ORIGINAL ARTICLE

Serum Soluble Interleukin–2 Receptor Alpha and RANTES Chemokine as Potential Biomarkers for COVID–19 Infection and Severity

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ABSTRACT

Background: *Even though it seems that the COVID-19 epidemic has ended, the threat of viral mutation and resurgence is still looming. The illness is characterized by acute respiratory distress and other symptoms caused by immune system disturbance and cytokine storm.* ***Objective:*** *We aimed to determine the serum level of soluble Interleukin – 2 Receptor alpha (IL-2Ra) and RANTES in COVID-19 patients.* ***Methodology:*** *A case-control study was performed on 60 patients with a positive nasopharyngeal swab tested by real-time PCR for SARS-CoV-2 and 25 healthy controls to measure serum levels of sIL-2Ra and RANTES using Enzyme-Linked Immunosorbent Assay.* ***Results:*** *The findings showed no significant difference in the serum levels of sIL-2Ra and RANTES between patients and healthy controls, nor was there a difference between severe and non-severe cases of COVID-19. A significant association was observed between the presence of a previous chest condition and the level of RANTES and a significant positive correlation was detected between serum sIL2Ra and RANTES levels.* ***Conclusions:*** *The study analyzed the correlation between the serum sIL2Ra and RANTES, being positively correlated means the same factors influence them and they can be therapeutically targeted in the same way. The effect of a pre-existing chest condition on RANTES level encourages regular monitoring of RANTES. Although the UN WHO has announced that the status of COVID-19 as a public health emergency has been lifted, the virus will always remain a worldwide threat. Therefore, comprehensive research should continue to be better prepared.*

INTRODUCTION

Coronaviridae is a family of large, enveloped viruses with single-stranded RNA that are known to cause illness in humans and other species. Members of this family are known to cause respiratory as well as enteric diseases. The SARS-COV-2 is classified as belonging to the *Nidovirales* order, the *Coronaviridae* family, and the *Coronavirinae* subfamily [1]. It has been complicit in the emergence of the COVID-19. The virus causes a highly contagious, rapidly spreading acute respiratory distress syndrome, varying in severity from mild disease to severe forms requiring ICU admission and occasionally ending in mortality. The seriousness of COVID-19 infection is largely determined by the effectiveness of the host's immune response [2]. The virus activates both adaptive and innate immunity, and a well-balanced immune response is required to eliminate infection with minimal tissue damage [2].

However, the uncontrolled immune response leads to more injury and a lengthened disease course. Certain immunological parameters have been identified as important biomarkers for diagnosis, predicting the disease's severity, and estimating patient survival rates, such parameters are the neutrophil-to-lymphocyte ratio (NLR) in peripheral blood and serum levels of interleukin-6 (IL-6) [3]. Other inflammatory markers as CRP, ferritin, procalcitonin, TNF alpha, and other cytokines have been accused of correlating with worse disease prognosis and poor patient outcomes. The blood levels of such markers have become crucial laboratory information for deciding treatment protocols and aiding in disease management [4].

IL-2 is essential for the growth, differentiation, and activity of T cells, including Tregs, CD4+, and CD8+ effector cells [5]. CCL-5, also referred as Regulated upon Activation Normal T Expressed and Secreted (RANTES) is a chemokine responsible for chemotaxis of inflammatory cells to combat infection. RANTES is important in controlling the immune response and speculations have been made on whether its serum level in individuals with COVID-19 can be relevant in the diagnostic process and the correlation with disease outcome [6].

Activated T lymphocytes are essential for defense against viral diseases and have on their surface the truncated protein IL2Ra which binds circulating interleukin 2 (IL-2) [7]. Soluble IL2Ra has been suggested to participate in lymphopenia associated with COVID-19 by blocking IL-2 signaling, hence inhibiting lymphocyte proliferation [8].

The objective of the study is to quantify the serum concentrations of soluble IL-2Ra and RANTES in COVID-19 patients and compare them to healthy controls and detect any correlation with disease severity to determine whether disease severity could be predicted by the levels of these cytokines, and the possibility of predicting disease outcome and possible therapeutic targets.

METHODOLOGY

The Ethics Committee of the Faculty of Medicine at Cairo University has approved the research **(# MS -121-2022)**. The study was done corresponding with the principles of the Declaration of Helsinki. All participants provided informed consent before enrollment.

