Altered expression levels of TAS1R2 and TAS1R3 genes among SARS-CoV-2 variants of concerns

Qendresa Hoti
Near East University Faculty of Medicine: Yakin Dogu Universitesi Tip Fakultesi
Gokce Akan
Near East University: Yakin Dogu Universitesi
Gulten Tuncel
Near East University: Yakin Dogu Universitesi
Emine Unal Evren
Girne Universitesi
Hakan Evren
Girne Üniversitesi: Girne Universitesi
Kaya Suer
Near East University Faculty of Medicine: Yakin Dogu Universitesi Tip Fakultesi
Tamer Sanlidag
Near East University: Yakin Dogu Universitesi
Mahmut Cerkez Ergoren (mahmutcerkez.ergoren@neu.edu.tr)
Near East University Faculty of Medicine: Yakin Dogu Universitesi Tip Fakultesi https://orcid.org/0000-0001-9593-9325

Research Article

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Abstract

Background

The most common symptoms of coronavirus infections are fever, cough, shortness of breath, headache, ache of joints, a loss of smell and loss of taste, and etc. Early studies suggested that smell and taste receptors were associated with pathogenic detection and immunity. Thus, we aimed to evaluate the expression profile of gene receptors that are related to taste, smell, and appetite control in COVID-19 patients and their putative correlation with SARS-CoV-19 variants.

Method

Gene expression levels of *TAS1R2, TAS1R3, TAS2R38, OR51E1, LEPR, GHRL* were analyzed in 100 COVID-19 patients and 100 SARS-CoV-2 RT-qPCR negative group.

Results

The expression levels of *TAS1R2* and *TAS1R3* genes were significantly decreased in COVID-19 patients who were infected with Delta variant. However, the *TAS2R38* gene expression level was significantly lower when compared to the control group. The *TAS1R2* gene expression was positively correlated with *TAS1R3*, and *TAS2R38 genes* ($p = 0.001$, $p = 0.025$, respectively).

Conclusion

TAS1R2, *TAS1R3*, and *TAS2R38* gene expression levels were decreased in the Delta variant compared to the Omicron BA.1 variant in the studied groups. These results provided a significant clue for the temporary taste loss, especially in patients infected with the Delta variant, which is the most disruptive and symptomatic variant causing hospitalizations, and deaths compared to other variants may be because ACE2 is expressed in the taste buds and high replication of SARS-CoV-2 in the infected gustatory cells in the taste bud generates inflammation and then could eventually destroy the cells. This gustatory cell damage may cause malfunction of the gustatory system.

Introduction

A cluster of viral pneumonia cases associated with a novel coronavirus (2019-nCoV), the disease was subsequently named Coronavirus Disease-2019 (COVID-19), upon the spread of cases on March 11th, 2020, by the World Health Organization WHO the situation was categorized as a pandemic (Barham et al., 2021, Taha et al., 2021, Cucinotta & Vanelli, 2020).

The most know common symptoms of COVID-19 include fever, cough, dyspnoea, sore throat, headache, myalgia, rhinorrhea, diarrhea, and many professional organizations recognized other symptoms as well such as olfactory and taste dysfunction (OTD) as symptoms of COVID-19 and included them in their diagnostic guidelines (Jain et al., 2021).

A common sequela after the infection is the loss of smell sense; as many as 68% of patients who experience the COVID-19 infection have a loss of smell (anosmia), and many others manifest loss of taste (hypogeusia and ageusia). These symptoms are usually temporary and might manifest in absence of any other clinical symptoms but a chronic loss of smell and taste is occurring in many patients (Henkin et al., 2021, Høier et al., 2021).

The role of the taste system and its taste receptors is not restricted to driving food preferences to taste sensory, it is much more, and it has been proved to play important role in analyzing the chemical composition of food before ingestion and in immunity and inflammatory processes (Behrens et al., 2019).

The sense of taste, smell, and chemesthesis are involved in flavor perception. For the sense of taste activation of gustatory receptors on the tongue is required, which receive innervation from cranial nerves and recognize the main five tastes; bitter, sweet, sour, salty, and umami. The gustatory indicate and combined with sensations provided by retronasal olfaction give rise to flavors. Then the chemesthesis takes part to percept different food flavors (Mastrangelo et al., 2021).

Taste buds, which are mostly found on the tongue, soft palate, and epiglottis in animals, detect soluble tasting compounds through 2.000–5.000 taste buds (Briand & Salles, 2016). Taste buds have specialized taste receptor cells (TRCs) that express unique taste receptors which sense the presence of food in the salivary. The taste buds innervate three kinds of nerves, the chorda tympani, the vagus, and glossopharyngeal nerves, which transmit taste information to the brain (Meunier et al., 2021).

