infection with positive SARS-CoV-2 PCR from nasopharyngeal, blood, stool and placenta.[5] Recently, 2 asymptomatic neonates with positive SARS-CoV-2 RT-PCR after 24 hours, and after 7 days have been reported.[6] Of note, SARS-CoV-2 spike protein mRNA was detected on the fetal side of the placenta of women who delivered these neonates.

The symptomatic preterm infant described in this report demonstrated SARS-CoV-2 virus in both placental tissue and nasopharyngeal samples, with exclusion of bacterial and other viral neonatal infections. Although the histologic placental findings of histiocytic intervillositis and chronic villitis are not specific to SARS-CoV-2 infection, the presence of cytoplasmic staining for the SARS-CoV-2 nucleocapsid protein by immunohistochemistry and demonstration of viral particles by electron microscopy in the syncytiotrophoblastic cells strongly suggest in utero transmission.

A classification system for SARS-CoV-2 infection in pregnant women, fetuses and neonates has been described by Shah et al.[10] This classification includes placental PCR swabs and infant nasopharyngeal swabs at birth as probable evidence of congenital SARS-CoV-2. It does not consider ultrastructural demonstration of coronavirus particles or immunohistochemical detection of SARS-CoV-2 in the placenta. However, the infant described here represents congenital infection given the immunohistochemical and ultrastructural evidence of SARS-CoV-2 infection in the fetal cells of the placenta, criteria that we believe should be added to the classification system to confirm intrauterine transmission.

Overall, intrauterine transmission of SARS-CoV-2 appears to be a rare event. In the infant described, transmission could have occurred either due to ascending infection with premature rupture of membranes and primary involvement of the maternal gastrointestinal tract, or by hematogenous spread if the mother was viremic during her initial infectious period. Further studies are needed to determine the risks of vaginal delivery of mothers with SARS-CoV-2.

Additional studies on the mechanisms and risk factors of in utero SARS-CoV-2 transmission and the outcomes of congenital infection are urgently needed. In particular, the susceptibility to intrauterine transmission by gestational age and the relation to maternal active disease needs to be explored. Improving access to molecular testing of amniotic fluid and breast milk, cord blood antibody testing and establishing biorepositories for respiratory and nonrespiratory samples from exposed infants will enable investigators to further describe the epidemiology of congenital and neonatal disease in the setting of maternal SARS-CoV-2 infection.

ACKNOWLEDGMENTS

The authors would like to acknowledge the excellent clinical care and support provided by the Obstetric services and NICU staff at Parkland Health & Hospital System, Dallas, Texas. We also would like to thank Deyssy Carillo for immunohistochemistry and Richard Olivarez for electron microscopy.

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OPEN

NASAL SWAB AS PREFERRED CLINICAL SPECIMEN FOR COVID-19 TESTING IN CHILDREN

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Abstract: The first pediatric study demonstrating significantly higher positivity rate of nasal (mid-turbinate) swab testing over oropharyngeal swab testing in detecting SARS-CoV-2 (Fisher exact test 0.046, Cohen K 0.43, confidence interval 95%, 0.014–0.855). Benefits might include lower collection-related hazard for healthcare workers. We recommend it as preferred choice for swab-based SARS-CoV-2 testing in children.

Key Words: swab, coronavirus disease 2019, severe acute respiratory syndrome corona virus 2, pediatric, child

Accepted for publication June 5, 2020.
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DOI: 10.1097/INF.0000000000002812

The coronavirus disease 2019 (COVID-19) outbreak has been declared a pandemic on March 11, 2020.[1] Although exhaustive case finding represents the first step in the preventive strategy,[2] the best type of clinical specimen for the initial diagnostic test remains controversial. As stated by the Center for Disease Prevention and Control, nasal (mid-turbinate), oropharyngeal (throat) and nasopharyngeal specimen collection are considered acceptable alternatives.[3] Nasopharyngeal specimen collection is usually recommended,[3–5] but its sensitivity has been questioned if compared with other clinical specimens,[6] and it is not always feasible in young children, since specific swabs and containment measures are required to reach the pharynx through the small opening of the nostril.

