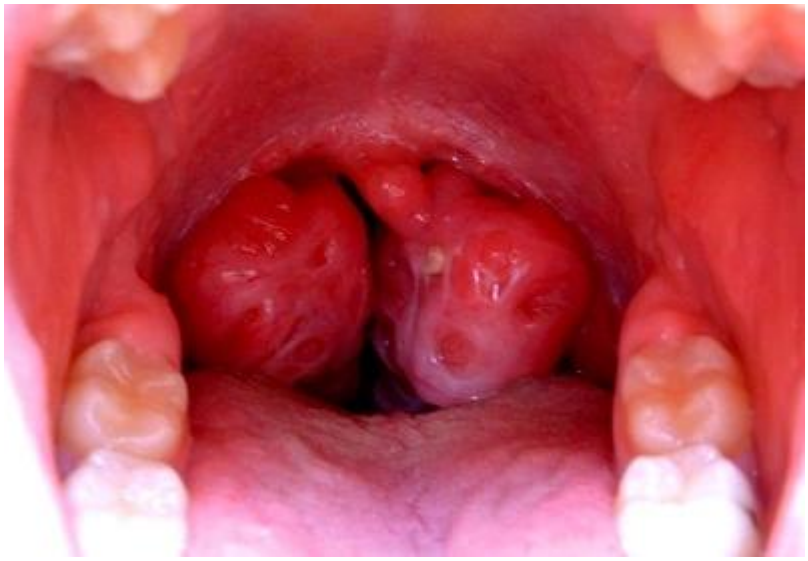
Children are more likely than adults to have mild or asymptomatic infections with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Previous studies have shown that tonsils from children who are convalescents from COVID-19 exhibit a persistent expansion of germinal centers and antiviral lymphocyte populations, as evidence of persistent tissue-specific immunity in the upper respiratory tract. In this study, the Brazilian authors investigated whether hypertrophic tonsils could serve as the SARS-CoV-2 reservoir in children. They analyzed samples of bilateral nasal wash, bilateral cytobrush of the olfactory area, and tissues of adenoid and palatine tonsils collected during adenotonsillectomy in children without COVID-19 symptoms.

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. Its genome encodes four structural proteins, namely the spike (S), envelope (E), nucleocapsid (N), and membrane (M) protein. Angiotensin-converting enzyme 2 (ACE2), which is bound by S-protein, and transmembrane serine protease 2 (TMPRSS2), which cleaves S-protein, allowing this binding to take place, are two host-cell factors important for SARS-CoV-2 viral entry into many cell types.

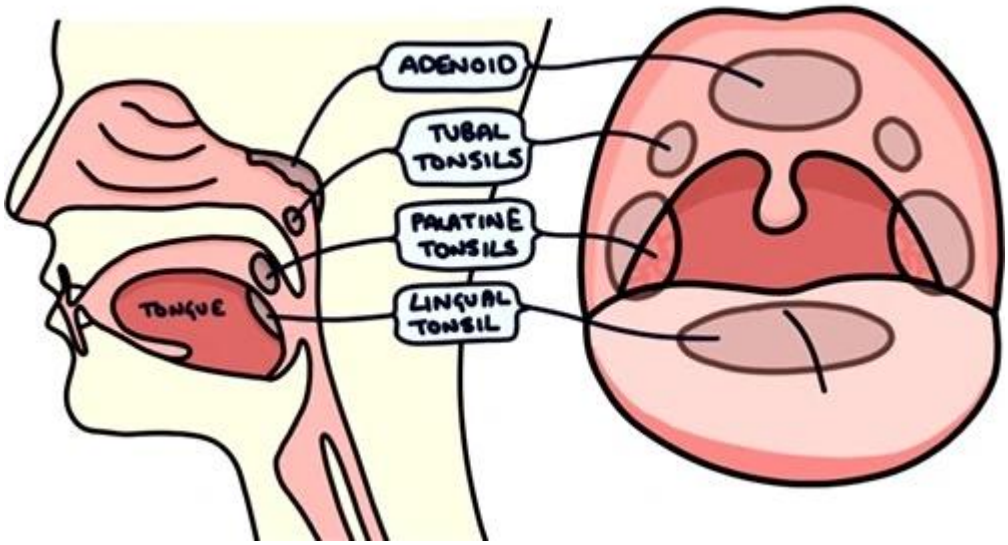


About the study

This cross-sectional study included children who had undergone adenotonsillectomy due to recurrent tonsillitis or obstructive sleep apnea. The exclusion criteria were symptoms of acute respiratory infections in the month before surgery, cranium facial malformations,

genetic syndromes, deposit diseases, immunodeficiencies, and suspected tonsillar cancer.

During surgery, the scientists collected samples of bilateral nasal wash, bilateral cytobrush of the olfactory area, adenoid and palatine tonsil tissues, and peripheral blood for serology. Nasal cytobrushes, nasal washes, and tonsillar tissue fragments were tested using the reverse transcription polymerase chain reaction (rt-PCR), immunohistochemistry, flow cytometry, and a neutralization assay.



