

Functional and Morphological Disorders of Taste and Olfaction in COVID-19-Patients

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Abstract

Objectives: To test the prevalence and evolution of acute olfactory and gustatory functional impairment and of their morphologic correlates in COVID-19 patients who require hospitalization due to COVID-19-related respiratory conditions. **Key-words:** COVID-19, taste, olfaction, electrogustometry, contact endoscopy **Design:** Electrogustometric (EGM) - thresholds at the tongue area supplied by the chorda tympani, at the soft palate and at the vallate papillae area were recorded bilaterally. Olfaction was examined by Sniffin' sticks. The patients' nasal and oral mucosa (fungiform papillae, fpap) were examined by contact endoscopy. **Setting:** Tertiary referral medical centre. **Patients:** 53 consecutive hospitalized patients (23 males, 30 females, age $42,54 \pm 10$, 95 yrs) with RT-PCR-confirmed COVID-19 diagnosis were included. Patients have been examined twice: just after hospital discharge and 4-6 weeks later. **Main outcome measures:** EGM-thresholds and taste strips, Schniffin-Sticks, Contact-Endoscopyresults: EGM-thresholds in patients were significantly higher at both instances than those of healthy subjects. EGM-thresholds at the second measurement were significantly lower than those at the first measurement. Accordingly, patient-reported gustatory outcomes were improved at the second measurement. The same pattern has been found using Sniffin' sticks. Significant alterations in form and vascularization of fPap have been detected in patients, especially at the first instance. **Conclusions:** COVID-19 affects both gustatory and olfactory functions. It also affects in parallel the structure and vascularization of both nasal and oral mucosa, although the nasal mucosa to a much less, non-significant, extent. Our findings suggest that COVID-19 may cause a mild to profound neuropathy of multiple cranial nerves.

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Key-points:

- COVID-19 affects both gustatory and olfactory functions.
- SARS-coronavirus affects more than one cranial nerves, namely the chorda tympani, the glossopharyngeal and the trigeminal nerves.
- It seems to disturb not only the peripheral receptors related to the chemical senses, but also the regeneration of their peripheral nerve fibers.
- The majority of the patients reports an improvement of taste and odour-disturbances within 4 weeks.
- The functional improvement of taste acuity does not correlate directly to the morphology (including the vascularization) of fPap.

1 INTRODUCTION

Coronavirus disease 2019 (COVID-19) due to SARS-CoV-2 evolved to a pandemic [1,2]. There are reports of early olfactory and/or gustatory dysfunction even before molecular confirmation of SARS-CoV-2 infection [1-7]. Smell and taste complaints in COVID-19 appear to have somehow different features compared to those related to other viruses [8-10].

The primary hypothesis of this study was that COVID-19-infection causes a functional deterioration of gustatory function, as measured by EGM, at the regions supplied by the trigeminal, facial and glossopharyngeal nerves. Secondary hypotheses of our study were: a. that COVID-infection causes structural alterations of the taste buds, more specifically of the fungiform papillae, b. that COVID-infection induces a functional olfactory decline, c. that COVID-infection causes structural alterations of the nasal mucosa, d. that structural and functional changes regarding olfaction and taste correlate and e. that the above structural and functional changes improve with time, more specifically 4 to 6 weeks after hospital discharge.

2 MATERIALS AND METHODS

2.1 Study design

This was a prospective controlled longitudinal study conducted in a tertiary general hospital setting. Patients, who had been tested positive for SARS-CoV-2 on PCR-test and were hospitalized were asked to participate in our study. 84 COVID-patients, hospitalized for severe COVID-19 were initially screened for inclusion in this study. After application of the above inclusion and exclusion criteria, 53 (23 males, 30 females, $42,54 \pm 10$, 95 yrs.; patients-group) were found appropriate for inclusion and were asked to participate in this study. The study protocol was reviewed and approved by the Institutional Review Board (Nr21-06022021).

Patients have been examined twice: 7 - 10 days after discharge and again 4-6 weeks later. Patients' findings were also compared to those of 53 healthy volunteers (23 males, 30 females, $42,90 \pm 10,64$ yrs; healthy

subjects-group), which served as controls. The study protocol was in agreement with the STARD 2015 guidelines.

2.2 Outcome measures

2.2.1 Electrogustometry

We have evaluated taste acuity with electrogustometry (EGM). Electrical stimuli were delivered using an electrogustometer (TR-06, Rion Co, Tokio, Japan) with a single, flat, circular stainless steel stimulus probe (5mm in diameter).