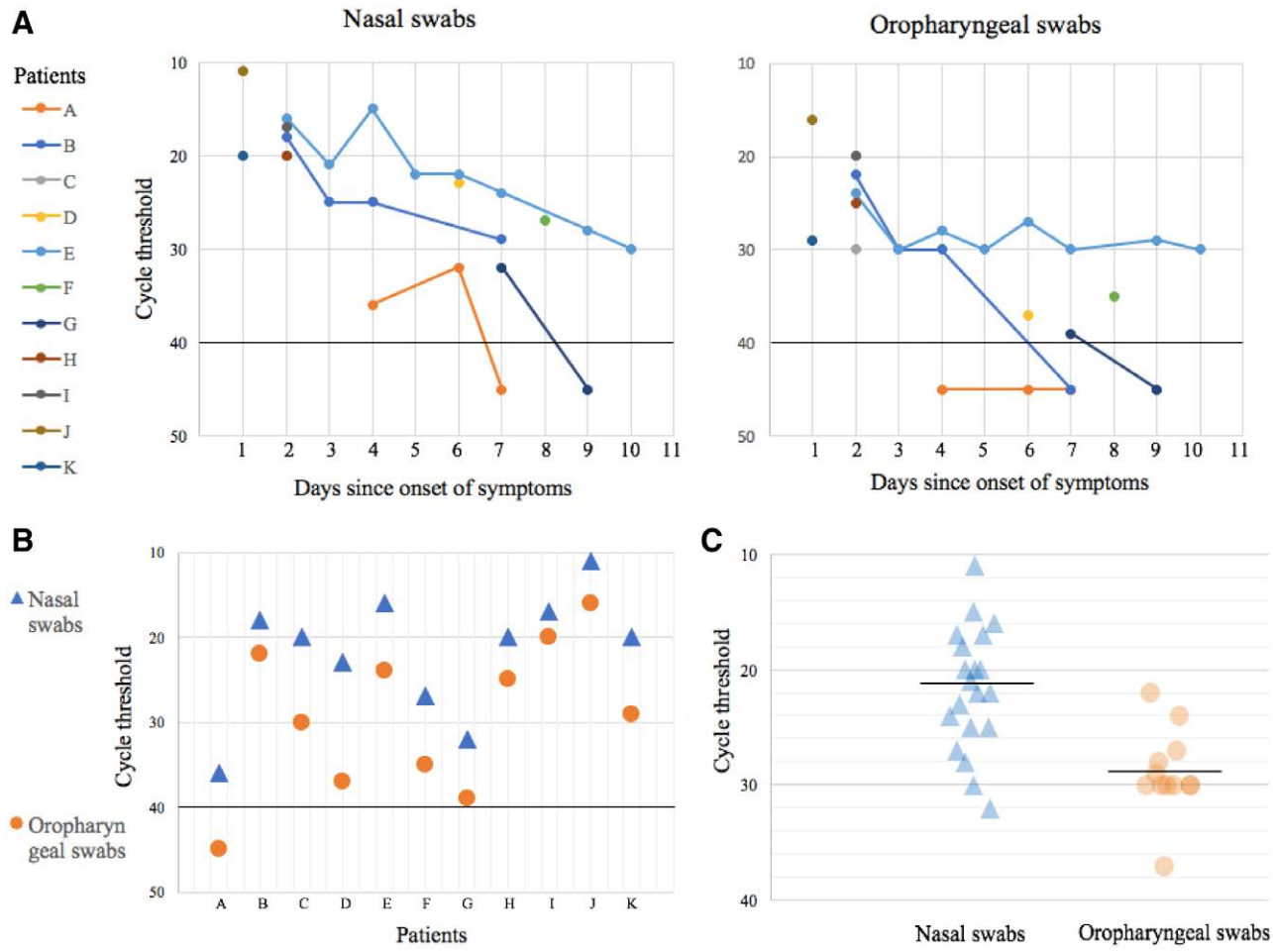

FIGURE 1. Comparison of cycle threshold (CT) values of nasal samples and oropharyngeal samples; (A) repeated sampling during hospitalization; (B) first paired samples collected for each inpatient on admission; (C) all positive paired samples.

The aim of this pediatric study is to compare the performance of nasal specimen testing and oropharyngeal specimen testing for severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) detection. Further potential benefits of nasal swabbing over the other upper respiratory sampling techniques are discussed.

