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Vitamin D And Omega-3 Long Chain Polyunsaturated Fatty Acids Lowering COVID-19 Cytokines and Anosmia

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ABSTRACT

Background: Several studies reported the association between olfactory dysfunction and the novel coronavirus disease in 2019 (COVID-19).

Objective: This study aimed to investigate the effect of vitamin D and omega-3 long-chain polyunsaturated fatty acids on anosmia and COVID-19-induced proinflammatory cytokines.

Subjects and methods: This is a supervised randomized prospective experiment performed at the Hospital of Faculty of Medicine, Benha University. It was performed on two groups; 110 cases per group were used; one was considered healthy control and the other showed anosmia after COVID-19 infection. Evaluation of the vitamin D and omega-3 impacts on decreasing anosmia and COVID-19-induced cytokines was performed using Sniffin' Sticks test and cytokines' serum tests, respectively.

Results: A number of 110 patients from each group had persistent anosmia; the smell scores with the Sniffin' Sticks test on the initial assessment were low. The levels of IFNγ, TNFα, IL4, IL6, and IL10 cytokines and CRP were higher in both groups than in the healthy control group ($p < 0.05$). On the 10th, 20th, and 30th days, there were improvements in the smell scores and decrease in the IFNγ, TNFα, IL4, IL6, and IL10 cytokines and CRP in the treatment group than in the control group ($p < 0.05$). The duration of recovering the sense of smell was shorter in the treatment group than in the control group (24.1 ± 1.35 days vs. 28.9 ± 0.93, $p < 0.05$).

Conclusion: Vitamin D and omega-3 long-chain polyunsaturated fatty acids may decrease the exaggerated cytokines and improve olfaction after COVID-19 infection.

Keywords: COVID-19, Anosmia, Cytokines, Vitamin D, Omega-3 long chain polyunsaturated fatty acids.

INTRODUCTION

In December 2019, an infectious outbreak started in Wuhan, Hubei, China. The novel etiology for this outbreak is the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is an enveloped RNA betacoronavirus and is responsible for coronavirus disease 2019 (COVID-19) [(1)]. Globally, as of June 16, 2021, there had been 176, 156, 662 confirmed COVID-19 cases, including 3, 815, 486 deaths [(2)]. Within the last two decades, COVID-19 in humans is considered the third pandemic after SARS-CoV in 2003 and the Middle East respiratory syndrome [(3,4)]. According to recent updates from the British Association of Otorhinolaryngology-Head and Neck Surgery and the European Rhinologic Society, sudden loss of smell might occur after COVID-19 infection. Indeed, anosmia is considered one of the COVID-19 symptoms [(5)].

Based on previous reports, anosmia can result from damage in the following three stages: 1) disturbance of oral or nasal mucosal olfactory receptors, which results in sensorineural loss of olfaction; 2) disconnection between the brain and afferent nerves from the receptors; and 3) damage to the brain tissue [(6)].

The COVID-19 infection stimulates innate and acquired immunity, which can recognize pathogen-associated
molecular patterns and viral antigens, respectively. Exaggerated cytokine production by activated immunologic cells leads to cytokine storm, which is an acute and severe systemic inflammatory response that can result in tissue injury and an unfavorable prognosis. With cytokine storm, high serum levels of TNFα, IFN-γ, IL2, IL4, IL6, IL-10, and CRP are detected [(7)]. COVID-19 is one of many viruses that can cause olfactory loss. A proposed explanation is the viral invasion of the olfactory system by neurotropic viruses [(8,9)].

Recently, some nutrients were suggested to play potential roles in the management of cytokine storm; these include vitamins B6, B12, C, D, E, and folate and trace elements, such as copper, iron, magnesium, selenium, and zinc [(10)]. In addition, long-chain polyunsaturated fatty acids (LC-PUFAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), have been considered noteworthy because of their direct effect on the host response to viral infectious diseases [(10,11)]. The improvement of olfactory function in patients after supplementation of omega-3 fatty acids was due to neuronal regeneration

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rather than the direct anti-inflammatory effect on the olfactory mucosa [12].

Low vitamin D levels in the blood have been linked to olfactory impairment. Vitamin D replacement therapy can help patients suffering from its deficiency and enhance their olfactory function [13]. The regulatory role of vitamin D had been confirmed to boost the innate immune response, resulting in reduced viral load, and decreased over-activation of the acquired immune response which suppressed cytokine production [14,15]. Vitamin D was suggested to play a role in reducing cytokine storm during the influenza pandemic from 1918 to 1919 and was reported to promote the immune response to various respiratory tract infections, such as influenza and coronaviruses [16,17].

