Prevalence of olfactory dysfunction in D614G, alpha, delta and omicron waves: a psychophysical case-control study*

Luigi Angelo Vaira[1,2], Jerome R. Lechien[3,4], Giovanna Deiana[2], Giovanni Salzano[1,5], Fabio Maglitto[1,5], Pasquale Piombino[5], Andrea Mazzatenta[6], Paolo Boscolo-Rizzo[7], Claire Hopkins[8,9], Giacomo De Riu[1]

[1] Maxillofacial Surgery Operative Unit, Department of Medical, Surgical and Experimental Sciences, University of Sassari, Sassari, Italy

[2] Biomedical Science Department, PhD School of Biomedical Science, University of Sassari, Sassari, Italy

[3] Department of Anatomy and Experimental Oncology, Mons School of Medicine, UMONS. Research Institute for Health Sciences and Technology, University of Mons (UMons), Mons, Belgium

[4] Department of Otolaryngology-Head Neck Surgery, Elsan Hospital, Paris, France

[5] Department of Maxillofacial Surgery, University of Naples "Federico II", Naples, Italy

[6] Neurophysiology, Olfaction and Chemoreception Laboratory, Physiology and Physiopathology Section, Neuroscience, Imaging and Clinical Sciences Department, 'G. d'Annunzio' University of Chieti-Pescara, Chieti Scalo, Italy

[7] Department of Medical, Surgical and Health Sciences, Section of Otolaryngology, University of Trieste, Trieste, Italy

[8] King's College, London, UK

[9] British Rhinological Society (President), London, UK

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Abstract

Background: The purpose of this study was to compare the prevalence of olfactory dysfunction (OD) at different stages of the COVID-19 pandemic by evaluating subjects diagnosed with SARS-CoV-2 infection during the Omicron wave with psychophysical tests and comparing the results with those obtained from patients infected during the D614G, Alpha and Delta waves and with those of a control group.

Methodology: The study included adult patients diagnosed with SARS-CoV-2 infection. Depending on the time of diagnosis, the subjects were divided into four study groups: D614G; Alpha, Delta and Omicron variant groups. A group of uninfected individuals was used as control. All subjects underwent psychophysical evaluation of the olfactory function with the Connecticut Chemosensory Clinical Research Center olfactory test (D614G and Alpha groups) or the extended version of the Sniffin'Sticks test (Delta, Omicron and control groups).

Results: 372 cases (134 D614G group, 118 Alpha group, 32 in Delta group and 88 Omicron group) were recruited and evaluated within 10 days of infection, alongside 80 controls. Patients self-reported olfactory loss in 72.4% of cases in the D614G group, in 75.4% of cases in the Alpha group, in 65.6% of cases in the Delta group and in 18.1% in the Omicron group. Psychophysical evaluation revealed a prevalence of OD: 80.6%, 83.0%, 65.6% and 36.3% in the D614G, Alpha, Delta and Omicron group respectively. The differences between the D614G, Alpha and Delta groups were not statistically significant. The Omicron group demonstrated a significantly lower prevalence of OD than the other variants but still significantly higher than the controls.

Conclusions: During the Omicron wave OD was less prevalent than during the D614G, Alpha and Delta periods. One-third of patients have reduced olfactory function on psychophysical evaluation during the Omicron wave. Our results should be considered with caution as the VOC has not been determined with certainty.

Key words: anosmia, ageusia, COVID-19, olfactory function, olfactory dysfunction, SARS-CoV-2, coronavirus, smell, taste, taste dysfunction, Omicron variant, variant of concern, Alpha variant, Delta variant, D614G, Maxillofacial surgery