This case-control study started in June 2022 and lasted for 6 months. It involved 60 individuals diagnosed with COVID-19 infection admitted to the isolation wards of Kasr Al Ainy Hospitals and recruited from the outpatient COVID-19 clinics. This study included both severe and non-severe cases. Patients receiving immunomodulatory drugs or suffering from malignancies were excluded. Twenty-five healthy individuals, matched for age and gender, were included in this research as a control group with no given history of immunological diseases, malignant diseases, chronic disorders, recent or current COVID-19 infection, or recent contact with patients infected with COVID-19.

All patients underwent a thorough history taking that focused on age, gender, any existing comorbidities, and the stay duration in the hospital or ICU if applicable. Data was obtained from medical records. All patients were diagnosed as having COVID-19 infection through a positive nasopharyngeal swab by Real-Time PCR (RT-PCR) to identify SARS-CoV-2 RNA *(Omicron variant)*. The following laboratory tests were done, complete blood count (CBC), C-reactive protein (CRP), ALT, AST, Creatinine, D-dimer, Procalcitonin, IL-6, and Ferritin. Patients underwent a CT scan of the chest to record any pathological findings related to COVID-19 infection such as ground glass opacities, pneumonic patches, or pleural fluid collection.

Patients were then categorized as having severe and non-severe illness based on the guidelines of COVID-19 treatment stated by the WHO "2020". There were thirty patients with non-severe illness, those who showed a positive COVID-19 test by RT-PCR with mild symptoms of headache, muscle pains, mild fever, cough, diarrhea, vomiting, malaise, and loss of smell and taste, but no greater symptoms. There were 30 severely ill patients, those who showed a positive RT-PCR test with the following clinical findings added to the previous symptoms: SpO2 less than 94% on room air at sea level, PaO2/FiO2 less than 300 mmHg., respiratory rate more than 30 breaths per minute and lung infiltrates exceeding 50%.

Measurement of Serum Level of Soluble IL-2Ra and RANTES was done as follows: Peripheral venous Samples were withdrawn from all participants in a completely sterile environment. For the preparation of serum, 4 ml of blood were drawn into sterile serum-separating plastic tubes and allowed to clot for 30 mins at room temperature, then spun in a centrifuge at 1000 g for 15 minutes. The separated serum was then located into separate containers and kept at -20˚C until use. Quantitative measurement of serum levels of sIL2Ra and RANTES for all patients and controls were done by a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kit designed for detection of human soluble IL-2Ra concentration (SinoGeneClon Biotech Co., Ltd. Catalog no.: SG-10674) and determination of human RANTES concentration (SinoGeneClon Biotech Co., Ltd. Catalog no.: SG-10704). The steps were done following the instructions of the manufacturer and finally, for the results, duplicate readings for each standard and sample were calculated and then averaged, with the average optical density of the zero standard being subtracted. A four-parameter logistic curve was plotted on a log-log graph, with the standard concentration on the x-axis and the optical density (OD) values on the y-axis.

The data was statistically summarized in mean ± standard deviation (± SD), median and range, or frequencies and percentages when applicable. The Kolmogorov Smirnov test was used to assess whether the numerical data followed a normal distribution. The Mann Whitney U test for independent samples was used to compare numerical variables between the study groups. To compare categorical data, a Chi-square (χ^2) test was conducted. The Exact test was employed when the expected frequency was less than 5. The correlation between the various variables was determined using Pearson's moment correlation equation for linear relations of normally distributed variables and Spearman's rank correlation equation for non-normal variables/non-linear monotonic relations. A two-sided p-value of less than 0.05 was considered statistically significant. All statistical analyses were conducted using IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

RESULTS

The demographic information of the groups is presented in table 1. No statistically significant difference was detected either in age or in gender between severe and non-severe patients (p-value = 0.102 and 0.299 respectively). Among patients, 50% received anti-COVID-19 vaccines, while 84% of the controls were vaccinated. This shows a significant difference in vaccination status between COVID-19 patients and the controls with a p-value of 0.004. Among the 30 patients suffering from severe disease, only 43.3% had received the vaccination, while within the 30 patients with non-severe illness, 56.7% were vaccinated with no significant difference between these 2 groups.