METHODS

Study Design and Participants

This prospective study included all children (age 0–18) with COVID-19 who were tested for detection of SARS-CoV-2 on both nasal and oropharyngeal specimens on admission to the Meyer Children's University Hospital between March 12 and March 31, 2020. The Meyer Children's University Hospital is a tertiary care referral pediatric hospital located in Florence, Italy. The screening consisted in collecting one of the recommended upper respiratory specimens (nasal, oropharyngeal or nasopharyngeal specimens)[3] and testing for the presence of SARS-CoV-2.

If initial diagnostic testing resulted positive for COVID-19 and the patient required hospitalization, a simultaneous collection of nasal and oropharyngeal specimen was performed on admission and was repeated every 1–3 days during hospitalization. Paired results were considered in the statistical analysis, to compare the positivity rate of the 2 sampling techniques and to describe changes in the viral load.

On admission, parents signed an informed consent, including specific approval to anonymous research activity. The study protocol was approved by the institutional ethics board.

Specimen Collection and Testing

Nasal specimens were collected by mid-turbinate swabbing of both nares. Oropharyngeal specimens were collected swabbing the posterior pharynx, avoiding the tongue. A flocked swab (ESwab Copan, Brescia, Italy) was used for the collection of all clinical samples and handled as recommended in international guidelines.[3]

The presence of SAR-CoV-2 RNA in the samples was evaluated through quantitative reverse transcription-polymerase chain reaction (qRT-PCR), as described in international guidelines.[7]

The cycle threshold (CT) values of qRT-PCR are inversely related to the copy number of SARS-CoV-2 RNA and are commonly used as a proxy of viral loads. Therefore, CT values were used to compare viral loads in different clinical samples. If no increase in the intensity of the fluorescent signal was observed after 40 cycles, the sample was classified as negative.

Statistical Analysis

Data were processed with the SPSS release 24 statistical package. Results were expressed as means and SDs or as median and interquartile range (IQRs), as appropriate. The Student T test

was used to assess group differences for continuous numerical variables, whereas the Fisher exact test and Kappa coefficient to assess group differences in categorical variables. *P* values <0.05 were considered statistically significant.

RESULTS

Eleven patients were identified as having laboratory-confirmed SARS-CoV-2 infection and were admitted for further evaluation. The median age was 4.5 (IQR 2–11) months. SARS-CoV-2 infected patients presented mild to moderate signs and symptoms, ranging from feeding difficulty to fever, rhinitis and cough.

A total of 52 paired clinical specimens (26 nasal swabs and 26 oropharyngeal swabs) were collected. The first paired samples were obtained on admission and, afterwards, up to 7 other paired samples per patient were obtained during hospitalization. Overall, 24 of 26 nasal specimens resulted positive, whereas 20 of 26 oropharyngeal specimens resulted positive. In particular, 20 nasal samples tested positive also on the 20 paired oropharyngeal samples; 4 nasal swabs resulted positive, but were paired to a negative oropharyngeal swab (Fig. 1A, patients A and B); the 2 remaining nasal swabs tested negative also on the 2 matched oropharyngeal swabs (Fisher exact test 0.046, Cohen K 0.43, confidence inteval 95% 0.014–0.855). The 2 patients with matched negative tests were tested 7 and 9 days after clinical onset, respectively (Fig. 1A, patients A and G).

Analysis of viral load based on CT values was also performed. As shown in Figure 1B, CT values of the first simultaneous collected materials were always lower in nasal specimens than in the paired oropharyngeal ones. The mean difference in CT values (delta CT) was 7, which corresponds approximately to a 100-fold difference in the viral load. Moreover, when considering the 40 positive matched samples, the mean CT value on nasal samples was significantly lower, 21.6 (SD = 5.1), if compared with that of oropharyngeal swabs of 28.7 (SD = 5.3, $P < 10^{-6}$), as represented in Figure 1C. A progressive increase in CT values on both nasal and oropharyngeal samples was recorded during hospitalization, indicating a progressive decrease in the viral load.