Through its receptors, vitamin D delivers its protection by regulating the immune system to lower the secretion of proinflammatory cytokines [15].

Recent data have shown a strong correlation between excessive cytokine production and the severity of COVID-19 [18]. Based on this ability of vitamin D to suppress cytokine production [19,20], we focused on vitamin D deficiency and its association with severe COVID-19. Determining the role of these inflammatory mediators on olfactory neuroepithelium damage may explain the mechanism of COVID-19-related anosmia.

This study aimed to evaluate the protective effect of vitamin D and LC-PUFAs, such as DHA and EPA, in decreasing the inflammatory mediators and ameliorating COVID-19 infection-induced anosmia.

SUBJECTS AND METHODS

Study design and patients

This was a supervised randomized prospective study performed at the Hospital of Faculty of Medicine, Benha University from September 2020 to December 2020. The study was conducted on cases that recently recovered from COVID-19 and had symptoms of hyposmia or anosmia. COVID-19 infection was confirmed by positive results of real-time reverse transcription polymerase chain reaction (rRT-PCR) on the patient's nasopharyngeal swab. Complete recovery of cases was defined as negative rRT-PCR on two consecutive tests.

The inclusion criteria were adults ≥18 years old, recently infected and recovered from COVID-19, and negative results of two consecutive rRT-PCR tests. The exclusion criteria were pregnancy, presence of olfactory dysfunction before the pandemic according to the case history, admission to the intensive care unit during the study period, no regular follow-up, intake of vitamin D or omega-3 LC-PUFAs during the treatment course, and mild hyposmia with high smell scores.

Preliminary evaluation and assessment

Information on sex, age, isolation place, the severity of COVID-19, and duration of anosmia was collected. According to the WHO 2021 [21], the COVID-19 disease severity classification was as follows; the mild degree when patients are symptomatic without evidence of viral pneumonia or hypoxia, the moderate degree when cases express clinical signs of non-severe pneumonia with SpO_2 equals 90% in the room air, and the severe degree when cases show clinical evidence of severe pneumonia, SpO_2 less than 90% in the room air, a respiratory rate greater than 30 breaths per minute, and acute respiratory distress.

Moreover, the presence of comorbidities, such as hypertension, chronic lung disease, chronic kidney disease, and diabetes mellitus was recorded. Suitable protective measures were conducted during the process of clinical examination to avoid infection.

Olfactory assessment

It was conducted using the olfactory Sniffin' Sticks test. A total of 16 odorants in filled pens were introduced to the patients; each pen was positioned about 2 cm in front of the nostrils for 2–3 treatments for the patients to sniff. The examinees were asked to choose a solution from a list of four descriptors. A score of one was given for each correct answer; the test identification score ranged from 0 to 16; ≥12 represented normal olfactory function, 9–11 represented hyposmia, and ≤8 represented anosmia.

The olfactory scores were evaluated upon initial assessment and after the 10^{th}, 20^{th}, and 30^{th} days [22], and the participants were returned to the clinic for the last three assessments. They were compensated by providing them with a sum of money that covered the fees of transportation and parking, one meal, and the vitamin D and omega-3 doses. The regimens of vitamin D and omega-3 doses during the study were listed on printed cards that were given to participants and they were informed to mark in front the taken doses throughout the study days. During each visit, the card of every participant was revised to confirm that patients in the treatment arm had followed the planned doses of vitamin D and omega-3.

Treatment regimen

According to the recent data from Egypt [5], the rate of persistent anosmia was estimated at 40/50 (80%) of COVID-19 positive cases, for the power calculation we used the free online power and sample size calculator available at (https://www.calculator.net/sample-size-calculator.html?type=1&cl=95&ci=5&pp=80&ps=113&x=62&y=15) to compute the minimum number of required samples to meet the needed statistical constraints. Hence, we found that a population size of 113 cases and 78 or more measurements were needed to have a confidence level of 95%.

The patients were assigned randomly into two groups of 113 patients. The first group was considered the control one that received standard COVID-19 treatment. Whereas the second one was the treatment group that was supplemented with vitamin D with a dose of 15 mcg/day, its concentration was 600 IU, and omega-3 LC-PUFAs such as DHA and EPA with a dose of 1.2 g/day during the standard COVID-19 treatment course. On the first day of signs, the (rRT-PCR) confirmed the positive infection with COVID-19. While, on the third and fifth days of signs the (rRT-PCR) was repeated and assured the first and the second negative result of COVID-19 infection, respectively. Based on these results, the study was started on the fifth day of signs, which is considered day zero.

