Proinflammatory Cytokines in the Olfactory Mucosa Result in COVID-19 Induced Anosmia

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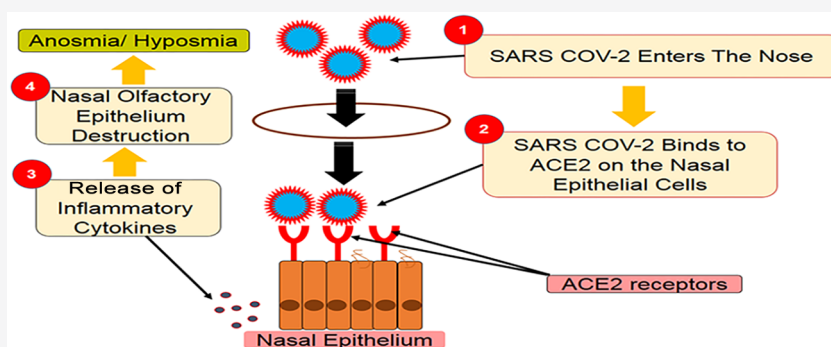
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ABSTRACT: Studies have found increased rates of dysosmia in patients with Novel Coronavirus disease 2019 (COVID-19). However, the mechanism that causes olfactory loss is unknown. The primary objective of this study was to explore local proinflammatory cytokine levels in the olfactory epithelium in patients with COVID-19. Biopsies of the olfactory epithelium were taken from patients with confirmed COVID-19 as well as uninfected controls. Levels of tumor necrosis factor α (TNF-α) and interleukin-1-beta (IL-1β) were assessed using ELISA and compared between groups. Average TNF-α levels were significantly increased in the olfactory epithelium of the COVID-19 group compared to the control group ($P < 0.05$). However, no differences in IL-1β were seen between groups. Elevated levels of the proinflammatory cytokine TNF-α were seen in the olfactory epithelium in patients with COVID-19. This suggests that direct inflammation of the olfactory epithelium could play a role in the acute olfactory loss described in many patients with COVID-19.

KEYWORDS: COVID-19, Anosmia, Inflammation, Tumor necrosis factor-alpha

Since the last days of 2019, Novel Coronavirus disease 2019 (COVID-19) has been spreading throughout the world (of 6 October 2019−11 December 2019 based on phylogenic evidence[1]), and as of this writing (17 April 2020) it has infected more than 2,000,000 individuals, caused about 140,000 deaths, and put a huge burden on the economies of nations.[2] SARS-COV-2 is not the first member of coronaviridea family to cause illness. In humans, after the severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) epidemics, this is the third one in the last two decades.[3,4]

COVID-19 patients have presented with various symptoms, from a simple fever or a mild cough to severe pneumonia and multiple organ failure (mortality rate = 2−4%).[5] According to recent evidence, sudden loss of taste or smell can also occur as a result of COVID-19 infection. Therefore, dysgeusia and anosmia might be considered as symptoms of COVID-19.[5]

To clarify the underlying cause of anosmia in COVID-19 patients, the entry pathway of this virus should be described at first. In general, coronaviruses enter the host cells via attachment of their specific glycoprotein spikes with matching host-cell receptors. These spikes involve some variable regions in their structure, named receptor-binding domain (RBD), which is responsible for direct interaction with host-cell receptors. Among members of every clade of coronaviruses, RBDs are identical; Therefore, the RBD in SARS-CoV-2 is identical to that in SARS-CoV (responsible for an outbreak in

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2003). The RBD of the SARS clade binds to angiotensin converting enzyme 2 (ACE2) on the surface of host cells.[6] As a result, theoretically, it can be concluded that tissues with high expression of ACE2 on their cell surface are more vulnerable to SARS-CoV-2. Lungs, heart, oral mucosa, kidneys, testis, and intestine are examples of high ACE2 expressing tissues.[7]

Another step to understand the mechanism of anosmia caused by SARS-CoV-2 infection is determining the pathophysiology. According to previous studies, anosmia can occur as a result of damage in three levels; (1) disturbance of olfactory receptors in oral or nasal mucosa (sensorineural olfactory loss), (2) disconnection of efferent nerves from receptors to the brain, or (3) brain damage.[8] Sensorineural olfactory loss occurs as a result of destruction of the olfactory neuroepithelium by toxic inflammatory factors (such as TNF-α and IL-1β) and infiltrating inflammatory cells that cause tissue damage.[9,10] Determining the role of these inflammatory mediators in tissue damage of olfactory neuroepithelium may explain the mechanism of COVID-19 related anosmia.

Briefly, although there is much evidence supporting the fact that anosmia occurs as a result of COVID-19, the underlying pathophysiology of this phenomenon is still unknown. In this study, we intended to determine if disturbance of the olfactory mucosa as a result of inflammation, caused by SARS-CoV-2, can explain the cause of this anosmia.