All subjects were instructed not to drink or eat for at least an hour before the beginning of the testing session. A 500-ms electric stimulus was applied, beginning at -6 dB and increasing up to +34 dB (3-400 μ A) in 2dB-steps [12,13,15]. Thresholds were measured randomly on both sides of the tongue to avoid any possible bias. All six areas were tested with the same stimulus duration before proceeding with the application of a stimulus of different duration. The subject was kept unaware of whether the current was applied (blind test) as previously reported [11,12]. A two-alternative forced-choice initially ascending single-staircase detection was employed using a two-down, one-up rule [11,12].

2.2.2 Contact endoscopy

Contact endoscopy exams (for the evaluation of the nasal and oral mucosa) were all performed with a contact endoscope (Karl Storz, 7215AA, 0°, 23 cm long, 4 mm in diameter; Tuttlingen, Germany) as an outpatient procedure. A contact endoscope (CE) was connected to a 150W xenon light source, a video camera, and an SVHS video recorder (Olympus, nCare medical recorder). Subsequently, the tip of the endoscope was gently put in contact with the mucosa (nasal-Locus Kiesselbachii-, or oral) and the mucosa was examined under 60x- and 150x- magnification, by adjusting the zoom switch on the endoscope. All findings were recorded digitally. The digital images were evaluated for blood flow dynamics, overall mucosal morphology, and density of the sub-epithelial vessels [11-14].

The form of the fungiform papillae was classified to one of four types in increasing order of damage as following: **type 1** , (egg-shaped or long ellipse type – without surface thickness), **type 2**(slight thicker surface compared to type 1), **type 3** (thick and irregular surface) and **type 4** (remarkably flat and atrophic surface). Due to their very light staining, fungiform papillae could be readily distinguished from filiform papillae, which stained dark [11,12,14].

The classification of the blood vessels' morphology at the tip of anterior tongue apex was performed according to a classification by Negoro et al. [14]. Five types of vessels' morphology, classified in increasing order of morphologic alterations were found, namely **type A** (clear loop and wooden branch shape), **type B**(unclear loop and wooden branch shape), **type C** (elongated blood vessels), **type D** (granular shape or dotted shape) and **type E** (unclear blood vessels).

Nonetheless, based on previous published experience and studies on description of the morphology and vascularization of the oral mucosa, a modification of Negoro's classification for the outline of the vascularization of nasal mucosa showed quite reproducible findings and therefore has been used [14,15]. According to this modified classification, there are five morphological types of nasal mucosa vascularization, which have been already described thoroughly above (Figure 1).

After carefully suctioning of the secretions, the anterior part of the nasal cavity was stained with methylene blue 1%. Then the mucosa was gently touched with the tip of the contact endoscope.

2.2.3 Sniffin' Sticks Test

For odor presentation, the cap was removed from the pen and the pen's tip was brought in front of the patient's nose and thoroughly moved from left to right nostril and backwards for 3s. Individual scores can be related to standard values for (a) normosmia (normal olfactory function), (b) hyposmia (impaired olfactory function) or (c) functional anosmia (residual or absent olfactory function) [16].

It was the patient's condition in most of the case (fatigue, exhaustion) which prevented us from applying the detailed TDI-battery.

2.3 Statistical analysis

Data were analyzed with IBM SPSS Statistics for Windows version 25.0 (IBM Corp., Armonk, NY, USA). The null hypothesis was that no statistical difference in vascularization-patterns in both oral and nasal mucosa existed between the two groups. The results of Sniffin' sticks have also been compared. For statistical analysis, if an EGM-threshold could not be measured at all, then it was assigned a numerical value of 36 dB. To examine if our results were normally distributed, we applied a quantile-quantile plot (QQ plot), which proved that the distribution was not normal. As a result, non-parametric tests were applied. The level of statistical significance was set at $p < 0.05$. On each occasion, the findings between two groups were compared by using Kruskal-Wallis and Mann-Whitney tests. The Bonferroni correction was applied when necessary. Tukey's multiple comparison was used to detect differences significant at the 0.05-level in mean thresholds for both groups.

