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Changes in Substance P and Calcitonin Gene-Related Peptide Serum Levels in COVID-19 Patients- For Better Understanding of Disease Biology

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ABSTRACT

Background: To win the ongoing battle against the emerging coronavirus, new strategies are needed for reliable diagnosis and more effective treatment, which requires a better understanding of disease biology. This study aimed to determine the changes in Substance P (SP), and Calcitonin Gene-Related Peptide (CGRP) serum levels as a result of SARS-COV-2infection, and to correlate these changes with the pathophysiological events affecting the severity of the disease.

Patients and Methods: Serum levels of SP and CGRP were measured for 20 healthy volunteers assigned as controls, and 64 COVID-19-positive patients, subdivided according to disease severity, into 3 groups assigned as asymptomatic (n = 19), hospitalized (n = 24), and ICU admitted (n = 21) groups. The results were statistically compared between the studied groups.

Results: The serum levels of SP were significantly elevated in COVID-19 patients (P<0.001) when compared to the control samples, with significant increases between COVID-19 groups due to disease severity. On the other hand, serum levels of CGRP in COVID-19 patients, were greatly decreased (P<0.001) as compared to normal controls, with no effect driven by disease severity

Conclusion: SP and/or CGRP can be measured as diagnostic and prognostic biomarkers in SARS-COV-2 infection. Adjusting CGRP level in COVID-19 patients is very important for proper angiogenesis, powerful immune response, and good epithelial repair, so may represent a novel therapeutic approach. Also, targeting NK-1Rs or TRPV-1, could modulate the inflammatory and immune responses in COVID-19, leading to better disease outcomes.

Keywords: Substance P, Calcitonin Gene-Related Peptide, COVID-19, Cytokine Storming

INTRODUCTION

As the world is (hopefully) emerging from the Covid-19 pandemic, which represented the world's biggest challenge in the past few years, that killed millions of people, along with its enormous impact on nations' economies, safety and religious practices[1], new strategies are needed for reliable diagnosis and conclusive treatment, which requires a good understanding of pathophysiology, and inflammatory pathways of the disease and its complications. This study attempted to correlate the neuroimmune functions of 2 neuropeptides (SP and CGRP), with the pathophysiology of COVID-19, and to investigate whether the cytokine storming (the actual killer in COVID-19)[2], is directly related to the changes in their serum levels subsequent toSARS-CoV-2infection.

Substance P (SP):

Substance P (SP), the first inflammatory neuropeptide (11 amino-acids), discovered by **Euler and Gaddum** in 1931[3], is the most common modulator of neuroimmunoregulation in the lungairways[4]. In addition to their abundant expression in central nervous system, peripheral nervous system, and gastrointestinal tract[5], SP and its selective receptors Neurokinin 1 (NK-1Rs), are widely expressed in the sensory neurons innervating the airways, lung parenchyma, and lymphoid organs, where SP is released as a neurotransmitter (NT), for neuroimmunoregulation (i.e., coordinates between the nervous and the immune systems)[6]. Several studies have reported elevated serum levels of SP with cancers and several viral infections including HIV/AIDS and respiratory syncytial virus (RSV)[7].Recent studies investigating the novel SARS-CoV-2, accuses the SP of being the main trigger of many inflammatory pathways in the course of the COVID-19 pandemic caused by this damn virus[8], as it is over secreted to direct the immune cells and other cells in the respiratory tract to release cytokine storming mediators responsible for many serious complications of the disease[6]. So, we can conclude that NK-1Rs may be a target for those interested in developing new therapeutic approaches for COVID-19.

Calcitonin Gene-Related Peptide (CGRP):

Calcitonin gene-related peptide (CGRP) is a potent vasodilating, angiogenic and immune modulating peptide (37 amino-acids)[9], primarily localized to C and Aδ sensory fibers, which have a dual sensory and motor functions, and displayed widely throughout the body, with extensive perivascular localization[10]. At present, little is known about its nonneuronal localization, as well as its role, despite excellent previous studies[11]. In the

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respiratory system, CGRP is abundantly present in the sensory nerves supplying the epithelium, neuroendocrine cells, and smooth muscle cells[12], with a wide spread of its receptors in the pulmonary blood vessels, epithelial and smooth muscle cells of large airways[13]. This wide respiratory distribution of CGRP and its receptors, allows it to play several important roles (closely related to the evolution of the pathogenesis of COVID-19), including vasoregulation, bronchoprotection, and anti-inflammatory actions, in addition to its importance for tissue repair as it promotes bronchial, alveolar, and epithelial cells growth following lung injury[14].