Evidence shows that many viruses produce temporary changes in odor perception due to inflammatory responses. Previous coronavirus infections (parainfluenza, rhinovirus, SARS, and others) have been linked to a loss of smell (hyposmia or anosmia), but this was a rare event (Hwang, 2006, Beltrán-Corbellini et al., 2020). While in COVID-19 patients it was reported acute and post-acute phases of the changes in sensory perception, appetite, and, food-related pleasure (Høier et al., 2021).

The sweet taste receptor genes *TAS1R2* and *TAS1R3* as well as the bitter receptor gene *TAS2s* are expressed on non-ciliated solitary chemosensory cells (SCCs) epithelial cells (Maina et al., 2018). The bitter taste receptors, *TAS2Rs*, assure defense responses, particularly for ciliated sinonasal epithelial cells in the initial layer of upper airway defense (Maina et al., 2018), which associates with SARS-CoV-2, destabilizing immune responses (Aoe, 2020). Evidence suggests that a specific bitter taste receptor the *TAS2R38* gene is involved in the upper airway innate immune defense and that variability in this gene contributes to differences in susceptibility to respiratory infection (Lee et al., 2012). From recent studies,

the bitter taste receptors (T2Rs) appear to be associated with the clinical manifestation and duration of symptoms of SARS-CoV-2 (Barham et al., 2021).

The olfactory receptor family 51 subfamily E member 1 (*OR51E1)* gene belongs to the large G protein-coupled receptor family (GPCRs). This receptor interacts with odorant molecules in the nose, triggering the neuronal response that causes smell perception, and they are expressed in the nasal epithelium (Pronin & Slepak, 2021). Moreover, to mediate the odorant detection the olfactory receptors are functionalized (Mombaerts, 2004).

Growth hormone secretagogue receptor ligand (*GHRL*) earlier was identified as a peptide appetite hormone that is mainly secreted from gastrointestinal cells, it has an important function for appetite stimulation and energy homeostasis (Müller et al., 2015). The *GHRL* gene effects are modulated by growth hormone secretagogue receptors (GHS-R) which are part of the large family of (GPCRs) (Müller et al., 2015). *GHRL* is expressed in different tissues, it was detected in saliva, tooth germ cells and tissues, mesenchymal cells, odontoblast, and other specific cells (Groschl et al., 2005).

The protein that binds to leptin is known as the leptin receptor (*LEPR)* gene is a receptor for the hormone leptin also known as the starvation hormone (Viesti et al., 2014) that belongs to the class I cytokine superfamily (Guglielmi et al., 2021), which is secreted by adiposities, and it controls body weight as well as plays a role in fat metabolism and the hematological system (Viesti et al., 2014).

There are different known variants of SARS-CoV-2 that have been named alphabetically by the World Health Organization WHO (Duong, 2021). The highly transmitted Delta variant (B.1.617.2) was firstly known in India, that became the most predominant variant around the world, the Omicron (B.1.1.529) had been quickly replaced Delta as the most frequent SARS-CoV-2 variant (Del Rio et al., 2022) and the Omicron variant has been prominent variant since firstly detected on December 2021.

The symptom of loss of smell and taste sense was found to be less common in the Omicron variant compared to the Delta variant, while the sore throat and hoarse voice are more common in the omicron compared to the Delta variant (Wise, 2022).

In light of the reports of COVID-19 patient's phases in acute and post-acute olfactory, and gustatory dysfunction, these changes are related to sensory perception, appetite, and food-related pleasure (Høier et al., 2021, Kanjanaumporn et al., 2020), hence it is important to analyze gene expression profiles that are related to these symptoms to fruitful understanding and disease progression.

In the current study, the association between the gene expression profiles of *TAS1R2, TAS1R3, TAS2R38, OR51E1, LEPR*, and *GHRL* genes that are related to gustatory, olfactory, and, appetite receptors in COVID-19 patients and SARS-CoV-19 Delta and Omicron variants in Northern Cyprus were aimed to investigate for the first time.

Materials And Methods

Demographics of studied subjects

The nasopharyngeal swabs obtained from individuals who attended the Near East Hospital for the SARS-CoV-2 RT-qPCR (UNIPLEX SARS-CoV-2 RT-qPCR Detection Kit, IKAS MEDICAL, Nicosia, Northern Cyprus) test whereas used for a golden standard technique for COVID-19 disease detection. 100 individuals who were positive for SARS-CoV-2 RT-qPCR (50 SARS-CoV-2 Delta, 50 Omicron BA.1 variants) and 100 individuals with SARS-CoV-2 RT-qPCR negative test results were included in this study. All results were confirmed twice for the result verification.

