCL3, and TNF delineate as cytokine storm. Interferons are among the ‘Primer Cytokines’ that initiate systemic inflammation cascade in the cytokine storm [1, 3].

Discussion

Type I Interferons (IFN-I) are crucial immune mediators in order to host responses to viral infection likewise those are major components of the innate immune system moreover serve as critical antiviral molecules. IFN-I refer to a set of cytokines including the ubiquitous α and β subtypes (themselves subdivided into various isoforms), in addition the ϵ , ω and κ subtypes. They are produced by diverse cell types, particularly plasmacytoid dendritic cells, on the identification of viral components. Thus, IFN-I are within the first cytokines secreted during a viral infection [4].

Until now, IFN-I response has been evaluated in patients with COVID-19 in a few publications and its contribution to the viral load control and inflammation is very little known. In those studies, the researchers have found that IFN-I deficiency which characterized by no IFN- β and low IFN- α production and activity is a hallmark of severe and critical COVID-19. Besides, they have emphasized that disrupted IFN-I response has affiliated with a consistent blood virus load and aggravated inflammatory response [5, 6].

Studies mentioned above have used SIMOA (Single Molecule Array) technology for measuring all subtypes of IFN-I family especially IFN- α (7). Until recently, measurement of circulating serum levels of IFN- α have been limited owing to the limits of conventional immunoassay technology. Limit of detection problems of immunoassays has started to figure out thanks to SIMOA. On the top of that, generally the platforms that use SIMOA technology is 10–100 fold more sensitive than most conventional immunoassays. In addition, this method can be integrated into high-throughput automated instrument which is SIMOA[®] HD-1 Analyzer by Quanterix[®] Corporation [8, 9].

Available immunoassays are capable of detecting a protein concentration above a certain level that is 10^{-12} M. Cancer, neurological diseases, and especially in the early stages of viral infections, the concentration of most of the serum proteins varies between 10^{-16} M– 10^{-12} M. Therefore, a high sensitivity methodology is required to measure analytes at these concentrations. SIMOA which is also called ‘Digital Enzyme Linked Immunosorbent Assay (ELISA)’ has been developed for this requirement. Thus, proteins that may be clinically important have begun to be measured at subfemtomolar concentrations [8].

The SIMOA technology based on the separation of individual immunocomplexes on paramagnetic beads utilizing ELISA reagents. The major difference between this technology and traditional immunoassays is enmeshing single molecules in femtoliter-sized wells, letting in order to a “digital” readout of each individual bead to assignee if it is attached to the target analyte or not [8].

Microscopic beads of 2.7 μ m diameter with specific antibodies are used for proteins in low concentrations in the blood. Each protein molecule is captured by only one bead, resulting in the formation of an immunocomplex. These complexes are enzymatically labeled to form a fluorescent product. Fluorescence obtained from each protein molecule is detected by a special imaging method and results are expressed attomolar (femtograms per milliliter) [8].

In a study where were being investigated IFN- α levels, in terms of sensitivity a 5000-fold increase in SIMOA was observed against commercial ELISA kits. In addition, the limit of detection was determined as 0.69 fg/mL. This value is stated in pg/mL even in the most sensitive commercial ELISA kit [7].

ELISA tests are the most popular tool for measuring protein concentrations in blood; yet, owing to a lack of sensitivity, ELISA tests cannot be able to detect protein biomarkers that are clinically relevant in serum or plasma at very low levels such as IFN- α . Besides, IFN- α 2 levels can also be determined with SIMOA in healthy controls [10]. Therefore, for such situations, SIMOA will be able to begin to replace the ELISA.

Apart from proinflammatory cytokines, IFN-I is definitely prominent in the diagnosis, follow-up and treatment of COVID-19. IFN-I is utmost importance in fighting the virus in COVID-19 battle. For that reason, various clinical trials have been initiated to enhance the host antiviral response (clinical trials NCT04315948 and NCT04293887). Corticosteroids, anti-interferon antibody, JAK1/Tyk2 inhibitors or chloroquine which are certain drugs used in the treatment of COVID-19, have been claimed to interfere with the IFN pathway. Therefore It has been supposed that the

use of these drugs can be deleterious in severe COVID-19 with insufficient IFN-I production. Hence, routine blood IFN-I monitoring will be able to become important in these patients in the following days.

It has been emphasized that it is substantial to demonstrate IFN-I deficiency in differentiating severe COVID-19 patients. More importantly, it has been stressed that in patients with low IFN-I serum levels, the symptoms of the patients would be able to be reduced by administering alone or combined IFN therapies [10].

SIMOA is going to be used not only for IFN-Is but also for other protein markers such as prostate specific antigen (PSA) and TNF- α . Because, it has been pointed out that SIMOA can be used for early evidence of recurrence in patients who have undergone prostatectomy by detecting the lowest PSA level of 14 fg/mL in those patients for the first time [8].

Conclusion

It has been suggested that severe disease is caused by virally-driven hyper inflammation. Nevertheless, We still have little information about what immunological properties and molecular mechanisms are involved in the severity of COVID-19 disease. So, We should strive to do research that includes IFN-I with using platforms that include SIMOA technology, especially in severe COVID-19. Unfortunately, COVID-19 seems to keep going in the coming months.

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