TRPV-1 in respiratory viral infections:

Transient receptor potential, vanilloid subfamily, member 1 (TRPV-1), is a nonselective cation channel (with 10 percent higher preference for Ca^{2+})[15], expressed in the immune cells and terminals of type C sensory neurons of upper and lower airways and lung parenchyma[16].Inflammation of the airways either due to viral infection or exposure to inhaled allergens, is mediated by crosstalk (i.e., signal transfer) between this TRPV-1 positive-neuronal fibers and the immune cells[17]. The respiratory expression of TRPV-1 receptors is extensively upregulated, in many respiratory viral infections including Respiratory Syncytial Virus (RSV), Human Respiratory Rhinovirus (HRV), and measles virus, and this can drive an inflammatory cascade, which may progress to airways hyperactivity, in a manner proportional to the viral load and the duration of infection[18].

How does activated TRPV-1, mediate local inflammation?

Activation of TRPV-1 by respiratory pathogens, triggers the release of pro-inflammatory neuropeptides including substance P (SP), cytokines such as IL-6, and anti-inflammatory somatostatin (SST), which mediate vascular events and local neurogenic inflammation[19]. Moreover, a recent study has demonstrated upregulation of TRPV-1 receptors, and pro-inflammatory substances with SARS-COV-2 infection, in a manner that reflects the severity of the disease[20], and this proves the important role of TRPV-1 in host-pathogen communication (i.e. binding, entry and replication of the virus)[21].

PATIENTS AND METHODS

This study was conducted on 20 healthy volunteers assigned as normal control group, and 64 PCR-confirmed COVID-19 patients, who attended Tanta Chest Hospital from March, 2022 to June, 2022. Depending on disease severity, COVID-19 patients were subdivided into 3 groups assigned as asymptomatic group: (n = 19 home isolated patients, did not need to be hospitalized), hospitalized group: (n = 24), and ICU admitted group: (n = 21, two of them eventually died of disease complications). There were no statistically significant differences between the studied groups with respect to the mean age, sex distribution or risk factors including obesity, hypertension, diabetes mellitus, and dyslipidemia, to avoid their influence on the main conclusions of the study.

Ethical approval:

All procedures performed were approved by Ethics Review Committee of the Faculty of Medicine, Al Azhar University, and were carried out in accordance with the tenets of the Declaration of Helsinki after obtaining informed consents from all participants in the study groups.

Serum samples for SP and CGRP measurement:

Peripheral venous blood was obtained, centrifuged at 3000 rpm for 10 minutes. The serum was collected in serum separator tubes and frozen at -70ºC until further analysis. Serum SP was measured using a chromatographic procedure for partial purification and sensitive enzyme immunoassay (Caymen Chemical Co., Ann Arbor, Mich.)[22]. While a commercial ELISA kit was used, to measure serum levels of CGRP (MyBioSource, San Diego, CA, USA; Cat# MBS2023906, RRID:AB_2877716)[13]. Both procedures were performed according to the manufacturer's instructions.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) version 22 (SPSS Software Inc., Chicago, IL, USA), was used to analyze the results for statistical significance. One-way analysis of variance (ANOVA) was used for calculation of significance between the studied groups followed by Tukey's range test (multiple comparison test). $\chi 2$ test of association was used to compare proportions between qualitative parameters. Quantitative data were presented as mean ± standard deviation (SD) and qualitative data were presented as frequency and percentage. Results were considered statistically significant when $P \leq 0.05$.

RESULTS

As shown in **Table 1,** no significant differences were observed between the studied population regarding the mean age or sex distribution. Also, the studied population showed no significant differences regarding the risk factors including obesity, hypertension, diabetes mellitus, and dyslipidemia. It is only noted that, the mean age and the number of cases with comorbidities were somewhat higher ($P > 0.05$) in patients with more serious disease (i.e., hospitalized and ICU admitted patients).

Table 1: Mean age, sex distribution, and clinical characteristics of the studied population

Groups / Parameters	Controls *(n = 20)*	PCR-confirmed COVID-19 Patients *(n = 64)*		
		Asymptomatic *(n = 19)*	Hospitalized *(n = 24)*	ICU admitted *(n = 21)*
Age (years)	41.03 ± 10.2	42.6 ± 2.9	44.8 ± 7.6	45.3 ± 11.2
Sex (male/female)	11 / 9 55% / 45%	10 / 9 53% / 47%	13 / 11 54% / 46%	11 / 10 52% / 48%
Obese	2 (10%)	1 (5.2%)	3 (12.5%)	4 (19%)
Hypertensive	3 (15%)	2 (10.5%)	2 (8.3%)	4 (19%)
Diabetic	2 (10%)	2 (10.5%)	4 (16.6%)	2 (9.5%)
Dyslipidemic	1 (5%)	1 (5.2%)	2 (8.3%)	3 (14%)
P-value	P > 0.05			

Data are expressed as mean ± SD or frequency (Percentage)

Table 2 shows the serum levels of SP and CGRP, in the studied groups. Our results revealed significantly higher SP levels in COVID-19 groups in comparison with the healthy controls. Furthermore, significant elevations in SP levels were observed between COVID-19 groups, due to disease severity.