Identification of SARS-CoV-2 Variants of Concern (VoCs) by mutation detection

Variant analysis of SARS-CoV-2 RT-qPCR positive patients was performed with the Multiplex SARS-CoV-2 VOC RT-qPCR detection kit (IKAS MEDICAL, Nicosia, Northern Cyprus). The specimens were screened for H69-70 deletion, N501Y, K417N, T478K, Y144del, and P681R mutations to differentiate the SARS-CoV-2 VoCs between Delta (B.1.617.2) and Omicron (B.1.1.529) using manufacturer's guidelines.

We considered specimens positive for the T478K and P681R mutations and negative for the H69-70 deletion, N501Y, K417N, and Y144del mutations as Delta. Specimens positive for the H69-70 deletion, N501Y, T478K, K417N, and Y144del mutations and negative for the P681R mutation were classified as Omicron BA.1 according to the manufacturer's instructions. Whole-genome sequencing has been performed on the specimens to confirm the VoCs which were detected (GISAID reference numbers EPI_ISL_12574367, EPI_ISL_12574374, EPI_ISL_12574370, EPI_ISL_12574375, EPI_ISL_12574368, EPI_ISL_12574373, EPI_ISL_12574369, EPI_ISL_12574371, EPI_ISL_12574372, EPI_ISL_12574000).

Gene Expression Analysis

Nucleic acid isolation from SARS-CoV-2 PCR RT-qPCR positive (COVID-19 patients) and SARS-CoV-2 RT-qPCR negative samples (control group) was performed using commercial Hibrigen nucleic acid isolation kit (Hibrigen, Istanbul, Turkey); following isolation, cDNA synthesis was performed using the Revert Aid First Strand cDNA Synthesis Kit (Cat No: K1622, Fermentas, Canada).

Gene expression levels of *TAS1R2, TAS1R3, TAS2R38, OR51E1, LEPR, GHRL*, and the *ACTB* as a house-keeping gene were detected using gene-specific primers by qRT-PCR in the Insta Q96™ Plus Real-time PCR Detection System (HiMedia Laboratories Pvt. Ltd., Mumbai, India). Five-fold dilutions of cDNA synthesized from total RNA were used as templates. All samples were studied in triplicates and the mean values were obtained for further calculations. Primers were designed using Primer3 software and primers synthesized by Macrogene (Amsterdam, Netherlands) (Table 1). The PCR cycling conditions were 95^{o}C for 15 seconds followed by 40 cycles at 60^{o}C for 30 seconds and 72 ^{o}C for 45 seconds for all genes.

Table 1
Representative the gene primers in the panel of PCR.

Gene	Sequence 5' → 3'	PCR Product
TAS1R2	F: ATACCTGCAACCAGGAGTGC	160
	F: ATCTTCTTCGACCCGCAAGG	
	R: CAGGTGCTTTTGTCACAGCC	
	R: GTTGATGGTGTGCCAGGAGA	
TAS1R3	F: ACGCCCTCTTCAACTACAGC	185
	F: GAAAATGGCCGTGGAGGAGA	
	R: CAGGTGCGTCTTCACGTACT	
	R: GGCTGGTACTGCGTGTAGTT	
TAS2R38	F: CTGCCTCAGCCTGCTTTACT	325
	F: TGGAGTTTGCAGTGGGGTTT	
	R: GGAGACAGTCAGCATCCCAG	
	R: CAGAGGTTGGCTTGGTTTGC	
OR51E1	F: GATTGTCCATGGTGCATCGC	138
	F: CCTGAACACATAGCCAGGCA	
	R: TGCGCTGTCGAATCTCCTTT	
	R: ATGAAAGGCTGCACATGGGA	
LEPR	F: TGCCTGCTGGACTCTCAAAG	139
	F: TTCTGACAAGTGTTGGGTCT	
	R: TGCTCACTCCGAAAGCAACA	
	R: GCAGCAGTACACTGCATCAT	
GHRL	F: CCTCCTCGGGAAGGTGTAGA	206
	F: GGAGCACATGCTTCTTCCCT	
	R: GTCTGGGTGCAGCTTTGTTG	
	R: TAAACCAGCAACCCCATCCC	
ACTB	F: GCACTCTTCCAGCCTTCCTT	111
	R: GTTGGCGTACAGGTCTTTGC	