Table 1: Demographic data of COVID-19 Patients and Controls:

Parameter		**Controls (25)**		**Patients (60)**		**Severe (30)**		**Non-severe (30)**	
		No.	%	No.	%	No.	%	No.	%
Gender	**Males**	13	52.0	27	45.0	16	53.3	11	36.7
	Females	12	48.0	33	55.0	14	46.7	19	63.3
Age (years)		**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
		36.32	13.152	54.3	20.721	58.93	20.708	49.67	20.007

Age (years) is represented as a mean ± SD, gender is represented as a number and percentage

The comorbidities of the 60 patients included are outlined in table 2 regarding Hypertension, Diabetes Mellitus (DM), cardiac disease, renal disease, hepatic disease, and a previously diagnosed chest condition. As for the personal habits, 40% of patients were smokers, 60% were non-smokers and one patient was an alcohol drinker.

Table 2 provides a summary of the laboratory results of all COVID-19 patients. A difference, which is statistically significant, was found between severe and non-severe groups of illness regarding total leucocytic count (TLC), lymphocytic count, CRP, creatinine, and ferritin (p-values = 0.029, 0.019, 0.000, 0.003, and 0.044 respectively). No such difference was observed regarding serum IL6, procalcitonin, ALT, and D-dimer (p-values = 0.770, 0.261, 0.122, and 0.150 respectively) (table 3).

Table 2: Laboratory Findings and Comorbidities in COVID-19 Patients:

COVID-19 Patients		
Parameter	**Mean**	**SD**
TLC (*10^3/cmm) (N: 4-10)	12.627	15.7996
CRP (mg/L) (N: <10)	93.293	77.1895
Ferritin (ng/mL) (N: 12-150/300)	528.75	754.906
ALT (IU/L) (N: 19-25)	44.78	78.468
Creatinine (mg/dL) (N: 0.59-1.04)	2.38	2.84356
IL-6 (pg/mL) (N: <43.5)	190.26	533.633
Procalcitonin (ng/mL) (N: <0.1)	4.78	18.37888
D-dimer (µg/mL) (N: <0.5)	9.830	25.28711
Comorbidity	**Percentage of Patients Affected**	
Hypertension	53.3 %	
Diabetes Mellitus	43.3 %	
Heart Disease	31.7 %	
Renal Disease	21.7 %	
Hepatic Disease	20.3 %	
Chest Disease	5.1 %	

Laboratory Findings are represented as a mean ± SD and comorbidities are represented as percentages.
TLC (Total leucocytic count), CRP (C-reactive protein), ALT (Alanine transaminase), IL-6 (Interleukin 6)

Table 3: Laboratory differences in different groups of COVID-19 patients:

Parameter	Severe (30)		Non-severe (30)		p-value
	Mean	± SD	Mean	± SD	
TLC (10^3/cmm)	15.897	20.8781	9.447	7.1029	**0.029**
Lymphocytes (%)	8.9114	7.42753	20.6875	11.47588	**0.019**
CRP (mg/L)	133.230	73.0932	53.355	58.9855	**0.000**
Creatinine (mg/dl)	3.0443	2.97004	1.7153	2.59147	**0.003**
Ferritin (ng/mL)	863.91	1008.171	254.53	292.972	**0.044**
IL6 (pg/mL)	289.25	677.420	31.89	16.499	0.770
Procalcitonin (ng/mL)	6.9183	23.27353	1.2702	1.78746	0.261
ALT (IU/L)	62.00	107.116	28.13	25.557	0.122
D-dimer (µg/mL)	13.9442	33.28334	5.0308	9.86933	0.150
sIL2Ra/Lymphocyte	0.4257	0.51198	0.1869	0.23985	0.082

Significant p-value <0.05.
TLC (Total leucocytic count), CRP (C-reactive protein), ALT (Alanine transaminase), IL-6 (Interleukin 6), sIL2Ra (Soluble interleukin 2 receptor alpha)

The serum sIL2R alpha and RANTES levels were quantified in the patients and the health controls. No difference was detected between the 2 groups (p-value = 0.881 and 0.531 respectively). Further analysis was done by comparing the level of Serum sIL2R alpha and RANTES in severe and non-severe cases of COVID-19 patients to detect if their levels differ with the disease severity. No difference was observed in the serum levels of both parameters in patients with different disease severity (p-value = 0.582 and 0.701 respectively) (table 4).