The mean level of IL-1β in the COVID-19 group was 189 pg/mL, compared to 157.5 pg/mL in the control group. However, this difference was not statistically significant (Figure 1A). The mean level of TNF-α in the COVID-19 group was
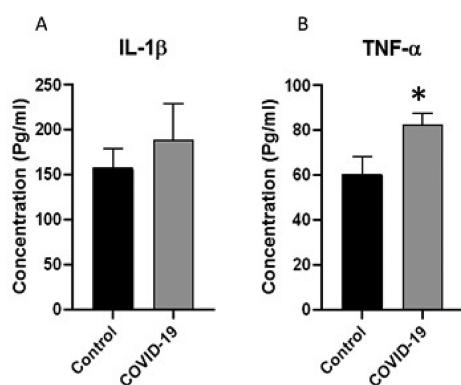

Figure 1. Concentration of inflammatory factors in the control and COVID-19 groups according to ELISA analysis. Although there is no noticeable difference between both groups in terms of the mean level of IL-1β (A), TNF-α concentration increased markedly in COVID-19 participants relative to control ones ($P < 0.05$) as indicated by an asterisk (B).

82.33 pg/mL, compared to 60.5 pg/mL in the control tissue ($P < 0.05$) (Figure 1B). There were statistically significant differences between study group and level of TNF-α in both crude ($P < 0.015$) and adjusted ($P < 0.026$) models. As shown in Table 1, the patient group showed an increase of the TNF-α concentration when adjusted for age, sex, general anesthesia, and outcome covariates. It means that the observed difference significance between the study group and serum level of TNF-α was not attenuated after adjusting for age, sex, general anesthesia, and outcome of study subjects (Table 1). The sample size was small, and the findings should be interpreted with caution.

Previously, other members of the coronaviridae family caused anosmia, so this presentation is not specific to the novel SARS-COV-2.[11,12] Neuropathology and medical imaging play a vital role in the detection of the abnormalities in cranial nerves, brain, and olfactory bulb of the patients with COVID-19 infection.[13] During the last few weeks, many patients have reported sudden hyposmia and anosmia as the concomitant symptoms of COVID-19 infection. Since the symptoms of anosmia or hyposmia were not specifically recognized in these patients, COVID-19 may not be diagnosed clearly.

There are a few studies on the appearance of olfactory dysfunction due to COVID-19,[14−18] and only one study has reported anosmia or hyposmia as a symptom of COVID-19 infection.[19] Recently, researchers from the University of California, San Francisco have used chemosensory function changes as pretest predictors for COVID-19 positivity, and they believe that relevant cohorts need to be done to refine classifier performance.[20] Moreover, a recent study considered sudden chemosensitive dysfunction as an important clinical implication.[21] If the major part of smell loss in these cases recovers within a few weeks, it can be supposed that the COVID-19 virus has led to an inflammatory reaction in the nasal cavity, which temporarily prevents odors from reaching the olfactory receptor neurons. Olfactory neuron injuries require a longer period of regrowth and regeneration of axons to produce beneficial synapses with the olfactory bulb.[22]

Angiotensin converting enzyme 2 (ACE2), which is considered as the receptor of SARS-CoV-2, may be specific to some individuals. Some investigation have proposed that some of the ACE2 variants can diminish the relationship between the SARS-CoV 2-protein and human ACE2.[23] In other words, the level of expression of ACE2 in various tissues may be vital for the outcome, sensitivity, and the symptoms of COVID-19 infection.[23] Additionally, a recent study which identifies ACE2-expressing cells (SARS-CoV-2 receptor) reveals that there are higher levels of ACE2 expressed in respiratory epithelial cells in comparison with the olfactory epithelium. Nevertheless, olfactory epithelial cells, but not olfactory receptor neurons (e.g., horizontal basal cells and supporting cells), express ACE2.[24] Virus transmission may be accompanied by other pathways, which are directly harmful for the olfactory bulb.[22] In this study, we hypothesize that the mechanism responsible for COVID-19 related anosmia is the impairment of olfactory receptors in the nasal and oral mucosa. According to a previous study, this impairment can occur as a result of inflammation, which can kill olfactory neurons.[21,25]

Table 1. Mean ± SD Values for IL-1β and TNF-α in COVID-19 Patients and Healthy Controls

	mean		adjusted mean[a]	
	case vs control	*P*-value	case vs control	*P*-value
IL-1β	189.16 ± 39.86 vs 177.50 ± 37.74	0.731	200.62 ± 18.96 vs 177.50 ± 17.88	0.44
TNF-α	82.33 ± 5.25 vs 60.50 ± 7.69	0.015[b]	83.62 ± 4.08 vs 60.50 ± 3.85	0.026[b]

[a]Adjusted mean was calculated with controlling covariates (sex, age, general anesthesia, and outcome). [b]$P < 0.05$.

Therefore, we measured IL-1β and TNF-α in the samples of three deceased patients who had COVID-19 infection.