Statistical Analysis

Statistical analysis was performed using SPSS software (Statistical Package for the Social Sciences 25.0, SPSS Inc, Chicago, IL, USA). The gene expression data were obtained as Cycle Threshold (CT) values (CT = cycle number at which logarithmic PCR plots cross a calculated threshold line). The expression of each gene was compared between depots using the $2^{\Delta\Delta CT}$ method ($\Delta\Delta CT$ = CT of the target gene-CT of the housekeeping gene), respectively. Quantitative variables were expressed as mean ± standard deviation (S.D.) or ± standard error (SE) whenever appropriate. The differences between normal and abnormal distributed continuous variables were compared using Student's t-test and Mann-Whitney U test respectively. To evaluate differences between the groups including patients infected with Delta and Omicron BA.1 variants, the data were undergo log transformation to satisfy ANOVA criteria and then subjected to one-way ANOVA with Tukey's post-hoc analysis. The correlations of the gene expression in the patient's group were evaluated with the Spearman correlation test. Statistical significance was taken as $p < 0.05$.

Results

The expression profiles of *TAS1R2, TAS1R3, TAS2R38, OR51E1, LEPR, and GHRL* genes among COVID-19 patients and the control group were evaluated (Table 2). To better understand the possible association between the expression profiles of the studied genes and SARS-CoV-2 variants in this study, we focused on SARS-CoV-19 Delta and Omicron variants. The expression levels of *TAS1R2* (2-fold) and *TAS1R3* (≈ 2-fold) genes were significantly decreased in COVID-19 patients who were infected with the Delta variant compared to the control group (p = 0.013, p = 0.025, respectively). Nevertheless, the expression level of the *TAS2R38* gene were four-fold lower in COVID-19 patients who were infected with the Delta variant compared to the COVID-19 negative individuals but the difference were not found as significant.

However, the expression levels of *LEPR* (four-fold), *GHRL* (three-fold), and *OR51E1* (two-and-half-fold) genes were significantly higher in COVID-19 patients who were infected with the Delta variant compared to the control group (p = 0.001, p = 0.001, p = 0.015, respectively). Moreover, COVID-19 patients who were infected with the Omicron BA.1 variant had two-fold higher *LEPR* gene expression levels compared to controls (p = 0.021).

On the other hand, the gene expression profiles among SARS-CoV-2 variants showed that *TAS1R2* (two-fold) and *TAS1R3* (~ two-fold) gene expression were increased in COVID-19 patients who were infected with the Omicron BA.1 variant compared to COVID-19 patients who were infected with the Delta variant (p = 0.001 and p = 0.001, respectively). The expression level of *LEPR* was down-regulated in COVID-19 patients who were infected with Omicron BA.1 variant compared to COVID-19 patients who were infected with Delta variant (two-fold) and the difference was significant (p = 0.019). There were no difference in the expression levels of *TAS2R38, GHRL*, and *OR51E1* genes between the Delta and Omicron BA.1 infected COVID-19 patient groups (p = 0.180, p = 0.701, and p = 0.883, respectively).

Table 2
Relative expression levels of *TAS1R2, TAS1R3, TAS2R38, OR51E1, LEPR, and GHRL* in COVID-19 positive patients and control group

Genes (mean ± SE)	Delta Variants	Omicron BA.1 Variants	Controls	p-Value
TAS1R2	0.97 ± 0.14	1.88 ± 0.17	2.07 ± 0.34	0.001 □ 0.013 Ψ 0.948 Ω
TAS1R3	1.98 ± 0.26	3.56 ± 0.29	3.45 ± 0.47	0.001 □ 0.025 Ψ 0.997 Ω
TAS2R38	0.70 ± 0.13	1.52 ± 0.41	2.69 ± 0.49	0.180 □ 0.139 Ψ 0.609 Ω
LEPR	5.80 ± 0.83	3.0 ± 0.52	1.40 ± 0.06	0.019 □ 0.001 Ψ 0.021 Ω
GHRL	3.94 ± 0.47	2.71 ± 0.14	1.16 ± 0.15	0.701 □ 0.001 Ψ 0.474 Ω
OR51E1	0.48 ± 0.07	0.39 ± 0.10	0.19 ± 0.06	0.883 □ 0.015 Ψ 0.290 Ω

SE: Standart Error. The values are calculated using a one-way ANOVA test. p-value ≤ 0.05 is considered statistically significant. p-values in bold have still remained their significance after Bonferroni correction. □: p-value for comparison of COVID-19 patients who were infected with Delta variants and COVID-19 patients who have infected with Omicron BA.1 variants, Ψ: p-value for comparison COVID-19 patients who were infected with Delta variants and controls, Ω: p-value for comparison of COVID-19 patients who infected with Omicron BA.1 variant and controls.