Table 4: Comparison of sIL2Ra and RANTES levels in COVID-19 patients and controls:

Parameter	Case (60)		Control (25)		p-value
	Mean	± SD	Mean	± SD	
IL2Ra	2.128	3.1177	2.893	3.3989	0.881
RANTES	570.718	205.839	914.252	924.5752	0.531
	Severe (30)		Non-severe (30)		p-value
	Mean	± SD	Mean	± SD	
IL2Ra	1.620	0.3585	2.367	4.3717	0.582
RANTES	559.647	123.6545	581.790	265.8127	0.701

Serum IL2Ra levels are in µg/L and serum RANTES levels are in pg/mL and they are expressed in mean ± SD.
Significant p-value <0.05.
sIL2Ra (Soluble interleukin 2 receptor alpha), RANTES (Regulated upon Activation Normal T Expressed and Secreted)

As regards the outcome and the stay duration in hospital in severe and non-severe COVID-19 patients, differences that are statistically significant were found between the 2 groups (p-value = 0.005 and 0.00 respectively). Within the 30 patients suffering from severe disease, 22 (73.3%) went into remission, while 8 (26.7%) died. All patients suffering from non-severe illness recovered with no mortality. The mean duration of hospital stay ± SD in non-severe cases was 5.6 ± 4.39 days. Whereas that of severe cases was 16.43 ± 25.716 days.

Associations and Correlations of the levels of sIL2Ra and RANTES with comorbidities and laboratory findings were done and shown in table 5. All results showed no significant associations and correlations between the presence of comorbidities and laboratory findings, and the levels of either sIL2Ra or RANTES, apart from the presence of a previous chest condition and the level of RANTES, expressing a p-value of 0.002. The ratio of sIL2Ra to lymphocytic count in severe and non-severe cases of COVID-19 was analyzed and there was no difference between both groups with a p-value of 0.082. Finally, a significant positive correlation was detected between serum sIL2Ra and RANTES levels in COVID-19 patients (r=0.626 and p value=0.000).

Table 5: Associations and correlations of serum levels of sIL2Ra and RANTES with comorbidities and laboratory findings in COVID-19 patients:

	sIL2Ra		**RANTES**	
Comorbidity	**p-value**		**p-value**	
Hypertension	0.160		0.257	
Diabetes Mellitus	0.224		0.190	
Cardiac Disease	0.300		0.148	
Renal Disease	0.495		0.395	
Hepatic Disease	0.548		0.801	
Previous Chest Condition	0.373		**0.002**	
laboratory findings	**r**	**p-value**	**r**	**p-value**
TLC	-0.087	0.510	-0.067	0.612
Neutrophils	-0.218	0.329	-0.182	0.418
Lymphocytes	0.141	0.520	0.242	0.265
CRP	-0.187	0.153	-0.114	0.387
IL-6	0.241	0.428	-0.167	0.586
Procalcitonin	0.052	0.790	0.159	0.411
ALT	-0.051	0.703	-0.048	0.721
D-dimer	-0.189	0.356	-0.139	0.498
Creatinine	-0.100	0.446	-0.062	0.636
Ferritin	0.240	0.308	0.143	0.548
sIL2Ra	-------	------	0.626	**0.000**

Significant p-value <0.05.

TLC (Total leucocytic count), CRP (C-reactive protein), ALT (Alanine transaminase), IL-6 (Interleukin 6), sIL2Ra (Soluble interleukin 2 receptor alpha)

DISCUSSION

Although currently, the pandemic of COVID-19 seems to have come to a halt with regressing cases, the threat of viral mutation and resurgence is still looming. This study was conducted during the crisis in Egypt in which 4,375 cases were detected (Johns Hopkins University, Centre for Systems Science and Engineering; COVID-19 Dashboard September 2022 Update).

In our study, there was an absence of significant difference in the results of sIL2Ra in the serum of patients against healthy controls. Also, there was no significant difference between severe and non-severe COVID-19 cases. This may be explained by the significant lymphopenia found in severe patients as opposed to non-severe cases and normal lymphocytic counts in healthy controls. To overcome this, sIL2Ra/Lymphocyte ratio was calculated and compared in severe and non-severe patients as done by Hou et al [9], who found a positive correlation between sIL2Ra/Lymphocyte ratio and disease severity. However, our study provided a non-significant difference.

Contrary to our results, a study done by Xie et al [10] revealed a statistical significance in serum levels of sIL2Ra in severe cases in comparison to non-severe cases. This difference in findings may be attributed in part to the greater sample size obtained by Xie et al (280 subjects), their methodology in using a mouse model to mimic acute infection, and their different approach in collecting data using bronchioalveolar lavage in obtaining their specimens.