On the other hand, all COVID-19 patients had significantly lower CGRP levels than healthy controls, with no significant differences among COVID-19 groups, suggesting no effect driven by disease severity.

Table 2: Serum levels of SP and CGRP, in the studied groups

Groups / Parameters	Controls *(n = 20)*	PCR-confirmed COVID-19 Patients *(n = 64)*			p-value
		Asymptomatic *(n = 19)*	Hospitalized *(n = 24)*	ICU admitted *(n = 21)*	
SP (pg/mL)	29.03 ± 3.1	43.9 ± 4.6[a]	56.2 ± 12.3[ab]	72.2± 16.3[abc]	<0.001
CGRP (pg/mL)	212.9 ± 16.8	98.4 ± 13.7[a]	91.5 ± 19.11[a]	87.1 ± 20.4[a]	<0.001

Data are expressed as mean ± SD; SP: Substance P; CGRP, Calcitonin Gene-Related Peptide; a: Significantly different from normal control group; b: Significantly different from asymptomatic group; c: Significantly different from hospitalized group.

DISCUSSION

The primary cause of death in Covid-19 patients, is the progressive respiratory failure[23], of which relatively little is known regarding its pathophysiology. In this study we investigated the changes caused by SARS-CoV-2 infection, in the serum levels of 2 neuropeptides (substance P and CGRP), expected to be implicated in the pathophysiology and inflammatory pathways of COVID-19.

In this study we have shown that, the serum substance P levels become significantly increased in COVID-19 patients, in degrees proportional to the severity of the disease. Substance P appears to be implicated in the pathologies of many viral infections, for example in encephalomyocarditis (EMCV) and RSV, it has been found to be directly related to bronchitis, myocarditis, and cardiac arrest following infection[24]. In HIV-AIDS, substance P accelerates infection and inflammation in immune cells, which can be blocked by NK-1R antagonists[25]. Substance P also have a pathogenic role in herpes simplex virus type 1and herpes simplex virus type 2[6], and also rat corona virus, and para-influenza virus 1 in rats[26].

In the immune response against COVID-19, the immune cells continue to release inflammatory mediators in an uncontrolled manner, (this is known as "cytokine storming"), which may be fatal as it causes acute respiratory distress syndrome (ARDS)[2].

Nahama *et al.* reported an elevated neuronal expression of TRPV-1 in COVID-19 patients, which is closely related to disease severity as it promotes increased levels of SP and IL-6[21]. This abnormal release of proinflammatory SP, may trigger the uncontrolled release of inflammatory mediators by the immune cells in the respiratory system, leading to cytokine storming, lung inflammation, injury, bronchoconstriction and may lead to cardiac failure in complicated cases[6]. So, it can be concluded that, targeting the SP/NK-1R or TRPV-1, may prevent or reverse this uncontrolled cytokine storming and may save COVID-19 patients.

On the other hand, our study revealed significant reduction in CGRP serum levels in COVID-19 patients, independent of disease severity. Previous studies demonstrated the important role of CGRP in viral infections. For example, with RSV infection, the respiratory expression of CGRP is reduced, leading to airway hyperresponsiveness, which improved when treated with CGRP[27].

Other studies have also demonstrated that CGRP may inhibit HIV-1 transmission[28].

One of the most serious complications of COVID-19, is the extensive pulmonary vascular occlusion[29], which may be related to the great reduction of the serum CGRP levels subsequent to SARS-CoV-2 infection, which in turn will lead to widespread pulmonary vasoconstriction, as a result of losing its potent vasodilator effect[9]. Also, CGRP is important for proper immune response against SARS-CoV-2, as it inhibits type 2 innate lymphoid cell (ILC2), responsible for lymphocyte sequestration in the lung and peripheral lymphopenia[30]. So, we can conclude that the observed reduction of CGRP levels in COVID-19 patients, may be responsible for lymphopenia and immune dysfunction.

Another critical role for CGRP in respiratory viral infections including COVID-19, is its contribution in tissue repair as it promotes bronchial, alveolar, and epithelial cells growth subsequent to damage caused by viral infection[31]. So, it may be exciting to test the CGRP as a treatment for COVID-19 patients, and to investigate whether it has any impact on the life cycle of this dreaded virus.

CONCLUSION

SP and CGRP serum levels can be measured as humoral biomarkers for SARS-COV-2 infection, to confirm the diagnosis, predict the clinical course, evaluate the prognosis, and to help in patient management.

Adjusting CGRP level may represent a novel therapeutic approach for COVID-19 patients, as it is important for proper angiogenesis, powerful immune response, and good epithelial repair.

Therapeutic approaches targeting the SP/NK-1R or TRPV-1, could modulate the inflammatory and immune pathways inSARS-COV-2 infection, leading to better COVID-19 outcomes with reduction of mortality rates.

Disclosure:

The authors declare no potential conflicts of interest with respect to this research in terms of its implementation, authorship, or publication.

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