The correlation analyses were done between expression levels of studied genes in COVID-19 patients who were infected with Delta and Omicron BA.1 variants of SARS-CoV-2 by the Spearman correlation test

(Fig. 1). The results showed that the *TAS1R2* gene expression was positively correlated with *TAS1R3, TAS2R38*, and *GHRL* (*r = 0.655*, p = 0.001, *r = 0.301* p = 0.025, *r = 0.354* p = 0.006, respectively). The expression of *TAS2R38* gene was positively correlated with *LEPR* and *GHRL* (*r = 0.378*, p = 0.012, *r = 0.672* p = 0.001, respectively). Moreover, the *TAS1R3* gene expression was positively correlated with the *GHRL* gene expression (*r = 0.333*, p = 0.009), however, the *GHRL* gene expression was positively correlated with the *OR51E1* gene expression (*r = 0.441*, p = 0.003).

Discussion

In the present study, we aimed to evaluate the association between gene expression levels of *TAS1R2, TAS1R3, TAS2R38, OR51E1, LEPR*, and *GHRL* genes which are linked to gustatory, olfactory, and, appetite receptors in COVID-19 patients and their correlation among SARS-CoV-19 Delta and Omicron BA.1 variants of concern (VoCs).

Different scenarios have been drawn to explain COVID-19 relation to loss of taste, one of them explains taste disfunction in the manifestation of the SARS-CoV-2 infection could be due to the damage to the taste nerves after the virus infects the central nervous system. While this appears improbable, evidence from the human study found that ageusia is associated with low severity in COVID-19 individuals once who do not have encephalitis (Meunier et al., 2021); although, a recent study observed that SARS-CoV-2 can directly infect the central nervous system but still it is unclear and the low incidence of SARS-CoV-2-related to central nervous system injury (Fabbri et al., 2022).

Another possible explanation is assumed that the regeneration of taste buds that express the taste receptors is limited after infection of the epithelial cells. Evidently, taste buds have large levels of Toll-like receptors (TLR) and interferon (IFN) receptors, and their activation may inhibit taste cell renewal (Wang et al., 2009). As a result of the “cytokine storm” (excessive or uncontrolled production of immune cells and cytokines) caused by SARS-CoV-2 in distant cells, ageusia could be the result of decreased taste bud renewal. The cytokine storm may also allow SARS-CoV-2 to infect taste buds cells. The angiotensin-converting enzyme 2 (ACE2) is identified as the main receptor for SARS-CoV-2 entrance into the host cell. Moreover, in the presence of IFN, the ACE2 has been demonstrated to be overexpressed. As a result, distant IFN production from infected keratinocytes might lead to the ACE2 expression in taste bud cells, which could then be infected with SARS-CoV-2 (Meunier et al., 2021).

Taste receptors are expressed in the upper airways. The non-ciliated solitary chemosensory cells (SCCs) epithelial cells, express the sweet taste receptors formed by two members of the taste 1 receptor family *TAS1R2* and *TAS1R3*, as well as the bitter taste receptors TAS2s (Maina et al., 2018). These cells have an important role in immunity response by activation of the taste receptors. (Imada et al., 2010).

Our results reveal that expression levels of the *TAS1R2* (two-fold) and *TAS1R3* (~ two-fold) genes were significantly decreased in COVID-19 patients who were infected with Delta variant compared to the non-infected individual. Additionally, the expression levels of *TAS1R2* (two-fold) and *TAS1R3* (~ two-fold) genes were increased in COVID-19 patients who were infected with Omicron BA.1 variant compared to

COVID-19 patients who were infected with Delta variant. This study demonstrated the possible scenario of down-regulation of sweet taste receptors involving SARS-CoV-2 Delta infection. These findings pointed to ACE2 being expressed in taste buds and high replication of SARS-CoV-2 in infected gustatory cells in the taste bud causing inflammation and eventually destroying the cells, especially with the SARS-CoV-2 Delta infections, which is the most disruptive and symptomatic variant causing hospitalizations and deaths in comparison to other variants. The injury to the gustatory cells could cause the gustatory system to malfunction.

Previous studies are revealed that the sweet taste receptors regulate energy balance, glucose hemostasis, and food intake, (Lee & Owyang, 2019). As sweet sensation encourages people to eat more, hence a study in the duodenum and parts of the mouse brain involved in energy regulation, observed that the *TAS1R3* gene is downregulated in obese patients' stomachs (Widmayer et al., 2011). The *TAS1R2* gene expression in the brain is reduced in obese mice whereas, the *TAS1R2* is downregulated by high glucose, while *TAS1R3* is downregulated by elevated leptin levels, both of which are outcomes of obesity (Chao et al., 2016). In our study, the *TAS1R2* gene expression was positively correlated with *GHRL*, as well as *TAS1R3* gene expression was positively correlated with *GHRL* gene expression among the COVID-19 patients. This might be associated with the individual's health condition which was, unfortunately lacking in our data.