Introduction

During the SARS-CoV-2 pandemic, olfactory dysfunction (OD) represented a key symptom COVID-19 affecting 50-70% of patients [1-4]. However, SARS-CoV-2 has changed over time. The first wave of COVID-19 pandemic in Italy was already dominated by a SARS-CoV-2 spike protein variant characterized by 23403A>G single nucleotide polymorphism (SNP), corresponding to D614G amino acid change (from here on, D614G variant) that quickly supplanted the ancestral wild-type virus worldwide [5]. The D614G mutation enhances cell binding of the SARS-CoV-2 spike protein to the ACE2 protein and was proposed to be responsible for the higher rate of OD observed in Western Countries compared to that reported in East Asian cohorts in the early phase of the pandemic driven by wild-type SARS-CoV-2 [6,7]. Subsequently, several variants of concern (VOC), containing the D614G SNP, plus many additional missense mutations, were identified including alpha (B.1.1.7) at the beginning of 2021, then replaced by the VOC delta (B.1617.2) in the summer of 2021 while the VOC omicron (B.1.1.529) became predominant in January 2022 [8,9]. Although the virological aspects have been well explored, the differences in clinical presentations between the different VOCs have not been specifically investigated. In particular, OD seem to be less frequent in subjects infected with omicron VOC with a prevalence estimated by the few studies published so far [10-14] between 2.5 and 24.6%, 3-10 times lower than the previous VOCs. However, these studies are based on the analysis of clinical records or on self-reported olfactory loss alone without psychophysical testing. To the best of our knowledge, the recent study by Hintschich et al. it is the only study to compare the olfactory function of infected patients between different VOC confirmed by next-generation sequencing. in the various pandemic waves with psychophysical tests and identifying the VOC involved [15-17], highlighting a decreasing trend in OD prevalence in later waves. This could be due to a lower pathogenicity of the virus, to a greater immunization of the population or both. While not including the current Omicron VOC in the analysis, the authors found a significantly higher prevalence of OD during the first wave of the pandemic in Germany compared to the VOCs Alpha and Delta periods. The greater severity of OD during the wild-type SARS-CoV-2 period compared to the Delta variant is also confirmed by the psychophysical study by Klimek et al. [18], where the VOC was again determined by next-generation sequencing.

The purpose of this study was to add value to these studies by evaluating subjects diagnosed with SARS-CoV-2 infection during the Omicron wave with psychophysical tests and to compare the results with those obtained from patients infected during the D614G, Alpha and Delta waves and with those of a control group.

Materials and methods

This cross-sectional case-control study was conducted at the University Hospital of Sassari, the protocol was approved by the institutional ethics committee (University Hospital of Cagliari, PG 2021/7118) and written informed consent was obtained from each participant. The study included adult patients (> 18 years old) diagnosed with SARS-CoV-2 infection confirmed by real time polymerase chain reaction on nasopharyngeal swab between March 2020 and May 2022 and who underwent psychophysical olfactory evaluation within 10 days of symptom onset. All patients were included at the COVID-19 Departments of the University Hospital of Sassari and at the Infection Control Center of the Prevention Department which monitored individuals in home isolation. The exclusion criteria were: COVID-19 second episode, previous olfactory dysfunction, previous surgery, radiotherapy or trauma to the nasal cavity, chronic rhinosinusitis, neurological or psychiatric comorbidities.

Depending on the time of diagnosis, the subjects were divided into four study groups:

1. D614G group: infection diagnosis from March 2020 to June 2020.
2. Alpha VOC group: infection diagnosis from February 2021 to April 2021
3. Delta VOC group: infection diagnosis from September 2021 to December 2021.
4. Omicron VOC group: infection diagnosis from March 2022 to May 2022

This temporal division was determined on the basis of the data provided by the Italian Ministry of Health when the circulation of each VOC was greater than 98% in the territory of the Sardinian region [8,9]. Only for the Alpha variant, in the period of maximum circulation the prevalence of the VOC was between 77 and 91% [9]. To avoid inclusion bias, patients were contacted consecutively on the basis of lists provided by the Prevention Department. Data from subjects included in the D614G(19,20) and Alpha VOC groups [21,22] were partly included in previous studies.

Furthermore, a control group was recruited with healthy subjects respecting the same exclusion criteria as the other three study groups. These individuals, who were part of the medical staff and therefore subjected to frequent control swabs, had never previously been diagnosed with SARS-CoV-2 infection, although they did not have PCR testing immediately prior to evaluation.

Epidemiological and clinical data

Some general data was collected for all individuals included in the study: age, gender, comorbidities. The prevalence and severity of COVID-19 symptoms were investigated with the COVID-19 symptom index, which is a 26-item patient-reported outcome questionnaire assessing common COVID-19 symptoms [23]. The symptom severity of general and otolaryngological symptoms

Table 1. Patients' characteristics.

