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ORIGINAL ARTICLE

The receptor for advanced glycation end product (RAGE) pathway in COVID-19

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ABSTRACT

Introduction: Coronavirus disease-2019 (COVID-19) with lung involvement frequently causes morbidity and mortality. Advanced age appears to be the most important risk factor. The receptor for advanced glycation end-product (RAGE) pathway is considered to play important roles in the physiological aging and pathogenesis of lung diseases. This study aimed to investigate the possible relationship between COVID-19 and RAGE pathway.

Materials and methods: This study included 23 asymptomatic patients and 35 patients with lung involvement who were diagnosed with COVID-19 as well as 22 healthy volunteers. Lung involvement was determined using computed tomography. Serum soluble-RAGE (sRAGE) levels were determined using enzyme-linked immunosorbent assay.

Results: The sRAGE levels were significantly higher in the asymptomatic group than in the control group. Age, fibrinogen, C-reactive protein, and ferritin levels were higher and the sRAGE level was lower in the patients with lung involvement than in the asymptomatic patients.

Conclusions: In this study, patients with high sRAGE levels were younger and had asymptomatic COVID-19. Patients with low sRAGE levels were elderly patients with lung involvement, which indicates that the RAGE pathway plays an important role in the aggravation of COVID-19.

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COVID-19; the receptor for advanced glycation end product (RAGE); aging; inflammation; infection; co-morbidity

Introduction

Currently, we are faced with the largest pandemic of the twenty-first century, and the rapid increase in the number of deaths and absence of a clear definition of a comprehensive pathophysiology that can be used to develop a treatment protocol have united scientists and even nations under a common purpose. The virus that causes coronavirus disease-2019 (COVID-19), which emerged in December 2019, is clinically similar to the atypical pneumonia virus and the Middle East Respiratory Syndrome virus, which are coronaviruses from the same family; however, it has posed a much more serious threat to the world because of its transmission and mortality rates (Wang and Ding 2020).

In children and young adults, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection causes a mild clinical presentation that is mostly asymptomatic or includes fever, dry cough, and shortness of breath; in the elderly population and in patients with comorbidities, it can lead to lung involvement that develops rapidly and is potentially fatal. Pulmonary involvement in COVID-19 manifests with severe pneumonia in 15% of patients and acute respiratory distress syndrome (ARDS) in 5% of patients (Chen *et al.* 2020).

Receptor for advanced glycation end product (RAGE) is a 35-kDa protein and is a member of the immunoglobulin superfamily. RAGE exists in two forms: membrane-bound RAGE (mRAGE) and soluble RAGE (sRAGE) (Neeper *et al.* 1992). Ligands such as advanced glycation end products (AGEs), S100/calgranulin protein, and high mobility group box 1 protein initiate intracellular signal stimulation when linked to mRAGE (Vistoli *et al.* 2013). This signal stimulation causes activation of various inflammatory transcription factors, primarily nuclear factor kappa B (Huttunen *et al.* 1999; Yeh *et al.* 2001). sRAGE contains only an extracellular domain and is a trap receptor for the sequestered RAGE ligands. It prevents ligands from binding mRAGE, thereby preventing the inflammatory response (Oczypok *et al.* 2017). The application of sRAGE in various animal models has been shown to reduce the inflammatory response (Hofmann *et al.* 2002; Ekong *et al.* 2006).

While RAGE expression is high in many embryonic tissues, it is significantly reduced in all tissues, except in the lungs, as an individual progress to adulthood (Bierhaus *et al.* 2005). Experimental and clinical trials have demonstrated that the RAGE pathway plays an important role in the pathogenesis of lung diseases such as chronic obstructive pulmonary disease (COPD), interstitial lung disease, and ARDS (Calfee *et al.*

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2008; Nakamura *et al.* 2011). In animal models, treatments that modulate the RAGE pathway have been shown to be significantly beneficial in the course of these diseases (Jabaudon *et al.* 2015; Blondonnet *et al.* 2017).