As time goes by and overproduction of cytokines continues, olfactory neurons start to die, which can lead to histologic changes in the neuroepithelium. Notably, if production of inflammatory cytokines is stopped, the basal stem cells (within the epithelium) are able to regenerate new olfactory neurons; this process may help to regain olfactory function.[25] Altogether, this evidence implies that inflammation can lead to sensorineural anosmia. Therefore, seeking new strategies for elimination of certain inflammatory mediators can be helpful in finding an effective treatment for COVID-associated anosmia.[26]

Among all of the cytokines that are involved in inflammatory diseases of the nasal mucosa, TNF-α is one of the most powerful and relevant inflammatory mediators associated with many cell types.[27,28] Although it has been proved that TNF-α can affect olfactory neuron function, the specific mechanism of this phenomenon has not been specified yet. TNF-α receptors and mRNA for TNF-α itself are expressed in normal epithelial cells.[29] Our results showed a considerable increase in the amount of TNF-α, which was one of the inflammation factors we assessed. TNF-α exposure in olfactory tissue can be accompanied by some specific histopathological consequences. Inflammatory infiltration occurs in response to local TNF-α expression; this incident can lead to a considerable expansion of the olfactory submucosa.[25]

Another factor we assessed in our study was IL-1β. When COVID-19 infection occurs in the upper or lower respiratory tract, it may cause a wide spectrum of symptoms, from mild to highly acute respiratory syndrome, and trigger the release of proinflammatory cytokines, including interleukin IL-1β. When COVID-19 binds to the toll-like receptor (TLR), pro-IL-1β is released. Furthermore, pro-IL-1β is cleaved by caspase-1, which leads to inflammasome activation and production of active mature IL-1β. Then, active mature IL-1β can mediate fever, lung inflammation, and fibrosis. Interestingly, it has been proved that when proinflammatory IL-1 family members are suppressed, they might have therapeutic effects on many inflammatory diseases (such as viral infections);[30] our results showed an increase in the amount of IL-1β. On the other hand, the test for the existence of coronavirus in cerebrospinal fluid was negative in our study (data not shown), so we think that the chance of central nervous damage is low in anosmic patients. Although overall different studies have presented different hypotheses on the underlying mechanism involved in COVID-19 anosmia,[22,31−35] the mechanism behind the anosmia is not fully understood. The evidence from this report in line with previous studies on TNF-α and IL-1β can be taken into consideration for future strategies for control and treatment of COVID-19.

METHODS

Sample and Data Collection. This was a case series of COVID-19 patients admitted in a university affiliated major hospital in Tehran. The olfactory epithelium of three deceased patients (due to COVID-19) with anosmia was extracted. The presence of COVID-19 was confirmed by real time polymerase chain reaction (RT-PCR) as well as spiral computed tomography (CT) showing findings of pneumonia (Table 2). Using a 30° rod telescope trough, an endoscopic transnasal approach, after middle turbinate resection, the olfactory epithelium in the left nasal cavity was harvested. In the control group, the nasal mucosa was obtained by biopsy during

Table 2. Demographic and Clinical Characteristics of Subjects in the Study Groups

variables	control N	case N	P-value[a]
sex			
male/female	3/0	2/1	1.00
outcome			
alive	3		0.014
death		3	
general anesthesia			
yes	3	0	0.014
no	0	3	
	mean ± SD[b]	mean ± SD	
age	40.66 ± 3.05	46.66 ± 5.68	0.183

[a]*P < 0.05. [b]Standard deviation.

routine nasal surgery with the patient under general anesthesia. The patients were undergoing surgery for septoplasty or turbinectomy.

Laboratory Process. Enzyme-linked immunosorbent assays (ELISA) was performed on the olfactory epithelium to evaluate the IL-1β (abcam, ab46052) and TNF-α (abcam, ab181421) levels.

Ethics and Informed Consent. Ethical approval was obtained by the ethics committee of the Shahid Beheshti University of Medical Sciences IRB. All procedures, including the informed consent process, were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Statistical Analyses. All statistical analyses were performed using SSPS version 22.0 (SPSS, Inc., Chicago, IL). Qualitative data and quantitative data were illustrated by mean ± standard deviation (SD) and frequency, respectively. Furthermore, making use of a χ^2 test, categorical data was analyzed. The independent t test was carried out to assess comparisons of numeric values. In order to control covariates, we calculated adjusted mean values in both studied groups using one-way ANOVA. The continuous variables such as sex, age, general anesthesia, and outcome were considered as the covariate in variance analysis. The level of statistical significance was set at $p <$ 0.05.

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Author Contributions

A.T. and E. M. contributed equally. A.T. and E.M. had a critical role in the acquisition of data and revised the manuscript. N.A.D., A.H.B., R.A., O.R., M.H., and M.R.H had a role in the acquisition of data. M.F. and K.V. revised the manuscript. L.S. had a role in the design of the study, conceptualized it, and drafted the manuscript for intellectual content. A.A. designed and conceived study, analyzed and interpreted the data, and revised the manuscript for intellectual content.

■ ABBREVIATIONS

COVID-19, novel coronavirus disease 2019; SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome; RBD, receptor-binding domain; ACE2, angiotensin converting enzyme 2; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1-beta; RT-PCR, reverse transcription polymerase chain reaction; ELISA, enzyme-linked immunosorbent assays; TLR, toll-like receptor

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