Collection of blood samples and laboratory examination

Blood (5 mL) was collected from the patients in both groups (33 cases each, with persistent anosmia) and placed into a Vacutainer tube on the fifth day of COVID-19 infection. Moreover, blood samples were collected from 33 healthy control cases without any signs of COVID-19 from the same governorate. The healthy control cases were tested using the olfactory Sniffin' Sticks test and proved no olfactory disorders. Furthermore, this healthy control group was implemented in this study to elucidate the difference between inflammatory cytokines and CRP on the initial assessment and on the 10^{th}, 20^{th}, and 30^{th} days after the initial evaluation. The blood samples were collected on the first day of examination (initial sample) and then after the 10^{th}, 20^{th}, and 30^{th} days.

The serum was separated by centrifugation of the blood samples at 2000 rpm for 20 minutes. The concentrations (pg/mL) of serum cytokines IFNγ, TNFα, IL4, IL6, and IL10 were determined using the BD FACSCalibur™ Flow Cytometer, BD Biosciences, CA, and human kits for Th1/Th2 cytokines (Ceger, Hangzhou, China), according to the manufacturer's instructions. Internal control of the recombinant protein for each cytokine was included. The BD FACS Calibur Flow Cytometer was used for cytokine detection at a range of 2.5 to 5000 pg/mL. The CRP concentration (mg/L) was measured using the i-CHROMA immunofluorescence analyzer and related kits (i-CHROMA Reader, Boditech Med Inc., Korea). The BD FACSCalibur™ Flow Cytometer, BD Biosciences, CA, and the i-CHROMA Reader, Boditech Med Inc., Korea machines were present at the Department of Clinical Pathology, Faculty of Medicine.

Ethical approval:

The patients signed written informed consent to take part in the study. The research was conducted in line with the 1975 Helsinki declaration and its regulations. The Research Ethics Committee of the Faculty of Medicine has approved the research protocol (approval number RC4.7.2021).

Statistical analysis

The obtained data were coded, processed, and analysed with SPSS V. 22 for Windows®. The Shapiro Wilk test was used to determine whether the data had a normal distribution. The qualitative data were reported as frequencies and relative percentages. The Chi square test (X^2) was used to calculate the difference between qualitative variables. The quantitative results were presented as mean±standard deviation (SD). The t-test for independent samples was developed to compare two independent groups of regularly distributed variables (parametric data). P value of 0.05 was judged significant.

RESULTS

A total of 226 patients were assigned randomly into two groups; 3 cases from each group were excluded because they died (Table 1). The 220 patients investigated had an age range of 18 to 40 years in the control group and 18 to 45 years in the treatment group. A total of 141 (62.4%) cases were men, and 79 (34.9%) were women. There was a significant difference between the 2 groups regarding grading of COVID-19 illness. The patients that showed mild COVID-19 signs were isolated in their homes; on the other hand, while cases with moderate and severe COVID-19 signs were isolated at the hospital. Regarding comorbidities, the difference between the 2 groups was insignificant except for chronic kidney disease. The degrees of olfactory loss in the control and the treatment groups are in an analogous manner (Table 1). On initial evaluation, the smell scores were worsened in both groups, without any significant difference. After 10, 20, and 30 days, the smell scores were significantly higher in the treatment group than in the control group. In addition, symptom duration was significantly shorter in the treatment group than in the control group (Tables 1 and 2; Figure 1).

Table (1): A detailed description of the investigated groups

Characteristics	Total (226)	Group I (Control, 113)	Group II (Treatment, 113)	*p*-value
Age: Range	-	18–40	18–45	-
Mean	-	29.5	29.55	0.0887
Sex	-			-
Male	141(62.4%)	73 (64.6%)	68 (60.2%)	-
Female	79 (34.9%)	37 (32.7%)	42(37.2%)	-
Dead	6 (2.7%)	3 (2.7%)	3(2.7%)	-
Grading of COVID-19 illness				
Mild	175 (79.5%)	85 (77.3%)	90 (81.8%)	<0.0001
Moderate	31 (14.1%)	21 (19.1%)	10 (9.1%)	<0.0001
Severe	14 (6.4%)	4 (3.6%)	10 (9.1%)	<0.0001
Isolation site				
Home	175 (79.5%)	85 (77.3%)	90 (81.8%)	-
Hospital	45 (20.5%)	25 (22.7%)	20 (18.2%)	-
Comorbidities				
Hypertension	20 (9.1%)	10 (9.1%)	10 (9.1%)	1.0000
Chronic lung disease	11 (5%)	5 (4.5%)	6 (5.5%)	0.08
Chronic kidney disease	13 (5.9%)	6 (5.5%)	7 (6.4%)	0.05
Diabetes mellitus	14 (6.4%)	7(6.4%)	7(6.4%)	1.0000
Olfactory disorders				
None	64 (29.1%)	32 (29.1%)	32 (29.1%)	1.0000
Mild (hyposmia)	60 (27.3%)	30 (27.3%)	30 (27.3%)	1.0000
Moderate (anosmia)	60 (27.3%)	30 (27.3%)	30 (27.3%)	1.0000
Poor (anosmia)	36 (16.4%)	18 (16.4%)	18 (16.4%)	1.0000
The day of the initial onset of olfactory disorders during COVID-19 infection		4^{th} day	4^{th} day	
The smell score: mean (Range)				
Initial score		1.45 (1–2)	1.36 (1–2)	0.97
10^{th} day		3.6 (2–5)	4.5 (2–7)	<0.0001
20^{th} day		7.1 (6–9)	11.6 (8–14)	<0.0001
30^{th} day		11.75 (10–14)	15.7 (15–16)	<0.0001
Duration of anosmia till recovered smell in days (Mean±SD)		28.9±0.93	24.1±1.35	<0.0001