	D614G group (N=134)	Alpha VOC group (N=118)	Delta VOC group (N=32)	Omicron VOC group (N=88)	Control group (N=80)	p-value
Age (years) Median (IQR)	46 (36.75-58.25)	47 (39-54.25)	46.6 (37.5-59)	48 (38-54)	49 (32-53)	0.949
Gender						
Male	43 (32.1%)	55 (46.6%)	15 (42.9%)	38 (43.2%)	34 (42.5%)	0.156
Female	91 (67.9%)	63 (53.4%)	17 (53.1%)	50 (56.8%)	46 (57.5%)	
Comorbidities						
Hypertension	15 (11.2%)	15 (12.7%)	2 (6.2%)	8 (9.1%)	9 (11.2%)	0.838
Heart disorders	6 (4.5%)	4 (3.4%)	1 (3.1%)	2 (2.3%)	2 (2.5%)	0.905
Asthma	11 (8.2%)	10 (8.5%)	3 (9.4%)	6 (5.1%)	5 (6.2%)	0.962
Diabetes	10 (7.5%)	10 (8.5%)	2 (6.2%)	7 (7.9%)	6 (7.5%)	0.215
Chronic renal failure	3 (2.2%)	2 (1.7%)	1 (3.1%)	1 (1.1%)	1 (1.2%)	0.838
Chronic pulmonary disease	7 (5.2%)	4 (3.4%)	1 (3.1%)	3 (3.4%)	3 (3.7%)	0.938
Depression	14 (10.4%)	12 (10.2%)	4 (12.5%)	6 (6.8%)	7 (8.7%)	0.861
Liver failure	2 (1.5%)	3 (1.7%)	1 (3.1%)	1 (1.4%)	0 (0%)	0.888
Smoker	31 (23.1%)	28 (23.7%)	9 (28.1%)	19 (21.6%)	20 (25%)	0.956
COVID-19 symptom index	27 (10-30)	28.5 (12-32)	17.5 (11-27.5)	13 (9-21.5)		< 0.001

was assessed as 0 (no symptom), 1 (mild symptom), 2 (moderate symptom) 3 (severe symptom) and 4 (very severe symptom), while loss of smell and taste were rated as total (2), partial (1) or absent (0). The total COVID-19 symptom index score ranges from 0 to 100.

Olfactory psychophysical evaluation

The psychophysical evaluation of smell took place within the first 10 days from the onset of symptoms in the four study groups. The Connecticut Chemosensory Clinical Research Center olfactory test (CCCRC) [20] was used for the D614G and Alpha VOC groups. The individuals of the Delta and Omicron groups and controls were instead evaluated with the extended version of the Sniffin'Sticks test (SST) (Medisense, Groningen, the Netherlands) [24]. Both of these tests are widely diffused and the CCCRC was used as the reference for validating the newer SST [25].

The CCCRC test includes an assessment of the olfactory threshold using solutions with decreasing dilutions of N-butyl alcohol and a 10-items odor identification test with common odorants. The methods of administering the test have been extensively described in previous studies [20,21]. The CCCRC test allows classification of the olfactory function as normal (score between 90 and 100), hyposmia (score between 20 and 80) and anosmia (score between 0 and 10).

The SST was administered following a previously established protocol [24] evaluating three domains of the olfactory function: threshold (T), odor discrimination (D) and odor identification (I). Each subtest was assigned a score of 1-16 (for T) or between 0-16 (for D and I). The sum of these scores yielded a composite TDI score which classifies the olfactory function as: normal (TDI score of ≥31), hyposmia (TDI score from 17 to 30.75) and anosmia (TDI score of <17).

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows version 26.0 (IBM Corp, Armonk, NY, USA). Categorical variables are reported in numerals and percentages of the total. Descriptive statistics for quantitative variables are given as the median (interquartile range (IQR)). For the purposes of the statistical analysis, the patients were classified into 3 categories of olfactory function according to the psychophysical scores obtained: normal, hyposmic and anosmic. Chi2-square test was performed to evaluate the differences between the groups in terms of proportion of normal, hyposmic and anosmic individuals. Differences between groups for continuous variables were analyzed using the Kruskal-Wallis test. The level of statistical significance was set at $p<0.05$ with a 95% confidence interval. The sample size was calculated on a predicted prevalence of OD of 62.4% in the D614G, Alpha VOC and Delta VOC groups [12], 24.6% in the Omicron VOC group [12] and 3.5% between controls [26], 80% power and 5% margin of error resulting in a minimum sample size of 41 individuals for each study group.

Results

Four hundred and fifty-two individuals were included in the study and analyzed. Of these 134 were part of the D614G group, 118 of the Alpha VOC group, 32 of the Delta VOC group and 88

Table 2. Olfactory function assessment results.

