An Empirical Cross-Sectional Study to Identify the Olfactory and Gustatory Effects of SARS-CoV-2 – A Social Analysis

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ABSTRACT: Severe acute respiratory syndrome coronavirus (SARS-CoV-2) is a glycan-coated coronavirus with an exposed receptor-binding domain (RBD), capable of binding to the angiotensin-converting enzyme-2 (ACE2) receptors and entering the cell through the cleavage of the spike proteins by the extracellular host enzyme Transmembrane serine protease 2 (TMPRSS2). The severity of the infection is directly associated with loss of taste (ageusia) and loss of smell, varying from hyposmia (reduced perception of smell) to anosmia (complete loss of smell). The mechanisms underlying the independent symptoms remain inconclusive; however, have been linked to sustentacular cell infection, changes in the olfactory bulb and subcortical regions of the brain, defects in the respiratory centres of the central nervous system and cerebral glucose abnormalities. The survey for this cross-sectional study was conducted in 2021 and involved 126 individuals who were exposed to or tested positive for COVID-19, through the Reverse transcription polymerase chain reaction (RT-PCR). 6.3% of covid-negative and 34.9% covid-positive individuals exposed to COVID-19, experienced anosmia and ageusia. This study aims to identify patterns in symptoms presented when an individual is infected with COVID-19; focusing on the correlation between anosmia and ageusia and associated visual impairments or cognitive difficulties. The understanding of varied symptom combinations observed amongst individuals differing in age, sex, comorbidities or vaccination status could aid doctors and healthcare practitioners to provide a prognosis specific to the subset to which the patients belong, depending on their symptom combinations and severity for the same.

KEYWORDS: Anosmia, Ageusia, Blood-Brain Barrier (BBB), Olfactory Epithelium (OE), Olfactory Sensory Neurons (OSNs).

1. INTRODUCTION

Severe acute respiratory syndrome coronavirus (SARS-CoV-2), a β-coronavirus, belongs to a family of enveloped positive-sense single-stranded RNA (+ss-RNA) viruses termed: coronaviruses (CoVs). This zoonotic virus was declared as a Public Health Emergency of International Concern (Cruz M. et al., 2021) on 30 January 2020, by the World Health Organization (WHO). The variants of SARS-CoV-2 are classified according to concern, high consequence and interest. Variants of Concern (VOC), such as Alpha: Beta: Gamma: Delta - include strains with increased transmissivity and virulence, and reduced effects of vaccines, medications or diagnostic techniques, leading to unpredictable modes of disease presentation and epidemiology. Variants of Interest (VOI), such as Eta: Iota: Kappa: Lambda: Mu - strains capable of evading immunological attacks through expected changes in specific genetic markers can potentially risk global public health (Chakraborty C. et al., 2021).

SARS-CoV-2 is a spike protein, with a complementary Receptor Binding Domain (RBD) to the angiotensin-converting enzyme-2 (ACE2). The spike (S) protein evades the immune response through glycan coating and a hinge-like motion at the hip, knee or ankle regions of the stalk. For the virus to be endocytosed into the cell, it must be proteolytically cleaved by cell surface TMPRSS2 enzyme or lysosomal protease cathepsin B/L, at the junction of receptor-binding subunit (S1) and membrane fusion subunit (S2) (Shang J. et al., 2020). Studies involving the radiolabelling of the S1 proteins in mice have revealed the entry of the virus into the brain endothelial cells (in close association with the brain capillary glycocalyx), across the blood-brain barrier (BBB), through the ACE2 receptor, leading to elevated levels of kynurenine, quinolinic acid, and glutamate, and neurotransmitter (dopamine, norepinephrine, serotonin) depletion. This is due to the activation of microglial cells, leading to elevated levels of cytokines (potentially escalating to a cytokine storm) (Boldrini, M. et al., 2021; Rhea E. et al., 2021). Irregularities in neurotransmission could lead to hypoxic injury and excitotoxicity (upregulation of N-methyl-D-aspartate receptors), causing
neuronophagia and synaptic pruning; the effects of which are observed through neuropsychiatric symptoms, accounting for injuries in the Brodmann areas. The S protein is activated by the removal of S1, leading to a conformational change in S2 through the association of the heptapeptide repeat sequence 1 (HR1) trimer and the heptapeptide repeat sequence 2 (HR2) domain, forming 6-HB (6-helix structure in a format, antiparallel to that of the core format). This increases the association between the host cell membrane and the viral envelope, promoting the entry of the virus into the host cell. This is observed in epithelial type II cells, present in the lung, kidney, intestine and heart. The high frequency of furin cleavage sites, specific to SARS-CoV-2 increases the probability of the virus being cleaved by furin-like proteases, thus increasing the likelihood of infection upon exposure to epithelial type II cells. A similar mutation to enhance infectivity has been observed in the influenza virus: H5N1, responsible for the outbreak of avian influenza in Hong Kong, 1997. Single-cell RNA sequencing has proven, the expression of ACE2 receptors to be highest amongst ciliated and goblet cells: clusters present in the nasal epithelium, compared to the rest of the respiratory trunk, indicating the primary route of the SARS-CoV-2 infection to be through the nasopharyngeal route.