DISCUSSION

This is the first pediatric study comparing nasal swabbing to other upper respiratory sampling methods. Results support the superiority of nasal over oropharyngeal swab collection, determined by a significantly higher positivity rate and a significantly higher mean viral load on nasal samples. In fact, the difference in CT suggests a 100-fold higher viral load in nasal specimens when compared with the oropharyngeal ones. This finding was recorded not only on the first combined analysis of infected inpatients but also on the repeated testing during hospitalization.

The clinical impact of our statistical analysis is most evident in patient A, which resulted positive on the repeated testing of nasal specimens, but never on oropharyngeal specimens. In fact, a diagnostic approach based on the evaluation of only oropharyngeal samples would have missed this SARS-CoV-2 infected patient (Fig. 1A).

The dynamic changes in CT following the initial detection support the above-mentioned data and show that the viral load progressively decreased over time for both clinical specimens. The same result has already been described and confirmed for other clinical specimens,[8] strengthening the representativeness of our sample.

Interestingly, nasal specimens tested positive for a longer time span. The virus was still detectable on nasal samples collected during the recovery phase of 2 patients, when the simultaneous oropharyngeal swab tested negative for the presence of the virus. Since our knowledge on the interruption of viral shedding is limited and relies on negative tests,[9] the above-mentioned findings would confer a preferential role to nasal sampling not only as a screening tool but also as a confirmatory sampling technique to end quarantine.

For the initial diagnostic testing of COVID-19, most international organizations indicate also nasopharyngeal swabbing as a possible alternative.[3–5] Although our study does not compare directly the performance of nasal swab with the nasopharyngeal one, we highlight several limitations to this approach, at least in the pediatric setting. In fact, it might be reasonable that sensitivity does not change between the two tests, considering the apparent higher viral load of the virus in nasal mid-turbinate samples as compared with oropharyngeal ones.[6] Furthermore, when dealing with the smaller upper airway diameter of neonates and infants, specific swabs are required, which are not currently available worldwide.[5] For this reason, many pediatric centers perform oropharyngeal swabbing instead of nasopharyngeal one. In addition, the sampling technique requires a deep and repeated insertion of the swab, which is not comfortable: less compliant patients, such as infants and young children, might oppose and cough during the collection, thus exposing the healthcare worker to a higher risk of viral transmission.[5] In fact, according to the European Centers for Disease Control and Prevention, pharyngeal sampling (nasopharyngeal and oropharyngeal) has to be considered an aerosol-generating procedure.[5]

The strength of this work is the simultaneous collection of both nasal and oropharyngeal specimens and the peculiarity of the selected population accounting many infants. The results are consistent with those on adult SARS-CoV-2 infection.[6,8] There are two main limitations: first, the small sample size, due to the low number of pediatric patients with known infection;[2] second, the single-swab-based screening of patients, due to shortages of specific materials in the first phase of the outbreak.

CONCLUSION

In conclusion, this study highlights the superiority of nasal specimen over oropharyngeal specimen collection in detecting SARS-CoV-2 in children, mainly due to a significantly higher positivity rate and a significantly higher mean viral load on nasal samples. Although larger studies are required to strengthen our conclusions, these results are of great importance, especially among the youngest, where the most recommended nasopharyngeal swabbing method is not always feasible. The benefits described might also extend to a lower collection-related exposure risk for healthcare workers.

We suggest nasal swabbing as the preferred clinical specimen for COVID-19 initial diagnostic testing and discontinuation of isolation strategy in children, as we are doing in the clinical practice of our pediatric hospital.

ACKNOWLEDGMENTS

On behalf of the Tuscany Pediatric Network for Molecular Surveillance of Infectious Disease: Agostiniani R, Pistoia; Astorino V, Firenze; Citera F, Firenze; Contimiglia M, Firenze; Mangoni G, Firenze; Nieddu F, Firenze; Pezzati M, Firenze; Valleriani C, Firenze; Vasarri P, Prato.

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AGE-DEPENDENT SENSORY IMPAIRMENT IN COVID-19 INFECTION AND ITS CORRELATION WITH ACE2 EXPRESSION

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Abstract: Among individuals who tested positive for coronavirus disease 2019, smell and taste sensations were significantly less impaired among children than among adults, in a stepwise manner. Sensory impairment was correlated with recent data of angiotensin-converting enzyme 2 expression in the corresponding age groups. This is the first report to compare sensory impairment in children and adults testing positive for coronavirus disease 2019.