3 RESULTS

3.1 Patient-reported outcomes

The patients have been asked to report on their subjective smell-related complaints or experiences prior to the Sniffin' Sticks-test. On the first examination, on the day of hospital discharge, 30 patients reported anosmia and 23 hyposmia (without being able to specify any specific odorant at all). Regarding patient-reported gustatory outcomes, 18 patients reported ageusia, 22 hypogeusia and 3 of them phantogeusia (an often metallic or salty taste in the mouth for which no external stimulus can be found), the rest 10 reported a normogeusia. On the second examination, 4-6 weeks later, fourteen participants reported normosmia, seven reported anosmia, and 32 hyposmia. Additionally, 22 of them reported hypogeusia, 4 patients reported dysgeusia and 8 ageusia. Interestingly 6 of them reported alloseusia. The rest 13 reported no taste alterations. The results are depicted in **Table 1**.

3.2 EGM-thresholds

We have found a significant difference between the measurements of the EGM-thresholds (both 1st and the 2nd measurement) and those of the healthy subjects. The values on the second measurement were clearly lower (i.e. better) than those on the first occasion.

The mean numerical values of EGM-thresholds for both groups are depicted on **Table 1**. The statistical differences between them are depicted on **Table 2**.

The statistical difference between the values at the 1st measurement and those of the healthy subject was $p=0.001$ for all six loci.

The p - values, estimated between the EGM-Thresholds recorded on the 2nd measurement and the control-patients-group are depicted on **Table 3**.

3.3 Structural changes related to the fungiform papillae

In comparison to the findings of the healthy subjects, changes in both structure and vascularization of fPap have been detected. It is interesting that the parameters of fPap (vascularization and form) seem to have in some patients a tendency to deteriorate after the end of the treatment (in 2 males: from 3/D to 4/A and 4/B). Findings of both measurements, as those of the healthy subjects are presented in Table 4. As it can be appreciated in this Table, despite the remarkable improvement in the EGM-thresholds in all six oral anatomic areas, the changes in form and vascularization of fPap are not completely synchronized.

3.4 Correlation between EGM-thresholds and morphology and vascularization of fPap.

We have used the Spearman-test to calculate any correlation between the EGM-thresholds and the morphology and vascularization of fPap. The null hypothesis was that there is no correlation between the above

parameters for all measurements of the study.

On the 1st measurement we have found a correlation coefficient (r) between the EGM-thresholds and the morphology, equal to 0.31. This correlation was significantly increased at the second exam ($r = 0.5$). Our findings indicate, that the functional improvement of taste acuity does not correlate directly to the morphology (including the vascularization) of fPap. Concerning the vascularization and the EGM-Thresholds, we have found a correlation coefficient (r) on the first measurement equal to 0.56. This correlation was found increased by the second measurement ($r=0.74$) This indicates that the vascularization of fPap is not directly correlated to the morphology of the fPap.

3.5 Sniffin' Sticks and patient-reported olfactory outcomes

The use of the Sniffin' sticks showed anosmia in the majority of the patients ($n=39$, 14 males, 25 females) on the first examination. Hyposmia was found in the remaining 14 patients (6 males, 8 females) on the first examination. The average of the right answers was 2,84. On the second exam, we have found that 4 patients had normosmia (3 males, 1 female), 17 had hyposmia (6 males, 11 females), but 32 still had anosmia (12 males, 20 females). The average of the correct answers was 6,62.

Comparing the results of the first measurement in the patients' group to those of the healthy subjects, a significant statistical difference ($p=0.0024$) was found. The difference between the results of the second measurement and those of healthy controls was less, at the $p = 0.02$ -level. The average of the correct answers by the Schniffin-Stick's Test was 11,62.

3.6 Contact endoscopy and nasal mucosa

Contact endoscopy allowed direct visualization of the superficial cell layers of the epithelium previously stained with methylene blue. This was the case in both examinations in patients-group and in healthy subjects-group.

By using contact endoscopy, we managed to observe the condition of the microvascular network correlated to any possible damage of the nasal mucosa. More specifically, on the first evaluation of patients-group, 6 patients had a type A microvascular network pattern, 35 had a type B pattern (granular or dotted shape) and 12 patients a type C pattern. On the second exam of patients-group, 15 patients had an A type, 32 exhibited a B type and 6 of them had a C type. In the control group, 38 healthy subjects showed a type B, 10 of them had an A type and the rest 5 had a type C pattern. No septal defect was observed in any subject of the study groups.

Although it is not quite clear, whether SARS-coronavirus directly affects the shape of vessels in the nasal mucosa, it is worth mentioning that the vessels in patients on the second examination and those of the healthy subjects tended to exhibit similar patterns in absolute numbers.