RAGE has been suggested to be the most important determinant of physiological aging (Frimat *et al.* 2019). The re-increase in mRAGE expression with age and the fact that mRAGE acts as a receptor for ligands such as AGEs, damage-associated molecular patterns (DAMPs), and pathogen-associated molecular patterns (PAMPs), which are responsible for aging, support this idea (Janeway and Medzhitov 2002, Bianchi 2007). Moreover, knockout of mRAGE in animal models has been shown to prevent age-related diseases such as atherosclerosis, nephrosclerosis, and Alzheimer's disease (Franceschi *et al.* 2017, Teissier and Boulanger 2019).

COVID-19 with lung involvement most often causes morbidity and mortality, and advanced age is considered to be the most important risk factor. The aim of this study was to investigate the possible relationship between COVID-19 and the RAGE pathway, which is considered to play an important role in the physiological aging and pathogenesis of lung diseases.

Materials and methods

This study included patients who presented from March 25 to April 25, 2020, with a clinical presentation leading to suspicion of COVID-19 and were diagnosed with COVID-19 using polymerase chain reaction of swab samples. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Approval was obtained from the local ethics committee (OMU/KAEK 2020/165).

Demographic, laboratory, radiological, and clinical data of all patients were recorded. Patients with COVID-19 were divided into two groups based on computed tomography (CT) findings: those with lung involvement and those without lung involvement (Figure 1). The appearance of bilateral distribution, diffuse distribution, multiple lesions, nodular shapes, patchy shapes, pure ground-glass opacities and vascular thickening sign in CT were accepted as lung involvement. Asymptomatic patients were determined by PCR analysis of individuals who had contact with the symptomatic patients with COVID-19. They had no clinical complaints, and did not develop any typical symptoms for 14 days (Figure 1). Age and gender-matched individuals were included in the control group.

This study is a cross-sectional study and serum samples were taken from patients at the first presentation. Other laboratory analyzes were also evaluated in the study at the first presentation. Peripheral venous blood samples were collected at presentation. The blood samples were centrifuged at 3000× *g* for 10 minutes and the sera were stored at −80 °C. On the evaluation day, the sera were melted at room temperature. When the samples showed higher concentrations, were diluted and measured in duplicate.

The levels of human RAGE in serum were measured using commercially available RAGE Enzyme-Linked Immunosorbent Assay (ELISA) Kit (Human Receptor for Advanced Glycation End Products, Cat. No. E0031Hu, Bioassay Technology Laboratory, Shangai, China). The enzymatic reactions were evaluated in an automatic microplate photometer. The concentrations of RAGE were determined by comparing the optic density of the samples to the standard curve. The mean interassay and intra-assay coefficients of variation percentage for RAGE were <10% and <8%, respectively. All assays were conducted according to the manufacturer's instructions. The expected values of the test is 0·05–20 ng/mL.

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS 19.0, Chicago, IL, USA). Normal distributions were tested with the Kolmogorov-Smirnov test with Lilliefors correction. Quantitative data were presented as mean ± standard deviation (SD). Parametric data were analysed by 3-way ANOVA followed by the Student's *t*-test *post hoc* analysis (age, haemoglobin, leukocyte, aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet, and fibrinogen). Nonparametric data were analysed by Kruskal–Wallis tests. *Post hoc* evaluations of between-group differences were conducted using Mann–Whitney *U* test (RAGE, neutrophil, lymphocyte, C-reactive protein, and ferritin). Serum sRAGE levels were evaluated using the ANCOVA model adjusting for age. Spearman Chi-square test was used to compare the categorical variables. P values less than 0.05 were considered as significant.

Results

This study included 23 asymptomatic patients and 35 patients with lung involvement who were diagnosed with COVID-19 as well as 22 healthy volunteers (control group) (Table 1). The sRAGE level was significantly higher in the asymptomatic COVID-19 group (17.5 ± 5.29) without lung involvement than in the control group (4.42 ± 2.92, $p < 0.001$) (Figure 1). The sRAGE levels were significantly lower in the COVID-19 group (2.05 ± 0.658, $p < 0.001$) with lung involvement than in the group without lung involvement; moreover, they were also significantly lower than that in the control group (Figure 2). After adjusting for age, serum sRAGE level was higher in the patients with lung involvement compared with the control group and the asymptomatic COVID-19 group (ANCOVA, for both $p < 0.001$).