Table (2): The results of smell scores and recovered smell.

Type of group	Time of analysis				Period of recovered smell (days)
	Initial	10^{th} day	20^{th} day	30^{th} day	
First group smell score (Mean±SD)	1.45±0.5	3.6±0.1	7.1±1.1	11.75±1.3	28.9±0.93
Treatment group smell score (Mean±SD)	1.36±0.18	4.5±1.6	11.6±2.01	15.7±0.46	24.1±1.35
p value	=0.4584	=0.0079	<0.0001	<0.0001	<0.0001

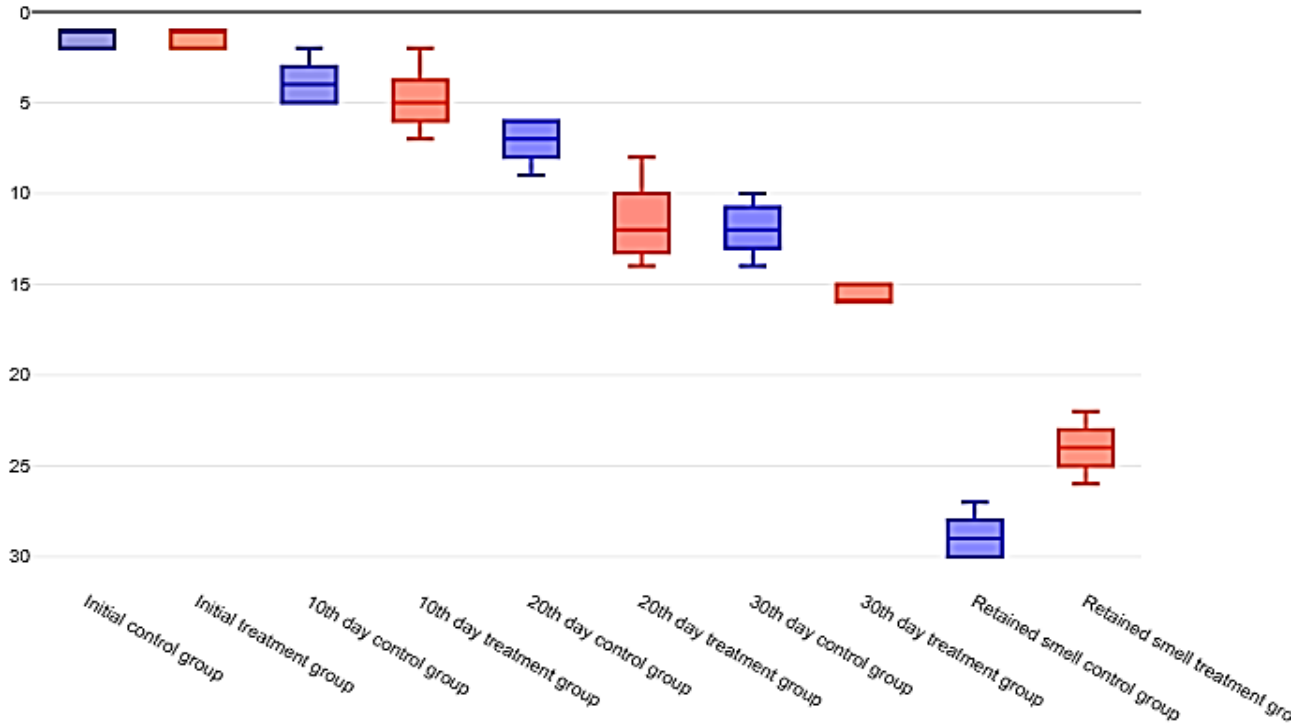

Figure (1): Results of anosmia and recovered smell in the control and the treatment groups