COVID-19 is identified through a multitude of respiratory and gastrointestinal symptoms such as fever, cough, cold, fatigue, conjunctivitis, discolouration of toes or skin rashes, diarrhoea, angina pectoris, shortness of breath, headache, sore throat, loss of movement or speech; however, its long-term neuropsychiatric symptoms and brain sequelae remain undetermined and inconclusive. Individuals who have tested positive have presented symptoms of anosmia, ageusia, depression, new-onset anxiety, cognitive deficits, seizures and psychosis, before, during or post the presentation of other physiological clinical symptoms, indicating the possibility of independent brain damage when infected. The recovery of the SARS-CoV-2 mRNA from the spinal cord cerebrospinal fluid in mice proves the virus to be capable of crossing the BBB. However, the impact of this virus is not only limited to its entry via the BBB but could also indirectly afflict the central nervous system (CNS) due to the invasion of cytokines into the BBB; as an extension of the cytokine storm. Anosmia and ageusia are characterized as cardinal symptoms in the detection of infection, however, the mechanisms leading to their clinical presentation remain inconclusive.

2. METHODOLOGY

2.1. Sampling

The survey was taken by individuals who either tested positive for COVID-19 through the RT-PCR test or lived with someone who had tested positive through the RT-PCR test.

2.2. Patient History and Exclusion Criteria

All participants are above the age of 18. The exclusion criteria included participants who had previously undergone radiotherapy in the oral or nasal cavities or who had a pre-existing manifestation of smell and taste alterations. Applicants who presented with a diagnosed history of head trauma, pre-existing rhinitis, chronic rhinosinusitis, or pre-existing olfactory, psychiatric or neurological disorders were also excluded from the study.

2.3. Methodology

This study commenced on August 8, 2021, and concluded on August 16, 2021. An online objective and subjective questionnaire (Google Forms – encrypted via institutional ID) was circulated for data collection. The form was made available to the participants through various social media platforms. This study was conducted under the guidance of the faculty of the Department of Chemistry at St. Xavier’s College (Autonomous), Mumbai. The study comprises 126 subjects who were provided with informed consent for the same. The detailed outline of the questionnaire is attached as Appendix I.

2.4. Measures

The diagrammatic representation of the statistically significant data was performed using Google Sheets. Comparison of recovery rates of anosmia vs ageusia amongst individuals who tested positive for COVID-19 was performed through the unpaired T-test (the confidence of the study is 95%), on Microsoft Office Excel 2007, Analysis ToolPak 2019 extension.

2.5. Limitations

This study used the RT-PCR test as the diagnostic tool for the determination of COVID-19, which is subject to personal, intentional, instrumental and operational error, depending on the efficacy with which the test is performed and the sterilization of the swabs. Due to the lack of objectivity in the tools to determine anosmia and ageusia, the findings of the same were acquired...
through inconsistent subjective observations, subject to individual recall bias. Future studies could enhance the accuracy of their findings through the implementation of pathophysiological and psychophysical tests to determine anosmia and ageusia. The mode of data collection may introduce chances of misinterpretation while answering the questionnaire. The participant pool was chosen through convenience and referring sampling techniques which might have been responsible for diminishing the potential outreach of this study. This study did not consider drugs to be an interference, however, there is potential for further research to analyze the correlation of certain medications to the severity of symptoms experienced during COVID-19. There is potential to study anosmia and ageusia specific to SARS-CoV-2 variants, to assess symptom severity with viral mutations in greater detail. The study was conducted through a self-evaluated survey. There was no significant medical test conducted to verify the authenticity of following medical norms. The study is to give a holistic perspective to our research question and place a perspective on the concept through self-conducted studies. The only reliability is the nature of our questionnaire and the study outcomes from it.