Age and levels of alanine aminotransferase, aspartate aminotransferase, fibrinogen, C-reactive protein, and ferritin were significantly higher (Table 1) whereas platelet levels were significantly lower in the patients with COVID-19 with lung involvement than in the asymptomatic patients without lung involvement. On the other hand, both groups were similar in terms of haemoglobin level and white blood cell and lymphocyte counts.

Discussion

This study investigated the relationship between lung involvement and serum sRAGE levels in patients with COVID-19. Compared with the healthy control group, sRAGE levels were higher in the asymptomatic group. On the other hand,

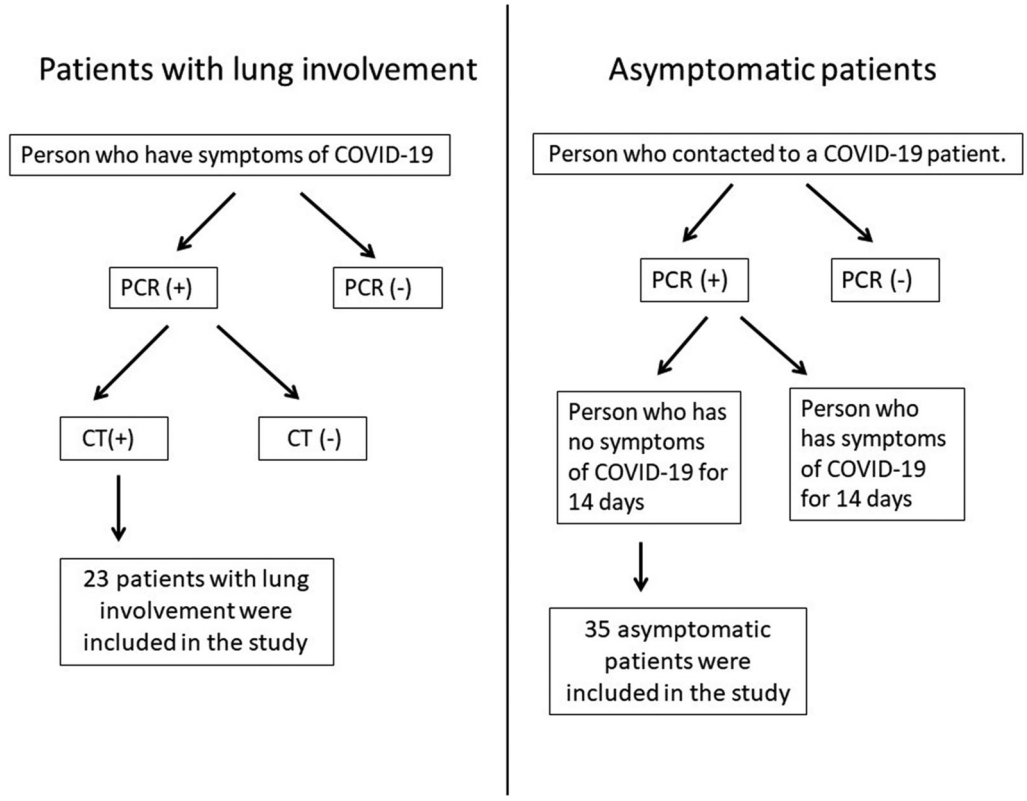

Figure 1. Patient flow diagram.

Table 1. Demographic and laboratory data of study groups.