Key Words: COVID-19, SARS-CoV-2, taste, smell, ACE2
Accepted for publication June 21, 2020.
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DOI: 10.1097/INF.0000000000002817

The coronavirus disease 2019 (COVID-19) pandemic has affected people of all ages. Initial reports were mainly in adults.[1] Subsequent reports confirmed contraction of the disease by children, although in lower frequencies and with milder clinical manifestations.[2] Most infected persons initially manifest with nonspecific symptoms of a common viral disease (eg, fever, myalgia, cough, vomiting). However, a unique symptom that has been commonly described in persons testing positive for COVID-19 is a temporary dysfunction in taste and smell, which has been reported in 49%–70% of infected persons.[3,4] Recognizing this impairment can alert individuals who lack additional symptoms, thereby leading to screening and preventing further distribution. Interestingly, discrepancies in the prevalence rates of olfactory and gustatory symptoms between European and East Asian cohorts have been reported.[3–6] Increased expression of angiotensin-converting enzyme 2 (ACE2) in European populations was suggested as a possible explanation. We analyzed sensory impairment in children and adults who tested positive for COVID-19, from the city of Bnei Brak in the center of Israel, during April 2020. Bnei Brak, one of the most crowded cities in the world and the city with the highest number of children per family in Israel, has been one of the main epicenters of COVID-19 infection in Israel. We correlated our findings with recent data regarding nasal epithelial ACE2 expression in corresponding age groups to examine whether alterations in ACE2 expression may explain differential sensory impairment.

METHODS

We evaluated sensory function in households in an outpatient setting in Bnei Brak, Israel, during an outbreak of COVID-19. COVID-19 was confirmed in all the participating individuals by documentation of SARS-CoV-2-positive polymerase chain reaction. Children (5–17 years of age; further divided to 5–10 and 11–17 years) and adults (18 years and older; further divided to 18–25 years and 26 and older) were evaluated for sensory impairment by responses to questions regarding the presence or lack of olfactory and gustatory dysfunction. A scoring system of 0–2 was attributed to each sense: smell and taste. Accordingly, 0 represented no loss of sense (taste or smell), 1 mild loss, and 2 complete loss, for a total score per individual of 0–4. Finally, scores of sensory impairment in the 4 age groups were correlated with published results of ACE2 expression in persons infected with COVID-19 of corresponding age groups.[7] Logarithmic values of relative expression of ACE2 according to age group were converted to actual numbers. Plots comparing sensory impairment (Fig. 1A) and ACE2 expression (Fig. 1B) in the 4 age groups were correlated. The study was approved by the ethics committee at the Mayanei Hayeshua Medical Center. Statistical evaluation was performed using the *t* test, chi square test, and the Pearson correlation test.

RESULTS

Sensory Impairment

Members of 20 families were evaluated. Of the 73 respondents (mean 3.7 per family), 31 were 5–17 years of age and 42 were 18 years and older (Table 1). In total, 37 (51%) reported having some impairment of taste or smell. This included 25.8% of the children and 71.4% of the adults ($P = 0.00014$; risk ratio, 0.39, 95% confidence interval, 0.23–0.65). The mean score (±SD) for the pediatric respondents was 0.55 ± 1.03 and for the adults was 2.01 ± 1.66 ($P < 0.0001$). Stratifying the adult group by age showed a mean score of 1.25 ± 1.54 for the young adults (18–25 years of age) compared with 2.43 ± 1.61 for the older adults ($P = 0.038$). Stratifying the pediatric group by age revealed no reports on altered sense of taste and smell in children of age 5–10 years, compared with an average score of 0.85 ± 1.18 in children of age 11–17 years ($P = 0.005$).

Correlation with ACE2 Expression

The mean scores of sensory impairment for the 4 age groups were correlated with published data[7] of expression of ACE2 in the corresponding age groups. The correlation between the 2 sets of values (sensory impairment scores and relative ACE2 expression) was 0.95 ($P = 0.05$; Fig. 1).

DISCUSSION

One of the proposed mechanisms of COVID-19-related altered smell and taste is the ability of SARS-CoV-2 to bind to