TAS2Rs are associated with the respiratory system (Shah et al., 2009). The TAS2Rs have been discovered to be capable of recognizing bacterial pathogens and triggering downstream reactions within minutes. One of the most studied isoforms of the TAS2R family is the *TAS2R38* gene, which induces a response to different bitter compounds such as bacterial bitter compound acyl-homoserine lactones (AHLs) (Maina et al., 2018). The *TAS2R38* gene is activated by the microbial bitter products, inducing canonical taste-signaling pathway immune response, releasing calcium Ca^{+2} from the endoplasmic reticulum. The Ca^{+2} increasing via nitric oxide synthase activates nitric oxide (NO) production. NO tends to increase ciliary beat frequency by activating protein kinase G and causing damage to infectious microbial compounds (Carey et al., 2017). TAS2Rs, ensure defense responses primarily for ciliated sinonasal epithelial cells occurring in the first layer of upper airway immunity (Maina et al., 2018) which associates with SARS-CoV-2, causing immune responses to destabilization (Aoe, 2020).

Hence, we analyze the expression of the *TAS2R38* gene, whereas activation of bitter taste receptors shows to have anti-inflammatory effects and its upregulation might have a protective role in some conditions (Orsmark-Pietras et al., 2013), controversially the findings showed that the expression levels of the *TAS2R38* gene were four-fold lower in COVID-19 patients who were infected with Delta variant. The comparison of this group to the control group was not as well as no significant differences found between the two COVID-19 variant groups. Supporting Risso et al., (2022), the *TAS2R38* genotypes do not alter infection-driven dysgeusia and are not responsible for variances in the presence or severity of COVID-19 (Risso et al., 2022).

Furthermore, TAS2s activate the enteroendocrine cells, resulting in ghrelin level elevation, and an increase in food intake, causing gastric fulfillment, which in long term reduces food intake, in mice. Our study confirmed a positive correlation of the *TAS2R38* expression with the *LEPR* gene and among COVID-19 patients. In another study the food avoidance behavior was observed in mice over-expressing *TAS2R38* receptors, suggesting a possible therapeutic target for appetite suppression medicines (Dalesio et al., 2018).

Olfactory receptors (ORs) are expressed in the olfactory epithelium in the nasal cavity and influence the sense of smell. Apart from nasal tissues, the ORs are observed in non-nasal tissues as well, however, the function of these ectopic ORs in cell signaling, survival, and proliferation is still not known well (Pronin & Slepak, 2021). The ORs were primarily identified from nasal epithelium tissue (Buck & Axel, 1991), and a few years ago the ORs genes expression in human and rodents cell lines and tissues was detected in the airways, brain, blood vessels and guts, and some other organs (Dalesio et al., 2018).

Loss of smell was observed in several COVID-19 patients who did not have any coryzal symptoms or substantial nasal infection (Levinson et al., 2020). This discovery is most likely the cause of virus-induced damage to olfactory receptor neurons in the olfactory epithelium. In some individuals, a cytokine storm occurs, affecting the neurological system and sensory organs of smell (Kanjanaumporn et al., 2020). Moreover, the presence of both receptors, ACE2 and transmembrane serine protease 2 (TMPRSS2), are essential for effective SARS-CoV-2 infection in humans in non-neural cells of the olfactory epithelium (Butowt & Bilinska, 2020).

Here, we report the expression level of the *OR51E1* gene in COVID-19 patients in comparison with non-infected individuals, the expression levels of the *OR51E1* gene (two-and-half-fold) were significantly higher in COVID-19 patients who were infected with SARS-CoV-2 Delta variants compared to the control group, this may be explained that the virus induces damage to olfactory receptor neurons as well as might be correlated with olfactory dysfunction symptoms in COVID-19 positive patients. Although, more clinical studies are urged in this area to elucidate the role of ORs in the context of COVID-19 diseases. A recent *in-silico* study that was performed to explain the messenger RNA expression profiles for various genes among COVID-19 patients showed that the *OR51E1* gene was upregulated within COVID-19 patients' groups (Jha et al., 2022). On the other hand, there was no statistical difference in the expression of this gene between COVID-19 variants in our study.