3. RESULTS

The incidence of anosmia was 8.51% greater compared to the incidence of ageusia among individuals who tested positive for COVID-19. Equivalent recovery rates for anosmia and ageusia amongst individuals who tested positive for COVID-19 were observed. The mechanisms for the same remain inconclusive, however, they can be narrowed down to olfactory inflammation, sustentacular cell damage, and neuronal pruning leading to neurotransmitter irregularities. Another significant observation was that headache was not only the most reported symptom but also the most severe for the subjects among all other symptoms. Approximately 8.8% of the respondents experienced post-vaccination gustatory and olfactory disorders. The severity of the disease was found to have a weak correlation to the Ct value.

4. DISCUSSION

4.1. Anosmia and Ageusia

The mention of ageusia and hypogeusia (reduced perception of taste) was first noted in China in 5% of COVID-19 patients (Mao L. et al., 2020). This proportion is much higher amongst those infected in Europe, America and the Middle East, indicating the possibility of variations in populations, due to lifestyle changes, quarantine protocols, screening methods, and mean population age.

Citing several studies, a greater than a third of the people who are infected with COVID-19 present with neurological symptoms. While these symptoms might be the only indicator of COVID-19 infection in some patients, the degree of severity of these symptoms is found to be in a direct correlation with the severity of the infection (Niazkar H. R. et al., 2020). While the symptoms and signs of COVID-19 lack uniformity and vary proportionally with their severity, a few reported CNS involving signs to include ataxia, epilepsy, cerebrovascular diseases, headache, dizziness and even mental confusion. Another subset of the population reported peripheral nervous system (PNS) related symptoms apart from impairment of olfactory and gustatory pathways, including muscle pain and in certain rare cases, the rapid progression of COVID-19 in a patient also presented with impaired proprioception and hyperreflexia (Carvalho F. M. et al., 2021).

The perception of taste and smell are closely associated. Damage to the ACE2 receptors in the olfactory epithelium (OE) could lead to sustentacular cell and OE cell apoptosis consequently damaging the peripheral neurosensorv-taste chemoreceptors complex. This could also damage the VII, IX, or X cranial nerves, associated with taste (Aanand, P. et al., 2021). The anterior two-thirds of the tongue is innervated by Facial Nerve dendritic effectors, which branch to form the chorda tympani. The damage to chorda tympani could lead to loss of gustation in the anterior portion of the tongue, leading to dysgeusia or ageusia (depending on the severity of damage and neuronal pruning).

4.2. Symptoms

Out of all the respondents 32.5% suffered from altered, reduced or complete loss of taste and 35.7% suffered from altered, reduced or complete loss of smell. The top 3 symptoms reported in the survey were: Headache (42.1%), Fever (40.5%) and Fatigue (39.7%) (Fig1.1). The majority of the respondents reported headaches as the most severe symptom as well (Fig1.2). Viral illnesses are commonly attributed to CNS invasion and infection. Other viral illnesses such as herpesvirus, rabies virus, influenza A etc. use the olfactory nerve as a pathway into the brain (Van Riel, et al., 2015). Mouse studies have demonstrated that SARS-CoV has the ability for transneuronal penetration and can spread to the areas of the brain connected to the olfactory bulb. The dysfunction of
neurons as a consequence of transneuronal penetration caused the death of the animal (Netland J. et al., 2008). Similarly, Headaches could be not just a constitutional symptom but could be a consequence of CNS invasion (Lee Y. et al. 2020).

4.3. Anosmia and Ageusia amongst individuals who tested negative (exposed to SARS-CoV-2) for COVID-19

While the research on post-vaccination anomic and ageusic ramifications in patients is limited, this study shows that the lack of existing literature does not eliminate the probability of anosmia and ageusia presented as symptomatic effects of the vaccination itself. In this study, it was found that 4.8% of the participants had altered/reduced/selective or complete loss of both taste and smell. Another 3.2% reported having only olfaction disorders post-vaccination including but not limited to anosmia, hyposmia, dysosmia and parosmia. 0.8% of subjects reported gustatory distress including but not limited to ageusia or hypogeusic or dygeusic variations. Phantosmia, a condition where the olfactory pathways are activated without any olfactory stimulus (or odorant) also known as olfactory hallucination and Phantogeusia, is an analogous counterpart for the gustatory system, where tastes are felt but their origin is indeterminate. These are a few conditions which demand consideration that might nudge a patient who is on the border of hypochondriasis into becoming a hypochondriac.

4.4. Ageusia and Anosmia amongst individuals who tested positive for COVID-19

Taste is primarily perceived through Gustatory taste buds present in the papillae of the tongue, however, ageusia or dygeusia associated with facial, glossoaryngeal, and vagus nerve damage remains inconclusive, due to the scarcity of histopathological tissue specimens and advanced neuroimaging studies (Ray P. S. 2020). The oral cavity, except for the tongue, is lined by cells expressing the ACE2 receptors on the plasma membrane; however, there are no studies that prove the involvement of the ACE2 receptor-binding, to be responsible for ageusia amongst COVID-19-positive individuals. Since the tongue does not express the receptors recognized by SARS-CoV-2, ageusia is a symptom, difficult to reason. However, studies have proven loss of taste to be a result of excessive pro-inflammatory cytokine production (cytokine storm).