	D614G group (N=134)	Alpha VOC group (N=118)	Delta VOC group (N=32)	Omicron VOC group (N=88)	Control group (N=80)	p-value
Self-reported olfactory loss	97 (72.4%)	89 (75.4%)	21 (65.6%)	16 (18.1%)	0	
Self-reported taste loss	90 (67.1%)	85 (72.3%)	20 (62.5%)	16 (18.1%)	0	< 0.001
Psychophysical evaluation						
CCCRC score	60 (IQR 20-80)	70 (IQR 32.5-90				
SST score			28 (IQR 14-32.5)	35.25 (IQR 29.5-39)	35.75 (IQR 33-39.5)	
Normosmic	26 (19.4%)	20 (16.9%)	11(34.4%)	55 (62.5%)	76 (95%)	< 0.001
Hyposmic	67 (50%)	58 (49.1%)	11 (34.4%)	25 (28.4%)	4 (5%)	
Anosmic	41 (30.6%)	40 (33.9%)	10 (31.2%)	7 (7.9%)	0 (0%)	

of the Omicron group. Eighty individuals were included in the control group. The five groups were homogeneous in terms of gender, age and comorbidities (Table 1). 93.7% of the patients in the Delta VOC group, 97.7% in the Omicron VOC group and 100% of the controls were fully vaccinated. The severity of COVID-19 symptoms, as determined by the COVID-19 symptom index, was significantly greater in the D614G and Alpha VOC groups compared to the Delta and Omicron VOC group (Table 1). Differences in COVID-19 severity between the latter two groups were also statistically significant (p=0.027).

Patients self-reported an olfactory loss in 72.4% of the cases in the D614G group, in 75.4% of cases in the Alpha VOC group, in 65.6% of cases in the Delta VOC group and in 18.1% in the Omicron VOC group (Table 2). Psychophysical evaluation revealed a higher prevalence of OD: 80.6%, 83.0%, 65.6% and 36.3% in the D614G, Alpha VOC, Delta VOC and Omicron VOC group, respectively.

The differences between the D614G, Alpha VOC and Delta VOC groups were not statistically significant (Figure 1). The Omicron VOC group demonstrated a significantly lower prevalence of OD than the D614G, Alpha VOC and Delta VOC groups but still significantly higher than the controls (p<0.001) (Figure 1).

Discussion

Early reports investigating the prevalence of OD in Omicron VOC SARS-CoV-2 infections seem to indicate that this variant largely spares the sense of smell over previous ones. The ability of SARS-CoV-2 to induce OD is presumably linked to the D614G spike mutation which is also contained by the Omicron VOC [27]. It has therefore been hypothesized that the lower prevalence of OD is related to a lower solubility in the mucus of the Omicron variant and to a lower affinity for TMPRSS2 receptors which are massively expressed by the supporting cells of the olfactory epithelium [28,29]. The results of this study confirmed a lower prevalence of OD during the Omicron VOC period compared to earlier waves. The prevalence of OD is however significantly higher than in controls. In the latter group, the prevalence of unrecognized OD was 5%, low but compatible with the median age of the cohort and with the exclusion of pre-existing OD and conditions known to cause OD [30]. The prevalence of anosmia in the Omicron VOC group (7.5%) was significantly lower than in previous waves. It is suggested that damage to at least 90% of the supporting cells of the olfactory epithelium is necessary for the onset of anosmia [28,31]. As Omicron VOC also contains the D614G spike mutation required for binding to the sustentacular cells, it is possible that the reduced prevalence is due to host rather than virus related factors. Local immunological factors have been shown to play a role in the onset and duration of OD [32,33]. Recent studies provide evidence that SARS-CoV-2 vaccines are able to induce a nasal and salivary secretory antibody response, especially after the second dose [34,35]. It is therefore possible that the high prevalence of vaccinated or previously exposed individuals in the Omicron VOC group has an influence on the prevalence of OD by inducing a more rapid, effective and organized immune response also at the level of the olfactory epithelium. However, viral factors are also likely to be implicated as previous studies which included patients from the first waves found a prevalence of OD greater than 60% even in reinfections [36] and in individuals who had completed the vaccination course [37].

18.1% of the patients in the Omicron VOC group self-reported an OD. Previous studies reported a prevalence ranging between 1.2 and 24.6% but the frequency was estimated from the analysis of clinical records in which minor symptoms may have been omitted [10-14]. Psychophysical tests revealed a twice as high prevalence of OD compared to self-reported olfactory loss alone, confirming that the latter is an imprecise measure of olfactory function.