	Healthy control	Patients with lung involvement	Patients without lung involvement	*p* Value
Gender (female/male)	9/10	9/14	13/22	0.879[&]
Age (years)	42.42 ± 10.88	62.2 ± 11.9**	42.7 ± 21.6	<0.001
Co-morbidities	–	6	4	0.792[&]
Haemoglobin (gr/dL)	13.99 ± 055	14.1 ± 2.2	13.3 ± 2.2	0.398
Leukocyte (/mm^3)	8024 ± 1001.9	7550 ± 3939	6588 ± 4191	0.682
AST (U/L)	17.80 ± 3.05	24.7 ± 6.4	36.9 ± 18.1**	<0.001
ALT (U/L)	15.68 ± 2.64	19.7 ± 7.9	28.5 ± 7.0**	<0.001
BUN	14.2 ± 5.0	14.6 ± 4.2	15.3 ± 3.8	0.896
Creatinine	0.66 ± 0.13	0.71 ± 0.09	0.68 ± 0.12	0.611
Neutrophil (/mm^3)	3962 ± 1240	4802 ± 3591	4058 ± 2026	0.566
Neutrophil (%)	62.05 ± 8.58	60.7 ± 5.7	67.7 ± 13.3*	0.023
Lymphocyte (/mm^3)	1503 ± 239	1857 ± 953	1405 ± 788	0.137
Lymphocyte (%)	28.68 ± 2.98	27 ± 12	23 ± 11	0.416
Platelet (10^3/mm^3)	254 ± 35*	227 ± 35	203 ± 64	0.018
Fibrinogen (mg/dL)	238 ± 13	316 ± 108	589 ± 179**	<0.001
C-Reactive protein (mg/L)	2 ± 1	14 ± 19	50 ± 32**	<0.001
Ferritin (ng/mL)	113 ± 58	138 ± 139	416 ± 178**	<0.001

AST: aspartate Aminotransferase; ALT: alanine Aminotransferase; BUN: blood urea nitrogen.
p value: ANOVA test or [&]Chi-square test.
Post hoc evaluations of between group differences were conducted using Student's *t* test (age, haemoglobin, leukocyte, AST, ALT, platelet, and fibrinogen) and Mann–Whitney *U* test (neutrophil, lymphocyte, C-reactive protein, and ferritin).
*$p < 0.05$ or **$p < 0.001$ compared with other groups.

patients with asymptomatic COVID-19 without lung involvement were younger and had higher serum sRAGE levels, whereas those with lung involvement were older, and although their acute-phase levels were higher, their serum sRAGE levels were reduced and even significantly lower than that in the control group.

Age is considered to be the most important risk factor associated with morbidity and mortality in COVID-19 (Yin Wong *et al.* 2020). Other important risk factors include comorbid conditions such as COPD, atherosclerosis, heart failure (Falcone *et al.* 2005), and hypertension (HT) (Geroldi *et al.* 2005) as well as smoking (Yin Wong *et al.* 2020; Zhou *et al.* 2020). In contrast, COVID-19 has an asymptomatic or moderate course in young individuals and in those without comorbidities. In the course of aging, some changes in RAGE pathway arise. While the proinflammatory mRAGE expression and its age-related ligands (AGEs, DAMPs, and PAMPs) have increased, the anti-inflammatory sRAGE level has reduced (Janeway and Medzhitov 2002; Bianchi 2007; Hallam *et al.* 2010). This resulting proinflammatory situation can explain