Taste abnormalities were observed amongst 51.76% of the individuals, reporting either complete or partial loss of taste (Fig 1.5). Dysgeusia (distorted perception of taste) and hypogeusia were experienced by 3.2% and 22.6% of individuals who reported a partial loss of taste (Fig 1.6). The mean recovery rate amongst individuals experiencing ageusia was 14.773 days (Fig 1.7).

Smell perception takes place through the nasal epithelium, subdivided into the inferior respiratory epithelium (RE), responsible for the humidification and purification of the inhaled air, and the olfactory epithelium (OE), responsible for the detection of odours, through the dendritic cilia of the olfactory sensory neurons (OSNs), which are further supported by sustentacular cells, responsible for maintaining homeostasis in the respiratory trunk. Bulk RNA-Sequencing data of the Whole Olfactory Mucosa (WOM) prove the absence in the expression of ACE2 and TMPRSS2 in OSNs, however, the same genes were presented in sustentacular cells. Anosmia is caused due to four underlying mechanisms in COVID-19-positive individuals, namely: rhinorrhea and nasal obstruction: loss of OSNs: invasion of olfactory centres of the brain: and damage to sustentacular cells in the OE (Aanand, P. et al., 2021; Sungnak W. et al., 2020; Brann, D. H. et al., 2020). Anosmia is a common symptom of various intranasal diseases such as rhinosinusitis and nasal polyposis. Olfactory symptoms are expressed amongst individuals who have tested positive for COVID-19 and are early determinants of exposure to the virus. 60.27% of the participants who tested positive for COVID-19 indicated anosmia as a symptom of the infection (Fig 1.8). This is 30.08% higher than the average incidence of anosmia recorded in India, however, there have been studies expressing varied proportions of anosmia cases recorded, due to the symptoms being objective and subjective, both, hence leading to inconclusive tests to determine the subjective incidence of the same. Studies conducted by Leichen et al. reported the incidence of anosmia in 86% of positive cases. A general trend of increased cases of anosmia in Europe, compared to Asia has been observed, which could be accounted for: by genetic differences due to varied gene pools between populations, affecting the overall expression of ACE2 receptor and TMPRSS2 genes and the binding of the S1 protein. The nasal mucosal lining of ACE2 receptors plays a crucial role in the regulation of Bradykinin. However, this inflammatory peptide is absent among individuals who tested positive for COVID-19. Parosmia (distorted perception of smell) and hyposmia were reported by 14.8% and 18.5% of individuals, respectively. The exact quantification of the number of individuals afflicted with the same disease however remains inconclusive due to the lack of objective testing possibilities for smell disorders (Fig 1.9).

Hyposmia could be the result of the inflammation caused due to the obstruction of the olfactory cleft, preventing receptor-binding actions, sustentacular cell infection, or the impediment of the olfactory neurons as a result of cellular invasion by SARS-CoV-2. Parosmia is a result of the olfactory neuron rewiring occurring after synaptic pruning or neuronal apoptosis. Odours are
detected by approximately 350 receptors in the brain, and hence a particular smell detected is not a singular event, but rather the amalgamation of a combination of neurons working in sync. Hence the process of rewiring would involve the central reattachment of the olfactory receptor bipolar neurons’ axons, however, this process is not definite and the reattachment could be misguided to abnormal brain territories (Saniasaya & Narayanan, 2021). This process is known to take place in a checkerboard manner, causing the individual to experience an altered perception of a smell.

The median time of recovery from symptoms varies substantially across different studies and can be mainly attributed to varied methodologies used to gather data and the lack of quantitative assessments (Mullol J. et al., 2020). The short span of the recovery from anosmia post-COVID-19 infection suggests probable pathologies for the symptom, such as local inflammation due to cytokine storm or interleukin accumulation, rather than central wide-spread inflammation; damage to non-neural OE cells; SARS-CoV-2 competitively binding on the olfactory receptors (Torabi A. et al., 2020). The average recovery rate of anosmia from this study was 15.91 days, per the mean recovery rate of 14 days (Fig 1.8) (Aanand, P. et al., 2021).