Inflammatory cytokines and CRP on initial assessment

The serum levels of IFNγ, TNFα, IL4, IL6, IL10, and CRP were significantly higher in patients with COVID-19 than in the healthy control cases. Upon initial assessment, the levels of IFNγ, TNFα, IL4, IL6, and IL10 were higher by 62%, 56%, 75%, 54%, and 68%, respectively, in the control group and by 63%, 59%, 75%, 57%, and 67%, respectively, in the treatment group, compared with the levels in the healthy control cases. In addition, the CRP levels were higher by 10-fold in the control and treatment groups than in the healthy control cases.

Inflammatory cytokines and CRP on days 10, 20, and 30 after initial evaluation

On days 10, 20, and 30 after initial evaluation, the levels of cytokines IFNγ, TNFα, IL4, IL6, and IL10 and the CRP were noticeably higher in the control group but lower in the treatment group. Furthermore, the levels of inflammatory cytokines and CRP significantly differed between the two groups (Tables 3, 4, and 5).

Table (3): Results of cytokine and CRP measurements in the control group

Type of cytokine	Time of screening				
	Healthy control	Initial assessment	10th day	20th day	30th day
IFNγ (pg/mL)	2.68±0.16	4.26±0.34	5.24±0.49	4.78±0.44	4.34±0.39
TNFα (pg/mL)	2.6±0.33	4.68±0.75	5.69±0.43	5.35±0.39	5.02±0.42
IL4 (pg/mL)	2.87±0.31	3.76±0.27	4.31±0.25	4.5±0.23	3.91±0.18
IL6 (pg/mL)	5.04±0.71	9.19±1.4	12.33±2.1	12.5±2.1	8.38±0.44
IL10 (pg/mL)	4.19±0.52	6.1±0.55	7.24±0.51	7.42±0.35	6.22±0.17
CRP (mg/L)	0.49±0.05	9.12±1.7	18.67±2.3	25.14±2.01	16.9±0.6

Table (4): Results of cytokine and CRP measurements in the treatment group

Type of cytokine	Time of screening				
	Healthy control	Initial assessment	10th day	20th day	30th day
IFNγ (pg/mL)	2.68±0.16	4.1±0.38	4.5±0.51	3.9±0.39	3.38±0.28
TNFα (pg/mL)	2.6±0.33	4.46±0.73	4.82±0.69	4.32±0.61	3.43±0.29
IL4 (pg/mL)	2.87±0.31	3.78±0.24	3.99±0.24	3.52±0.23	3.18±0.19
IL6 (pg/mL)	5.04±0.71	8.78±1.5	11.01±2.2	11.85±2.19	7.51±0.54
IL10 (pg/mL)	4.19±0.52	6.16±0.55	6.61±0.44	6.89±0.41	5.38±0.21
CRP (mg/L)	0.49±0.05	9.25±1.6	13.97±0.98	22.92±3.1	11.17±0.66

Table (5): Comparison of the cytokine and CRP levels among groups

Type of cytokine	Healthy control	Control group				Treatment group			
		Initial assessment	10th day	20th day	30th day	Initial assessment	10th day	20th day	30th day
IFNγ (pg/mL)	<0.0001	0.1799	<0.0001	<0.0001	<0.0001	0.1799	<0.0001	<0.0001	<0.0001
TNFα (pg/mL)	<0.0001	0.3625	<0.0001	<0.0001	<0.0001	0.3625	<0.0001	<0.0001	<0.0001
IL4 (pg/mL)	<0.0001	0.7902	<0.0001	<0.0001	<0.0001	0.7902	<0.0001	<0.0001	<0.0001
IL6 (pg/mL)	<0.0001	0.3265	0.0368	0.037	<0.0001	0.3265	0.0368	0.037	<0.0001
IL10 (pg/mL)	<0.0001	0.7064	<0.0001	<0.0001	<0.0001	0.7064	<0.0001	<.0001	<0.0001
CRP (mg/L)	<0.0001	0.7874	<0.0001	0.0029	<0.0001	0.7874	<0.0001	0.0029	<0.0001