4 DISCUSSION

We show that COVID-19 impairs the epithelial structure and vascularization of both nasal and oral mucosa, although the nasal mucosa seems to be altered to a less extent than the oral mucosa is. We suggest that COVID-19 may cause multiple cranial neuropathies of quite varying degree (i.e. mild to profound) involving the nerves responsible for the innervation of fPap and the transmission of the chemical stimuli. Most patients reported a concomitant loss of smell and taste, and fewer patients reported only loss of either smell or taste [15,17]. 65.71 % of the patients reported a total recovery of both taste and olfaction within 4 weeks after the discharge from the hospital, and 74.29 % had recovered within 6 weeks after hospital discharge (both olfactory and gustatory function). However, the reported recovery time (median: 17 days) was not as short as reported in previous studies, probably because the hospitalized patients included in our study had more severe disease [17].

A significant study limitation is the lack of objective data on taste and olfaction in the study participants before hospitalization and even before the infection. Although all participants reported no history of gustatory or olfactory disorder in the past, any objective measurements to compare are missing. Another limitation is

the lack of subjective methods for the evaluation of gustatory function. In our study electrogustometry was preferred, because of its reliability and the ability to take repeated results on the same loci. It should be mentioned that the control group was not measured twice, as these subjects remained healthy throughout the study the study, without corresponding pneumonia or any other medication.

The nasal cavity plays an important role in COVID-19 infection; it is probably the major entry point for SARS-CoV-2 and the site of intense viral replication [17,18]. SARS-CoV-2 seems to have its own mechanisms of aggression to the olfactory neuroepithelium, with a greater predilection for neural structures' involvement over the nasal mucosa [19]. Subjective olfactory dysfunction in COVID-19 patients has been extensively reported in a number of studies [1,4,8-9].

The exact mechanisms underlying olfactory and gustatory disorders among patients with COVID-19 infection remain unclear. SARS-COV-2 seems to enter the central nervous system (CNS) via the olfactory or trigeminal route. Initially, the CNS infection or inflammation could be mild and cause olfactory damage [23-25]. However, olfactory impairment after upper respiratory tract infection is a common occurrence in clinical settings [21-24]. Another hypothesis is that COVID-19 infection could be related to the involvement of the olfactory bulb or to peripheral damage of the olfactory receptor cells in the nasal neuroepithelium. This assumption is based on the potential neurotrophic features of SARS-CoV-2 [17,18].

The role of innervation in the regeneration of the fungiform papillae has been extensively studied. The impact of denervation on fungiform taste buds appears to be less severe than on circumvallate; while the former are reduced in number, many albeit smaller taste buds remain [21,24]. Differentiated taste buds disappear or are reduced in density in denervated taste epithelium. Progenitor taste cells also require the trophic and / or electrical signals provided by the afferent taste nerve fibres, in order to continue to provide immature taste cells to the taste buds [24,25].

5 CONCLUSIONS

Our findings suggest that SARS-coronavirus affects more than one cranial nerves, namely the chorda tympani, the glossopharyngeal and the trigeminal nerves. It also seems to disturb not only the peripheral receptors related to the chemical senses, but also the regeneration of their peripheral nerve fibers.

Figures and Tables

Figure 1. The modified Negoro's Classification, there are 5 morphological types of nasal mucosa vascularization, which have been already described thoroughly in text.

Table 1 . The means of EGM-thresholds for both groups are depicted in this table. The threshold of the right side of the soft palate is **threshold A (A in Table)** , the one of the vallate papillae is **threshold B (B in Table)** and the right side of the tongue apex **threshold C (C in Table)** . The corresponding thresholds of the left side of the tongue are **threshold D (D in Table)** , **threshold E (E in Table)** and **threshold F** (F for the left side of the soft palate in Table). The reader can also find information about smell dysfunction and its recovery.

Table 2. The statistical differences between the 1st and 2nd measurement in patients' Group . The statistical difference between the values of the 1st measurement and those of the healthy subject is $p=0.001$ for all six loci.

Table 3. The p- values, estimated between the EGM-thresholds recorded in patients-group by the 2nd measurement and the control-group (healthy subjects-group). There is an improvement of taste acuity by the COVID- the patients.

Table 4. It is interesting that the parameters of fPap (vascularization and form) seem to have in some patients a deteriorating tension after the end of the treatment (2 men, from 3/D to 4/A and 4/B). Despite the remarkable improvement in the EGM-thresholds in all 6 anatomic areas, the form and vascularization of fPap do not follow in parallel.

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