4.5. Analysis of recovery rate for Anosmia and Ageusia amongst individuals who tested positive for COVID-19

The recovery rate amongst individuals who tested positive for COVID-19 and experienced anosmia (Fig 1.10) and ageusia (Fig 1.7) was the same (confidence 95%, α=0.05), providing evidence for the mechanisms behind the two symptoms to be correlated. Action potential signals are transmitted to the trigeminal and facial nerves and are excited when odorants bind to gustatory and olfactory receptors. However, this does not mean that anosmia will indefinitely lead to ageusia or vice versa. The average overall period of recovery in terms of the absence of symptoms was 17.42 days amongst individuals who tested positive for COVID-19 (Fig 1.11).

4.6. Cyclic threshold (Ct) value observed in individuals who tested positive for COVID-19

The reverse-transcription polymerase chain reaction (RT-PCR) test is the gold-standard diagnostic test used for COVID-19, which involves taking nasopharyngeal and oropharyngeal swabs. The swabs are then screened (upon amplification) for viral target genes expressed by SARS-CoV-2. The cyclic threshold (Ct) value is a numerical representation of the amplification required in terms of the number of PCR cycles for the viral load to be detected upon reaching the threshold value. The average Ct value of the participants in this study was 24 (21-30) (Fig 1.12).

Aranha C. et al proposed the significance of the Ct values, where the intensity and the duration of the infection vary inversely as a function of the Ct values, thereby determining the degree of viral load on the patient taken into account (Aranha, C. et al., (2021)). The Indian Council of Medical Research (ICMR), however, deems there to be inadequate data to conclusively support the correlation between the viral load and the Ct value. While none of the participants with a Ct value of 0-10 experienced hospitalization in the general ward or the ICU, 15.4% and 7.7% of the participants with a Ct value of 11-20 experienced hospitalization in the ICU and the general ward, respectively. Individuals in no other Ct value range required hospitalization in the ICU, however, 10.3% of the individuals with a Ct value of 21-30 required hospitalization in the general ward (Fig 1.13).

5. FUTURE SCOPE

The authors wish to explore the same areas of analysis to evaluate other changes experienced by individuals who have suffered from COVID-19 symptoms. We wish to carry out a mixed-method analysis to validate the studies explored both qualitatively and quantitatively. An expected exploration into this study via interviews aims at understanding the experienced conditions of individuals who experienced symptoms of SARS – CoV – 2.

6. CONFLICT OF INTEREST

The authors of this paper declare no conflict of interest associated with the research method, its data collection and analysis.

7. ACKNOWLEDGEMENTS

The authors would like to thank the Department of Chemistry, St. Xavier’s College (Autonomous), Mumbai, for the support and guidance provided to complete this project. The faculty associated have constantly furnished us with all the necessary resources and time for conducting the survey study. Special gratitude goes to our respondents who wholeheartedly submitted their views by answering our survey questionnaire.
REFERENCES


2. Chakraborty C et al, (2021), Present variants of concern and variants of interest of severe acute respiratory syndrome coronavirus 2: Their significant mutations in S-glycoprotein, infectivity, re-infectivity, immune escape and vaccines activity; Reviews in Medical Virology, e2270, https://doi.org/10.1002/rmv.2270


16. Torabi A et al, (2020), Proinflammatory cytokines in the olfactory mucosa result in COVID-19 induced anosmia, ACS chemical neuroscience, 11(13), 1909-1913; https://doi.org/10.1021/acschemneuro.0c00249


A. Figures

**Fig 1.1:** Diagrammatic representation of symptoms experienced: COVID-19 positive and negative (exposed) individuals.

**Fig 1.2:** Symptom severity: COVID-19 positive and negative (exposed) individuals.

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Fig 1.3: Diagramatic representation of Anosmia and Ageusia amongst individuals who tested negative for COVID-19.

Fig 1.4: Recovery rate of Anosmia and Ageusia amongst individuals who tested negative for COVID-19.
**Fig 1.5:** Diagramatic representation of individuals who experienced a loss of taste and tested positive for COVID-19.

**Fig 1.6:** Diagramatic representation of the degree of loss of taste experienced.
Fig 1.7: Recovery rate of individuals who tested positive for COVID-19 experiencing Ageusia.

Fig 1.8: Diagramatic representation of individuals who experienced a loss of smell and tested positive for COVID-19.
Fig 1.9: Diagramatic representation of the degree of loss of smell experienced.

Fig 1.10: Recovery rate of individuals who tested positive for COVID-19 experiencing Anosmia.
**Fig 1.11:** Diagrammatic representation of recovery rates amongst individuals who tested positive for COVID-19.

**Fig 1.12:** Diagrammatic representation of Cyclic threshold value amongst individuals who tested positive for COVID-19.
Fig 1.13: Correlation between Cyclic threshold value and disease severity.