DISCUSSION

The olfactory system has long been essential for the ability of individuals to sense their internal and external environment. The sense of smell is vital for humans in several aspects, including ingestion, social communication, and evasion of environmental hazards [23]. Several cross-sectional studies have elucidated that 33.9% to 68% of patients with COVID-19 showed olfactory dysfunction; these patients usually have both anosmia and dysgeusia [24]. This vast range could be attributable to racial differences, sample size, patient age, hospitalization, the existence of additional symptoms and comorbidities, and the disease severity [25]. Upper airway viral infections had been well established to result in sensorineural olfactory changes due to damage to the sensory olfactory epithelium. Functional dysfunction of the olfactory and gustatory systems has been identified as a hallmark and may be a significant predictor of the clinical outcome of COVID-19. One study confirmed that noticeable anosmia was caused by COVID-19 [26]. The sensorineural olfactory loss from damage to the olfactory neuroepithelium results from the toxic inflammatory mediators, such as IL-1β and TNFα, and infiltration of inflammatory cells [27].

In this study, 33 of 78 (42.3%) cases expressed persistent anosmia for up to 30 days even after recovery from COVID-19. Similarly, **Otte *et al.*** [28] reported that 50% of patients had persistent olfactory dysfunction seven weeks after the start of COVID-19 symptoms and despite complete recovery. Like the results of **Abdelalim *et al.*** [5] **and Santos *et al.*** [29]**,** our results showed that the onset of anosmia was after four days of COVID-19 infection and that the smell scores on the Sniffin' Sticks test were low. This result comes consistent with **Babaei *et al.*** [30], who confirmed that anosmia was experienced as a first symptom after COVID-19 infection. In the current study, the age of cases was near 30 years, which was like **Babaei *et al.*** [30], who reported that the risk of olfactory dysfunction was more prevalent in younger cases than in older ones. The explanation for this fact could be that elderly people have more comorbidities and olfactory impairment that may not be noticed. In line with the earlier investigation of **Babaei *et al.*** [30], most of the patients in this study were men. On the other hand, our result comes in contradiction with **Abdelalim *et al.*** [5], who found that females are more prone to COVID19-caused anosmia.

Our results showed significantly higher levels of IFNγ, TNFα, IL4, IL6, IL10, and CRP levels on initial assessment in patients with COVID-19 than in the control group with healthy cases. **Han *et al.*** [7] reported that COVID-19 infection resulted in a

cytokine storm caused by exaggerated and excessive secretion of cytokines. This cytokine storm correlated directly with the tissue injury caused by the infection. The selected serum inflammatory markers IFNγ, TNFα, IL4, IL6, IL10, and CRP were chosen because all of them show increased levels in COIVD-19 patients compared with non-infected cases, and some of them as IL6 and IL10 are predictors of disease severity [(7)]. Moreover, some of them are found to be increased during COVID-19-associated anosmia as TNFα, IL6, IL10, and IFNγ [(31)].

The higher cytokine levels resulted from the stimulation of innate and acquired immune responses that activate several immunologic cells [(32,33)]. During sepsis and acute organ injury, pleiotropic cytokines are generated at sites of tissue inflammation and are released into the bloodstream by many different cell types, including macrophages, lymphocytes, endothelial cells, epithelial cells, and fibroblasts [(34)]. The elevated cytokine and CRP levels in the infected patients lasted for more than 21 days. Moreover, we noticed periodical regulation of cytokine and CRP levels along with the disease progression in the control group of patients who received standard COVID-19 treatment. Similarly, **Han *et al.*** [(7)] reported that cytokines were indicators of COVID-19 severity and that some cytokines, such as IL6 and IL10, can be used as predictors for fast detection of severe disease. Our results showed that the cytokine and CRP levels were lower in the treatment group compared with the control group.

Vitamin D has been reported to have a regulatory role on the immune system as it is considered an important vitamin in lowering the proinflammatory cytokines and cytokine storm [(20)]. Moreover, vitamin D replacement therapy can improve olfactory function in patients with vitamin D deficiency [(13)]. In addition, **Weill *et al.*** [(35)], suggested that omega-3 LC-PUFAs are precursors of specialized pro-resolving lipid mediators and could aid in the establishment of the inflammatory equilibrium, thereby, reducing the severity and duration of the crucial inflammatory phase. Moreover, omega-3 LC-PUFAs can interact with viruses at various stages of infection, especially during entry and replication. Furthermore, omega-3 supplementation may improve olfaction through neuronal regeneration [(12)].

In this study, the smell scores on days 10, 20, and 30 were significantly higher in the treatment group than in the control group. Moreover, the duration of recovering smell was significantly shorter in the treatment group than in the control group. The rational interpretation of these results is the positive impact of vitamin D and omega-3 LC-PUFAs supplementation on olfactory system dysfunction and anosmia would include a decrease in excessive cytokine production, inflammatory resolution, neuronal regeneration, and less virus entry and replication [(35)]. Although the magnitude of difference observed among the control and treatment groups was low and might affect results, the authors couched their results with a bit more caution because this is a small study in a homogenous population. But the clinical findings and the effective therapy are important and could be confirmed in a larger sample. Furthermore, these results will be beneficial in directing the attention of healthcare professionals to lower the irrationalized utilization of corticosteroids during COVID-19 infections that predispose to serious life-threatening fungal infections.

