Neurosensory Rehabilitation and Olfactory Network Recovery in COVID-19-related Olfactory Dysfunction

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Case report

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Abstract

Background

Non-conductive olfactory dysfunction (OD) is an important extra-pulmonary manifestation of coronavirus disease 2019 (COVID-19). Prolonged COVID-19-related OD is a serious neurosensory disability. Treatment for the restoration of smell is urgently needed.

Case presentation

Two patients presenting with prolonged COVID-19-related OD underwent structural and resting-state functional magnetic resonance imaging (rs-fMRI) brain scans. Two healthy controls were recruited for radiological comparison. One patient received olfactory treatment (OT) by the combination of oral vitamin A and smell training via the novel electronic portable aromatic rehabilitation (EPAR) diffusers. After four-weeks of OT, clinical recuperation of smell was correlated with interval increase of bilateral OB volumes [right: 22.5mm³ to 49.5mm³ (120%), left: 37.5mm³ to 42mm³ (12%)] and the enhancement of mean olfactory functional connectivity [0.09 to 0.15 (66.6%)].

Conclusions

Olfactory network functional defects and OB volume loss were identified in patients presenting with prolonged COVID-19-related OD. Preliminary evidence demonstrated that the combination of oral vitamin A and smell training may induce neurogenesis at the olfactory apparatus and achieve olfactory neurosensory rehabilitation. This observation should be validated in large scale randomized-controlled trials.

Background

Non-conductive olfactory dysfunction (OD) is an important extra-pulmonary manifestation of coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).¹ Immunohistochemical analyses confirmed the presence of angiotensin-converting enzyme 2 (ACE2) receptors in the human olfactory epithelium (OE), supporting the pathological potential for SARS-CoV-2 binding and infection.² Furthermore, the neurotropic properties of SARS-CoV-2 have been demonstrated in U251 cell line, Tuj1⁺ Pax6⁺ Nestin⁺ human brain organoids, and hamster models.^{3–5}

OE is a pseudostratified columnar epithelium which consists of olfactory sensory neurons, non-neuronal supporting cells (for example: sustentacular cells, Bowman's glands and ducts), as well as progenitor and stem cells. The OE is maintained continuously by globose basal cells (GBCs) throughout life. After substantial tissue injury, OE regeneration is further supported by the differentiation of multipotent horizontal basal cells (HBCs).⁶ In an attempt to harness the regenerative potential and neuroplasticity of the OE, oral vitamin A (VitA) in combination with the novel electronic portable aromatic rehabilitation

(EPAR) diffuser (SOVOS, Hong Kong, China) technology were used in the treatment of prolonged COVID-19-related OD.

Case Presentation

In this case study, two patients diagnosed with prolonged COVID-19-related OD were recruited to undergo structural and resting-state functional MRI (rs-fMRI) brain scans. Non-clinical staff were invited to participate as controls (Table 1). COVID-19 patients received nasoendoscopic assessments. Detailed functional olfactory evaluations (supplementary tables 1 and 3) and SARS-CoV-2 virologic assessments can be found in supplementary notes 1.

Table 1
Demographic and clinical characteristics of COVID-19 patients and controls.

COVID-19 patients (n = 2)			Controls (n = 2)	
Characteristics	Patient 1 (P1)	Patient 2 (P2)	Control 1 (C1)	Control 2 (C2)
Age (years)	19	41	26	29
Gender	Male	Female	Male	Female
COVID-19 Disease Status	Mild	Mild	Not applicable	Not applicable
Respiratory condition				
SpO ₂ (room air)	98%	97%		
Dyspnoea	0	0		
Cough	0	0		
Sputum production	0	0		
Radiological lung changes	Nil	Bilateral patchy ground glass opacities		
Systemic symptoms				
Fever	0	1		
Chills	0	0		
Rigor	0	0		
Malaise	0	0		
Myalgia	0	0		
Vomiting	0	0		
Diarrhoea	0	0		
SARS-CoV-2 Investigations				
RT-PCR (Ct value)	Positive (23.79)	Positive (22.06)	Negative	Negative
Date of RT-PCR virologic diagnosis	31 March 2020	16 March 2020		

BTT: butanol threshold test; COVID-19: coronavirus disease 2019; Ct value: cycle threshold value; GPH: good past health; MN titre: microneutralization assay titre; RT-PCR: reverse transcription polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SpO₂: oxygen saturation.