COVID-19 related persistent OD are proving to be a difficult challenge that smell specialists will face in the future and for which large numbers of patients are seeking assistance [38,39]. Several studies have investigated the prevalence of persistent OD with psychophysical tests and follow-ups between 6 and 12 months [40-43]. These disabling morbidities affect a significant percentage

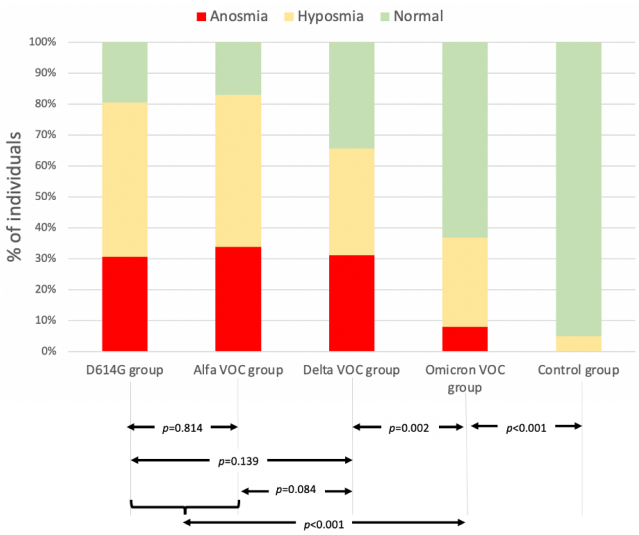

Figure. 1 Prevalence of olfactory disorders in the different study groups.

of patients ranging between 18 and 60%. As severity of initial loss is associated with longer duration [26,38,40], it is encouraging that the prevalence of anosmia is reduced in the Omicron VOC period, however long-term recovery rates are not yet known. The strength of the study was the use of validated psychophysical tests and the evaluation of subjects belonging to a single center, which minimises the possibility that ethnicity or genetic factors affect the prevalence of OD. However, it has some limitations: the psychophysical test used differs between groups as the SST was not available at our center in the first waves of the pandemic, although the self-reported OD rate was also significantly lower in the omicron and control groups. Psychophysical tests should lead to standardized and therefore comparable results and the SST was validated using the CCCRC test [25]. In the validation study, Hummel et al. found that the CCCRC test had, in its I component, a lower ability to detect the reductions in olfactory function and for this reason, the SST may be more sensitive in detecting OD [25]. For this reason, the SST may be more sensitive in detecting OD. If this were the case, the differences between the first waves and the Omicron VOC group could be underestimated while the differences between the latter groups and the controls can be considered reliable as both groups were evaluated with the SST. The VOC has not been determined with certainty and the groups were allocated based on timing of infection alone. In particular, in Sardinia, even in periods of maximum circulation, the Alpha variant never exceeded 91% of cases and was therefore considered a period in which the prevalence of VOC was between 77 and 91% [8,9]. Although subjects were recruited consecutively from lists of infected, it cannot be excluded that subjects with more prominent symptoms were more likely to be included in the study. Individuals enrolled in the control group were not tested for SARS-CoV-2 infection at the time of olfactory evaluation. However, all controls were part of the hospital staff and then subjected to regular antigenic swabs and immunoglobulin assays, which were consistently negative. Another limitation is that the groups are not homogeneous in severity of COVID-19 and less severe cases are included in the Delta and Omicron group. However, previous studies have found higher rates of OD in milder forms of COVID-19 in the past [44] or have found no association with the severity of the infection [19]. A recent study on hamsters infected with D614G and Gamma, Delta and Omicron VOC have found that the latter largely spares upper and lower respiratory tract (including the olfactory epithelium), which were extensively damaged with the previous variants [39]. It is therefore likely that the lower severity of COVID-19 symptoms in Omicron group is related to virological or immune factors rather than inclusion bias. Finally, the patients included in the Delta VOC group are fewer than the calculated sample size and the statistical analysis could be underpowered for this group.

Conclusion

During the Omicron VOC period OD was less prevalent than during the D614G, Alpha and Delta VOC waves but, when evaluated with psychophysical tests, one-third of patients have a reduction in olfactory function. OD therefore remains an important symptom to keep in mind for raising suspicion of SARS-CoV-2 infection. It will be important, in the future, to monitor the recovery of the olfactory function to establish whether OD can persist in a significant number of cases even in the Omicron variant. Our results should be considered with caution as the VOC has not been determined with certainty and it is not possible to exclude that there have been contaminations.

Authorship contribution

LAV and GDR have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conceptualization and Methodology: LAV, JLC, GDR. Acquisition, analysis and interpretation of the data: LAV, GD, FM, GS. Statistical analysis: LAV, PP. Critical revision of the literature: JRL, PP, AM. Provision of resources: SB, AGF, FB, AP. Original draft preparation: LAV, GD, GS, FM, GDR. Critical revision of the manuscript for important intellectual content: JRL, PP, AM, PBR, CH. Supervision: GDR. All the authors read and approved the final version of the manuscript.

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Conflict of interest

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Luigi Angelo Vaira
Viale San Pietro 43/B
University of Sassari
Sassari
Italy

Tel.: +39-3401846168
Fax: +39-079229002
E-mail: lavaira@uniss.it