One of the merits of the current study was the implementation of a uniform and widely available anosmia reporting technique, which allows comparison between current and future investigations. The study's follow-up time was long, which was another added strength. Moreover, the comparison between the smell scores and serum inflammatory markers IFNγ, TNFα, IL4, IL6, IL10, and CRP in the control and treatment groups in association with the recovery of normal smell rates was another merit. This study has some limitations; the first limitation was the inability to examine an extra number of cases covering different localities of Egypt. That is to confirm the gained clinically important and statistically significant findings in the larger sample. The second one was the inability to use immunological and molecular techniques to study cases that recovered smell fast to benefit from these changes in the treatment of cases suffering from persistent anosmia. The risk of COVID-19 infection was a serious limitation to the early follow-up of cases from the beginning of infection to find out some evidential clues about the onset of persistent anosmia. The subgroup analyses of patients with mild, moderate, and severe COVID-19 infection were not performed. The comparison between subjective and objective olfaction was not made. It was difficult to objectivate quantitative assessment of the smell test using the University of Pennsylvania smell identification test (UPSIT), that’s because the complete closure of many countries and the test was not available at Egypt during this period.

CONCLUSIONS

The inflammatory effects and the toxic mediators after COVID-19 infection could be the direct causes of anosmia. In this study, the association of elevated cytokines with worsened smell scores was confirmed. Standard treatment lowered the cytokine levels, which was reflected in improved smell scores. Supplementation with vitamin D and omega-3 LC-PUFAs, along with standard treatment, could have a positive impact on lowering inflammation and lowering cytokines.

This in turn lowered the disturbance of nasal mucosal olfactory receptors, which results in sensorineural improvement of olfaction; and improved the connection between the brain and afferent nerves from the receptors. These could lead to a marked

improvement in smell scores and early resolution of anosmia.