COVID-19 patients (n = 2)			Controls (n = 2)	
Date of RT-PCR virologic clearance	2 May 2020	6 April 2020		
Serology (MN titre)	Positive (1:40)	Positive (1:40)	Negative (< 1:10)	Negative (< 1:10)
Nasoendoscopy Assessments	No anatomical abnormalities	No anatomical abnormalities	Not available	Not available
Olfactory Function Assessments				
Onset of Olfactory Dysfunction	25 March 2020	23 March 2020		
BTT score (baseline)				
Baseline	0.5	0.5	6.5	4.5
2 weeks	1.0	Not available	Not available	Not available
4 weeks	2.0	Not available	Not available	Not available
Smell Identification Test				
Baseline	Ansomia	Severe Microsmia	Not available	Not available
4 weeks	Severe Microsmia	Not available	Not available	Not available
Smoking Status	Non-smoker	Non-smoker	Non-smoker	Non-smoker
Drinking Status	Social drinker	Non-drinker	Non-drinker	Non-drinker
Past Surgical History				
Head injuries	Nil	Nil	Nil	Nil
Neurosurgery	Nil	Nil	Nil	Nil
Maxillofacial surgery	Nil	Nil	Nil	Nil
Past Medical History	GPH	GPH	GPH	GPH
BTT: butanol threshold test; COVID-19: coronavirus disease 2019; Ct value: cycle threshold value; GPH: good past health; MN titre: microneutralization assay titre; RT-PCR: reverse transcription polymerase chain reaction; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SpO ₂ : oxygen saturation.				

All participants underwent MRI brain scans using a 1.5T MR scanner (SIGNA; GE Healthcare, Chicago, Illinois, United States). Structural and three-dimensional (3D) arterial spin labeling images were acquired. Volumetric analyses of the olfactory bulbs (OB) were performed. MR spectroscopy was performed using the single voxel point resolved spectroscopy (PRESS) at the gyrus rectus (GR) and superior frontal cortex (SFC).

rs-fMRI brain scans were collected using a gradient-echo echo-planar sequence sensitive to blood-oxygen-level-dependent (BOLD) contrast. Hypothesis-driven region of interest (ROI) approach was applied. The olfactory network seed regions were defined at the caudate nuclei.⁷ With reference to the automated anatomical labeling template, rs-fMRI data were segmented into 90 regions, of which 28 out of 90 regions were associated with the functional olfactory cortical networks (OCN; Fig. 1A). The functional connectivity (FC) between seed regions and OCN ROIs were obtained by extracting the average time series from individual ROIs and calculating the correlation. Detail methodology of the MRI data acquisition can be found in the supplementary notes 2.

Serial rs-fMRI FC images of the olfactory network are shown (Fig. 1B–D). Olfactory network FC were reduced in COVID-19 patients when compared to controls. FC were homogenously decreased in the right and left caudate seed regions for patient 1 (P1) and patient 2 (P2), respectively (supplementary tables 4).

Structural MRI brain scans showed OB volume defects in COVID-19 patients (supplementary table 5) when compared to controls. MR spectroscopy confirmed neuronal loss (supplementary Fig. 1C), as evident by reduction of *N*-acetylaspartate levels at the GR and SFC.

After baseline rs-fMRI assessments, olfactory treatment (OT) was initiated for patient 1 (P1). Oral Vita 25,000IU [7,500µg retinol activity equivalents (RAE); Carlson Laboratories, Illinois, USA] soft gels were prescribed daily for two weeks in combination with smell training (ST) thrice daily for four weeks via the novel EPAR diffusers. Video demonstrations and methodological details of ST can be found in the online supplementary information (supplementary note 3; supplementary materials 2 and 3).

Clinical improvements in olfaction were documented serially by subjective questionnaires (supplementary tables 1 and 2) and objective olfactory quantitation (Table 1) for P1 after OT. Butanol threshold test (BTT) revealed improvement in olfactory sensitivity from more than 4–1%. Smell identification test (SIT) confirmed categorical improvement in olfaction from anosmia to severe microsmia. Notably, there were measurable increase in the bilateral OB volumes [supplementary table 5; right side: 22.5mm³ to 49.5mm³ (120%), left side: 37.5mm³ to 42mm³ (12%)] in P1 after OT.

Olfactory recovery in P1 was correlated with significant improvements in the mean olfactory FC [0.09 to 0.15 (66.6%); supplementary table 4], when compared with pre-OT baseline scans. FC improvements were documented in both primary [left piriform cortex (PC), and right amygdala] and secondary (bilateral GR, and medial orbitofrontal cortices) OCN areas (Fig. 1B–D). Importantly, there were corresponding increase in regional CBF at the bilateral PC (left: 17.43%, right: 10.99%) and multiple secondary OCN regions (supplementary table 6), demonstrating robust neurovascular coupling.

Discussion And Conclusion

In this case study, COVID-19-related OD was correlated with OB volume loss and abnormal MR spectroscopy findings, indicating neuronal destruction in the central nervous system (CNS) secondary to COVID-19 infection. Furthermore, rs-fMRI demonstrated olfactory FC impairments, thereby providing further insights into the underlying neuropathological process of COVID-19-related OD.