The other gene that was targeted in this study was the *GHRL*, which regulates various physiological processes such as nutritional intake, metabolism, sleep, inflammation, and memory. As the *GHRL* gene is expressed in different tissues (Groschl et al., 2005), in many inflammatory diseases, it was reported that the level of ghrelin in plasma was increased, including sepsis, ulcerative colitis, rheumatoid arthritis, ankylosing spondylitis, pancreatitis, Crohn's disease (Baatar et al., 2011). The changes related to sensory perception, hunger, and food-associated pleasure, has been reported in COVID-19 patient (Høier et al., 2021), (Kanjanaumporn et al., 2020). Therefore, it is important to understand the expression level of the *GHRL* gene and the possible correlation with SARS-CoV-2 infection. Thus, our results emphasized that the

expression levels of the *GHRL* (three-fold) gene were significantly higher in COVID-19 patients who were infected with Delta variant compared to the control group; this might be associated with the high expression level of this gene in various inflammatory diseases and its role in the inflammatory response, as well and can be explained as upregulation in ghrelin receptors in response to inflammation may serve as a mechanism of protective feedback to initiate as an inflammatory response.

Comparing the expression profile of the *GHRL* gene among the SARS-CoV-2 variant, in this study, however, there was no significant difference between the studied groups, although this gene is observed to be down-regulated in different conditions, further studies might be needed to understand the accurate association with COVID-19 patients. Nevertheless, the current findings indicated that the *GHRL* gene expression was positively correlated with the *OR51E1* gene expression in the positive cases. Overall, the OR51E1 protein plays an important role in physiological function (Dalesio et al., 2018), more studies are needed for further understanding of this correlation in expression.

The *LEPR* gene encodes the leptin hormone (LEP) that is mostly produced in white adipose tissue and is linked to the amount of body fat. The *LEPR* gene is located in many tissues other than the hypothalamus, which is its classical target organ such as the placenta, lung, stomach mucosa, endometrium, immune cells, liver, and (Al-Shibli et al., 2019). It works by activating *LEPR* in the hypothalamus and peripheral organs like the liver to regulate food intake. LEP affects the central nervous system, causing a decrease in food intake and an increase in energy consumption (Viesti et al., 2014).

On the other hand, leptin protein and the *LEPR* gene have shown to have an important role in inflammation, whereas the *LEPR* gene is expressed all over the immune system (Guglielmi et al., 2021), and a study of COVID-19 patients shows increased levels of leptin, especially in severe cases in comparison with non-infected individuals (Wang et al., 2021), this might offer a reliable correlation with our study, as it was reported in COVID-19 patients with appetite destruction symptoms, hence our findings interestingly indicated that the expression profile of the *LEPR* (four-fold) genes was significantly higher in COVID-19 patients who were infected with Delta ($p = 0.001$).

Also, COVID-19 patients who were infected with Omicron BA.1 variants had two-fold higher *LEPR* gene expression levels compared to the control group ($p = 0.021$). Moreover, the comparison of two SARS-CoV-2 variants showed that the expression level of the *LEPR* gene was found to be slightly significantly down-regulated within COVID-19 patients who were infected with Omicron BA.1 (two-folds) ($p = 0.001$). Given the potential arguments of the COVID-19 infection might induce neurological damage and it is conceivable that the virus could have a significant impact on food intake, driven by taste and smell dysfunctions, and perhaps the virus spreading to brain areas involved in the hedonic regulation of food intake (Meunier et al., 2021).

There are notable limitations in our study, the limited sample size, and the unknown gene expression level of the detected genes in those individuals before infection. The other limitation is unavailable data on the health condition of those individuals and their clinical history. Despite that, a follow-up study for the

same patient after COVID-19 recovery may give insight into the disease progression and relation with gene expression.

Conclusion

The present study reported for the first time the expression level of *TAS1R2, TAS1R3, TAS2R38, OR51E1, LEPR*, and *GHRL* genes in COVID-19 patients that are infected with different SARS-CoV-2 variants with strong evidence of the genetic link. The study findings suggested that the expression levels of the *TAS1R2* and *TAS1R3* genes were significantly decreased in COVID-19 patients who were infected with the Delta variant whereas offering an additional key to understanding the genetic association between the loss of smell and taste as temporary symptoms of COVID-19 diseases caused by SARS-CoV-2 infection. On the other hand, the expression levels of *TAS1R2* and *TAS1R3* genes were increased among COVID-19 patients with SARS-CoV-2 Omicron BA.1 variant. The findings of our study provided an urgent clue for temporary taste loss, particularly with the Delta variant, which is the most disruptive and symptomatic variant, causing hospitalizations and deaths when compared to other variants, possibly because ACE2 is expressed in taste buds, and the high replication of SARS-CoV-2 in infected gustatory cells in the taste bud generates inflammation, which could eventually destroy the cells. This gustatory cell damage may be caused by a malfunction of the gustatory system. Further studies are needed to offer an additional key to understanding COVID-19 infection progress.