By measuring RANTES levels in COVID-19 patients and comparing them to healthy controls, no statistical significance was observed between both groups. As for RANTES levels in distinguishing disease severity, no statistical difference was observed either between severe and non-severe cases. Although not statistically significant, higher serum RANTES levels were found in healthy controls and milder cases than in more severe cases of infection.

A study conducted by Zhao et al [11] which was performed on 71 patients found significant results of higher RANTES levels among the non-severe cases in comparison to severe cases. Contradictory to the above findings, a study done by Patterson et al [12] conducted on 10 terminally ill patients with severe COVID-19 disease found a profoundly higher serum level of RANTES in those patients which was correlated to elevated levels of other inflammatory markers as IL-6, higher plasma viremia, and a repressed CD4/CD8 ratio.

The correlation of serum levels of sILR2a and RANTES in all 60 COVID-19 patients with previously existing comorbidities was done, and statistical significance was observed indicating a higher serum level of RANTES in patients with a pre-existing chest condition. This can support the hypothesis made by Patterson et al [12] that RANTES is involved in the aberrant immune response seen in COVID-19 disease

that primarily affects the lungs as a target organ and causes the occurrence of a cytokine storm.

We also studied the correlation between sIL2R alpha and RANTES levels in COVID-19 patients. A positive correlation was detected between the two parameters (r=0.626 and p-value=0.000). Because these two different variables move in the same direction, they theoretically are affected by the same factors.

Among the sixty COVID-19 cases, there was a significant increase in the levels of TLC in patients with severe illness compared to patients with non-severe illness. This was also linked to significant lymphopenia in severe cases when compared to non-severe cases. Similarly, the findings of Anurag et al [13] described higher leucocytic counts and lower lymphocytic counts in severe cases of COVID-19. This can be due to the cytokine storm that occurs in severe cases causing neutrophil activation, migration, and the release of granular content. The observed lymphopenia is accompanied by an inverted CD4/CD8 ratio found in severe COVID-19 disease to eliminate virally infected cells, this was also described by Assal et al [14].

Higher levels of CRP were found in severe cases which is in agreement with the research of Assal et al [14] who found higher levels of CRP among severe cases of COVID-19. CRP, being an acute phase reactant, is directly linked to the level of inflammation and the disease intensity.

In our investigation, serum creatinine levels in COVID-19 patients were statistically elevated in severe disease in comparison to non-severe disease. This was similarly recorded by Hachim et al [15] who discovered a higher rate of acute kidney injury (AKI) and elevated renal functions in more severe COVID-19 cases. The virus itself is suggested to cause direct tubular injury. It is also proposed that the presence of ACE-2 receptors necessary for SARS-CoV-2 binding and entry in the renal tubular cells participates greatly in renal pathology.

During this study, serum ferritin levels in patients suffering from severe COVID-19 illness were significantly higher in comparison to non-severe cases. Similarly, Dahan et al [16] found higher levels of serum ferritin in severe cases of COVID-19 than in non-severe cases. It is thought that ferritin is deeply involved in any inflammatory process as a pathogenic mediator, stimulating inflammatory pathways [17].

8 patients out of 60 died (13.3%) during the study. All the 8 patients suffered from severe COVID-19 disease. Similarly, Omran et al [18] found a more likely death outcome in severe cases vs. non-severe cases of COVID-19. The cytokine release syndrome and cytokine storm observed in severe COVID-19 illness can lead to multiorgan system affection and subsequent failure, eventually leading to death.

There was an observed prolonged hospital stay in patients with severe illness than in non-severe cases. This was also observed by Birhanu et al [19] during their study of 394 hospitalized patients. This prolonged duration of stay can be due to the more morbid nature of symptoms in severe COVID-19 illness requiring more intensive care and treatment.

CONCLUSION

The higher level of RANTES in cases with a pre-existing chest condition supports that RANTES is involved in the aberrant immune response seen in COVID-19 disease that primarily affects the lungs as a target organ and this encourages the regular monitoring of RANTES through the course of the disease. The positive correlation between the serum sIL2Ra and RANTES means that they are affected by the same factors and they can be therapeutically targeted in the same way. There is still a danger of new variants appearing that could cause a spike in the number of cases and fatalities. So, it is advised that thorough studies and efforts should remain the foundation of the response to a pandemic to be better prepared.

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

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