REFERENCES

1. **van Dorp L, Acman M, Richard D *et al.* (2020):** Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. Infect Genet Evol., 83:104351. doi: 10.1016/j.meegid.2020.104351.
2. **World Health Organization (2021):** WHO coronavirus disease (COVID-19) dashboard. https://covid19.who.int/.
3. **de Wit E, van Doremalen N, Falzarano D *et al.* (2016):** SARS and MERS: Recent insights into emerging coronaviruses. Nat Rev Microbiol., 14(8):523–534.
4. **Guan W, Ni Z, Hu Y *et al.* (2020):** Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med., 382:1708–1720.
5. **Abdelalim A, Mohamady A, Elsayed R *et al.* (2021):** Corticosteroid nasal spray for recovery of smell sensation in patients with COVID-19: A randomized controlled trial. Am J Otolaryngol., 42:102884.
6. **Torabi A, Mohammadbagheri E, Akbari Dilmaghani N *et al.* (2020):** Proinflammatory cytokines in the olfactory mucosa result in COVID-19-induced anosmia. ACS Chem Neurosci., 11:1909–1913.
7. **Han H, Ma Q, Li C *et al.* (2007):** Profiling serum cytokines in patients with COVID-19 reveals IL6 and IL10 are disease severity predictors. Emerg Microbes Infect., 9:1123–1130.
8. **Suzuki M, Saito K, Min W *et al.* (2007):** Identification of viruses in patients with postviral olfactory dysfunction. Laryngoscope, 117:272–277.
9. **Xydakis M, Mulligan L, Smith A *et al.* (2015):** Olfactory impairment and traumatic brain injury in blast-injured combat troops: a cohort study. Neurology, 84:1559–1567.
10. **Calder P, Carr A, Gombart A *et al.* (2020):** Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. Nutrients, 12:1181-86.
11. **Messina G, Polito R, Monda V *et al.* (2020):** Functional role of dietary intervention to improve the outcome of COVID-19: A hypothesis of work. Int J Mol Sci., 21(9):3104. doi: 10.3390/ijms21093104.
12. **Yan C, Rathor A, Krook K *et al.* (2020):** Effect of omega-3 supplementation in patients with smell dysfunction following endoscopic sellar and parasellar tumor resection: A multicenter prospective randomized controlled trial. Neurosurgery, 87(2): 91-98.
13. **Baki A, Yıldız M, Gündoğar S (2021):** Effect of vitamin D on olfactory function. B-ENT., 17(2): 86-91.
14. **Aranow C (2011):** Vitamin D and the immune system. J Investig Med., 59:881–886.
15. **Daneshkhah A, Agrawal V, Eshein A *et al.* (2020):** Evidence for possible association of vitamin D status with cytokine storm and unregulated inflammation in patients with COVID-19. Aging Clin Exp Res., 32:2141–2158.
16. **Grant W, Giovannucci E (2009):** The possible roles of solar ultraviolet-B radiation and vitamin D in reducing case-fatality rates from the 1918–1919 influenza pandemic in the United States. Derm Endocrinol., 1:215–219.
17. **Goncalves-Mendes N, Talvas J, Dualé C *et al.* (2019):** Impact of vitamin D supplementation on influenza vaccine response and immune functions in deficient elderly persons: A randomized placebo-controlled trial. Front Immunol., 10:65-69.
18. **Huang C, Wang Y, Li X *et al.* (2020):** Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet, 395(10223):497–506.
19. **Khare D, Godbole N, Pawar S *et al.* (2017):** Calcitriol [1, 25[OH]2 D3] pre- and post-treatment suppresses inflammatory response to influenza A (H1N1) infection in human lung A549 epithelial cells. Eur J Nutr., 52(4):1405–1415.
20. **Parlak E, Ertürk A, Çağ Y *et al.* (2015):** The effect of inflammatory cytokines and the level of vitamin D on prognosis in Crimean-Congo hemorrhagic fever. Int J Clin Exp Med., 8:18302–18310.
21. **World Health Organization (2021):** Living guidance for clinical management of COVID-19. https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-2
22. **Lin S, Chu S, Yuan B *et al.* (2009):** Survey of the frequency of olfactory dysfunction in Taiwan. J Chin Med Assoc., 72:68–71.
23. **Sollai G, Melis M, Mastinu M *et al.* (2021):** Olfactory function in patients with inflammatory bowel disease (IBD) is associated with their body mass index and polymorphism in the odor binding-protein (OBPIIa) gene. Nutrients, 13(2):703-7.
24. **Meng X, Deng Y, Dai Z *et al.* (2021):** COVID-19 and anosmia: A review based on up-to-date knowledge. Am J Otolaryngol., 41:102581. doi: 10.1016/j.amjoto.2020.102581.
25. **Karthikeyan P, Sivanand N, Vijayan N *et al.* (2021):** A clinical study of smell disorders in COVID- 19 patients in a tertiary care hospital in Pondicherry: a cross sectional study. Indian J Otolaryngol Head Neck Surg., 13:1- 6.
26. **Paolo G (2020):** Does COVID-19 cause permanent damage to olfactory and gustatory function? Med Hypo., 143:110086. doi: 10.1016/j.mehy.2020.110086.
27. **Sultan B, May L, Lane A (2011):** The role of TNF-α in inflammatory olfactory loss. Laryngoscope, 121:2481-2486.
28. **Otte M, Klussmann J, Luers J (2020):** Persisting olfactory dysfunction in patients after recovering from COVID-19. J Infect., 81: 58-62.
29. **Santos R, da Silva M, do Monte Silva M *et al.* (2021):** Onset and duration of symptoms of loss of smell/taste in patients with COVID-19: A systematic review. Am J Otolaryngol., 42:102889. doi: 10.1016/j.amjoto.2020.102889.
30. **Babaei A, Iravani K, Malekpour B *et al.* (2021):** Factors associated with anosmia recovery rate in COVID-19 patients. Laryngoscope Investig Otolaryngol., 6(6):1248-1255.
31. **Gori A, Leone F, Loffredo L *et al.* (2020):** COVID-19-related anosmia: The olfactory pathway hypothesis and early intervention. Front Neurol., 11:956. doi: 10.3389/fneur.2020.00956
32. **Ferrero-Miliani L, Nielsen O, Andersen P *et al.* (2007):** Chronic inflammation: Importance of NOD2 and NALP3 in interleukin-1beta generation. Clin Exp Immunol., 147:227–235.
33. **Takeuchi O, Akira S (2009):** Innate immunity to virus infection. Immunol Rev., 227:75–86.
34. **Gadient R, Patterson P (1999):** Leukemia inhibitory factor, interleukin 6, and other cytokines using the GP130 transducing receptor: Roles in inflammation and injury. Stem Cells, 17:127–137.
35. **Weill P, Plissonneau C, Legrand P *et al.* (2020):** May omega-3 fatty acid dietary supplementation help reduce severe complications in Covid-19 patients? Biochimie., 179:275–280.