Declarations

Conflict of Interest

The authors do not have any conflict of interest to declare.

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Data Availability Statement

The data is available upon request.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (*Approval Number YDU/2021/98-1448*). Informed consent was obtained from all individual participants included in the study.

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Figures

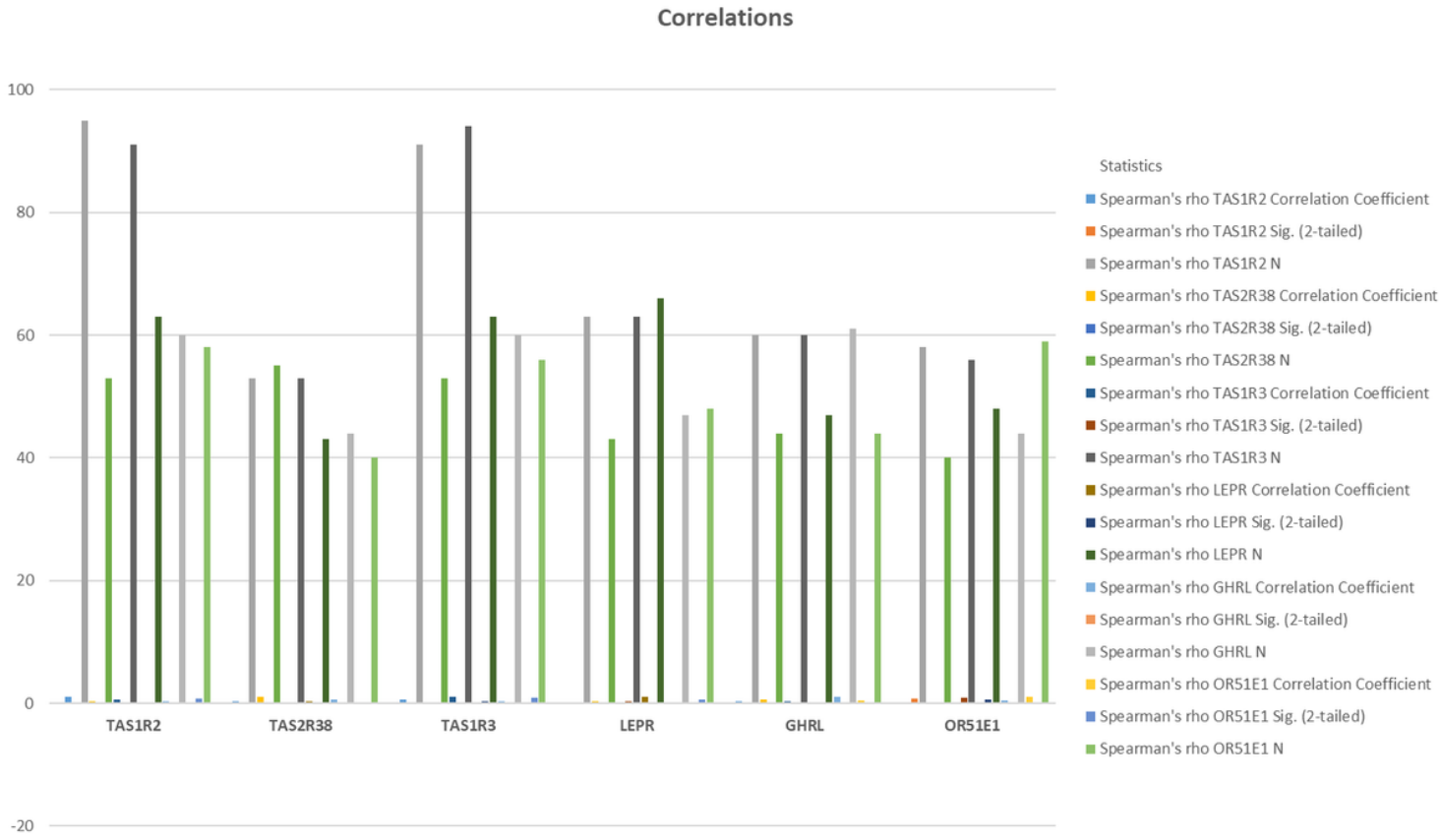

Figure 1

Correlation graph of the expression levels of the studied genes in COVID-19 patients