In the treatment for COVID-19-related OD, one patient was successfully challenged with oral VitA and ST, as demonstrated by (1) interval improvements in olfactory function; (2) structural restoration in OB volumes; as well as (3) olfactory network recovery and (4) enhanced cerebral perfusion. We hypothesize that VitA is an important metabolic substrate for robust neurogenesis at the olfactory apparatus and enhancement of olfactory functional neural connectivity. However, due to the limited scope of this report, the therapeutic efficacy of oral VitA and ST in the restoration of smell should be validated in large scale randomized-controlled trials.

The integrity of the mammalian OE is preserved by the mitotically active GBCs and dormant HBCs. The dormancy of HBCs is maintained by Notch1 signaling which is correlated with the expression of transcription factor protein 63 ($\Delta Np63\alpha$). During extensive OE injury, Notch1 signals and $\Delta Np63\alpha$ expressions are downregulated, leading to HBCs activation and differentiation.⁸ Retinoic acid (RA), the active metabolite of VitA, has been shown to reduce $\Delta Np63\alpha$ expression in HBCs, thereby promoting the differentiation of multipotent Sox2⁺ and Pax6⁺ progenitors via the canonical Wnt signaling pathway in the OE.⁶ Furthermore, the neurophysiological importance of RA signaling in the OE has been further demonstrated in the maintenance and survival of Ascl1⁺ GBCs.

Within the CNS, RA machineries have been identified in the murine and human brains, especially at the hippocampus and dentate gyrus. RA increases neurogenesis in the rodent subventricular zone (SVZ)–OB pathway, as demonstrated by increased bromodeoxyuridine-positive (BrdU⁺, proliferating cell marker) cells in SVZ neurospheres and altered cellular migration to the olfactory bulbs.⁹ In addition, depletion of doublecortin-positive (DCX⁺, immature neuronal marker) cells in the adult murine dentate gyrus was evident in the retinoid-deficient mouse model, indicating the crucial role of RA in the survival of neural progenitors.¹⁰

Olfactory stimulation via ST, as non-pharmaceutical intervention for post-infectious OD, was used to complement the therapeutic effects of oral VitA. MRI studies in ST-treated patients have demonstrated functional network improvements and structural increase of cortical thickness in the frontal cortex, where the olfactory apparatus is located. The exact therapeutic mechanism of ST is unknown; however, olfactory stimulations remain essential to the neuro-rehabilitation processes. Reversible and irreversible olfactory occlusion experiments in murine models have shown that olfactory stimuli are contributory to olfactory neurogenesis and glomeruli maturation. In this report, ST was delivered by the novel EPAR diffusers, which utilized ultrasonification to generate aerosolized essential oils for olfactory stimulation. The EPAR diffuser technology enhances the ST experience by facilitating the physiological penetration of

aromatic molecules through the olfactory meatus to the OE at the roof of the nasal cavity, therefore providing potent olfactory stimulation and neurosensory rehabilitation.

In conclusion, structural and functional olfactory defects were identified in patients presenting with prolonged COVID-19-related OD. Preliminary evidence suggested that combination treatment by oral VitA and ST may induce robust neurogenesis at the olfactory apparatus, attain restoration of functional network connectivity, and achieve olfactory neurosensory rehabilitation. The therapeutic efficacy of oral VitA and ST for COVID-19-related OD should be validated in large scale randomized–controlled trials.

Declarations

Ethics approval and consent

This study was approved by the institutional review board (IRB) of the University of Hong Kong/Hospital Authority–UW 20-454. Written informed consents were obtained.

Competing interests

S.S. has received speaker's honoraria from Sanofi-Aventis Hong Kong Limited and Abbott laboratories Limited. The other authors declare no conflicts of interest.

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Authors' contributions

T. W.-H. C., I. H.-F. N., and H. K.-F. M. conceived the study. T. W.-H. C., H. Z. and H. K.-F. M. designed the experiments and wrote the manuscript draft. T. W.-H. C. and F. K.-C. W. performed the olfactory assessments. H. Z. and H. K.-F. M. conducted the rs-fMRI scans and analyses. T. W.-H. C. and K. H. C. performed SARS-CoV-2 RT-PCR and serology tests and analyses. S.S, V. C.-C.-C, I. H.-F. N., and K. Y. Y. were involved in the patient recruitment, data analysis, and critically reviewed the manuscript.

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Figures

Figure 1

(A) Three-dimensional (3D) representations of the primary and secondary olfactory cortical network (OCN) areas. Olfactory network functional connectivity (FC) images using the left caudate as the seed region, as represented in the (B) sagittal, (C) coronal, and (D) axial planes.

Supplementary Files

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