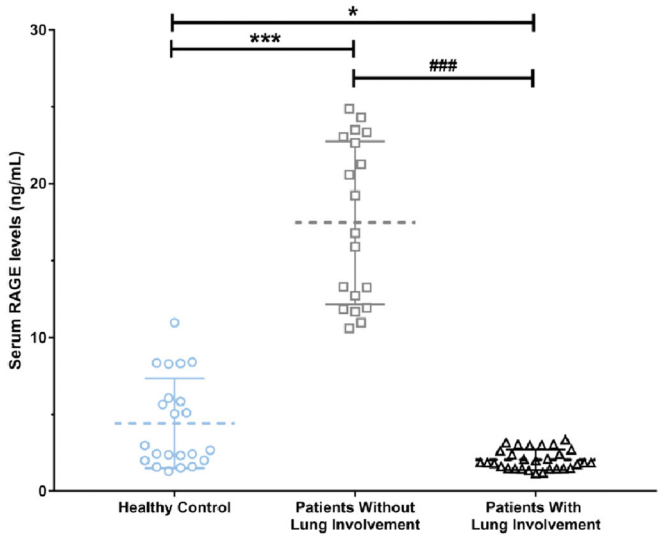

Figure 2. Serum sRAGE levels in all groups. All values were expressed as mean ± SD. $^{***}p < 0.001$, $^{*}p < 0.05$ versus control group and $^{###}p < 0.001$ versus patients without lung involvement. *Post hoc* evaluations of between group differences were conducted using Mann–Whitney *U* test.

the sustained and exaggerated immune response to COVID-19 in elderly patients with comorbid conditions. Individuals with asymptomatic COVID-19 in this study were significantly younger and had high sRAGE levels. This may have protected them from aggravation of the disease and occurrence of an exaggerated inflammatory response. On the other hand, as expected, symptomatic patients with lung involvement were older and their anti-inflammatory sRAGE levels were significantly lower, while their levels of acute-phase reactants were significantly higher. We believe that these results support our theory that the RAGE pathway plays a role in the aggravation of COVID-19.

In the initial studies of COVID-19, it was believed that the penetration of the virus into the cell through epithelial ACE receptors has a negative role in the pathogenesis of the disease (Kuba *et al.* 2006; Yan *et al.* 2020). However, this theoretical opinion cannot be supported by clinical trials. Moreover, the lower ACE2 levels in older individuals, men, hypertensive individuals, and obese individuals, who are affected more by SARS-CoV-2 infection, does not support this theory. On the contrary, it has been suggested that increased ACE2 activity has a protective effect against COVID-19 (Brojakowska *et al.* 2020). A previous survey reported that the use of ACE inhibitor and statin reduced morbidity and mortality in patients with COVID-19, thereby confirming this theory (Yin Wong *et al.* 2020). In fact, this occurred despite the presence of comorbid conditions such as coronary artery disease (CAD) and congestive heart failure (CHF), which are risk factors for morbidity and mortality in COVID-19 (Yin Wong *et al.* 2020). In a clinical study, Forbes *et al.* showed that treatment with ACE inhibitor increased the sRAGE level (Forbes *et al.* 2005). Experimental studies have also shown that sRAGE enhances ACE2 expression. In this study, the asymptomatic course of COVID-19 in individuals with high sRAGE levels may be associated with the increase in ACE2 expression induced by sRAGE.

While high RAGE expression occurs in all tissues during the embryonic period, RAGE expression in non-lung tissues is minimal or absent in adult life (Bierhaus *et al.* 2005). In a previous clinical trial, an increase in mRAGE expression was observed in patients with interstitial and postobstructive pneumonia (Morbini *et al.* 2006). Reynold *et al.* showed that mice lacking RAGE were protected from hyperoxia-induced ARDS and mortality (Reynolds *et al.* 2010). In this study, patients with COVID-19 without lung involvement had high sRAGE levels, which may have protected them from lung involvement.

This study has some limitations. It would make an extra contribution to the study to evaluate AGE estimation beforehand.

The RAGE pathway, which has been shown to play a key role in the biology of aging (Kuba *et al.* 2006), is also closely associated with comorbidities such as COPD, CAD, CHF, atherosclerosis, smoking, and HT. We think that the RAGE pathway plays a key role in the pathophysiology of COVID-19, which has similar risk factors in its morbidity and mortality. The relationship of the RAGE pathway with ACE also supports this idea. In this study, the asymptomatic course of individuals with COVID-19 who had high sRAGE levels indicated that the RAGE pathway may play an important role in the aggravation of COVID-19. Treatments that modulate the RAGE pathway may be promising in the reduction of morbidity and mortality of COVID-19, for which no effective treatment has been found yet, and perhaps other coronavirus infections.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data that support the finding of this study are available from the corresponding author upon reasonable request.

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