Results

The study included 48 patients aged between 3 and 11 years (mean 5.9 ± 2.2). The majority of the children (63%) were male. 50% of the children had a history of associated diseases, such as allergic rhinitis in 13 children (27%), recurrent otitis media in 3 children (6%), mild asthma in 2 children (4%), and two other diseases in the remaining 6 children (12.5%). As per the parents/guardians, the last acute infection of the upper airways occurred in an average of 9 months (1 to 24) before surgery. Eight children (17%) were exposed to COVID-19 1 to 13 months before surgery. Two patients had previous laboratory-confirmed SARS-CoV-2 infection 3 to 5 months before surgery.

According to the rt-PCR test, 27% of the patients (23/48) were positive for SARS-CoV-2. Also, 27% (13/48) of the palatine tonsil samples, 28% of adenoid samples, 20% of nasal cytobrush samples, and 12% of nasal washes were positive for SARS-CoV-2. The viral loads

of SARS-CoV-2 displayed significant variation. The median viral load in tonsillar tissues was about two-fold higher than in nasal tissues, but there was no significant difference between samples.

Anti-SARS-CoV-2 IgM and IgG antibodies were assessed in 12 of 13 children who tested positive for SARS-CoV-2. IgG antibodies were detected in five patients, while no patient was positive for IgM antibodies.

Analysis of adenoid and palatine tonsils

Immunohistochemistry showed positivity for the SARS-CoV-2 structural N protein in 11 of 13 (85%) tonsil tissue samples positive for SARS-CoV-2 by qRT-PCR. The epithelial surface and lymphoid compartment in extrafollicular and follicular regions of both, adenoids and palatine tonsils were positive for the SARS-CoV-2 N protein.

Also, 54% of samples (7/13) positive for the SARS-CoV-2 by rt-PCR were positive not only for the SARS-CoV-2 N protein but also for the non-structural protein NSP16. The robust indicator of SARS-CoV-2 replication, NSP-16, plays a crucial role in the process of viral RNA capping, which is crucial for efficient viral replication. The identification of NSP-16 in lymphoid tissues provided strong evidence for the ongoing replication of SARS-CoV-2.

The SARS-CoV-2 structural N protein was not solely restricted to tonsils but it was also detected in nasal cells from the olfactory region in two of five patients who tested positive for SARS-CoV-2 by qRT-PCR**.** The presence of the SARS-CoV-2 structural N protein in cells from the olfactory region indicates that prolonged SARS-CoV-2 infection is not limited to tonsillar cells.

The ACE2 and TMPRSS2 were more expressed in tonsillar samples positive for SARS-CoV-2 than in SARS-CoV-2 negative samples. The same regions of increased ACE2 and TMPRSS2 expression were also positive for the SARS-CoV-2 S protein. According to the authors, the higher expression of ACE2 and TMPRSS2 in SARS-CoV-2-infected tonsils may suggest that tonsillar infection promotes increased expression of ACE2 and TMPRSS2.

Flow cytometry showed that CD20+ B lymphocytes were the most frequently infected phenotypes in tonsil tissue samples, with median values of 22.9% and 28.1% in palatine tonsils and adenoids, respectively. They were followed by CD4+ T lymphocytes, CD123 dendritic cells, and CD8+ T lymphocytes. At the same time, CD14+ macrophages were the least abundant infected cell type, with median values of 6.9% and 10.6% in palatine tonsils and adenoids, respectively. The authors noted that infection of tonsillar monocytes,

macrophages, and dendritic cells by SARS-CoV-2 may enhance inflammation in an already chronically inflamed tissue. They also said that it is not surprising that B lymphocytes constitute about one-quarter of SARS-CoV-2-infected cells in tonsils since they are the most abundant cells in secondary lymphoid organs, but, surprisingly, CD8+ T lymphocytes, which are central cells for the combat of viral infections, were also infected.

Nanopore sequencing revealed the presence of different SARS-CoV-2 variants in 12 samples (9 tonsils and 3 cytobrushes) from 10 patients.

Conclusion

This study has shown that different lineages of SARS-CoV-2 were found in the upper respiratory tract of one-quarter of children who had undergone adenotonsillectomy. Five of the 13 children were positive for SARS-CoV-2 IgG, but none were positive for SARS-CoV-2 IgM. In 23% of children of the whole sample, and 85% of children who tested positive for SARS-CoV-2 by RT-qPCR, tonsil tissue samples were positive for the SARS-CoV-2 N protein. Apart from epithelial cells, all major types of lympho-mononuclear cells were positive for the SARS-CoV-2 N protein. The authors noted that the detection of the viral RNA could be considered a remnant of a previous infection. However, the discovery of the structural SARS-CoV-2 protein *in situ* in adenoids and palatine tonsils, in both epithelial and lymphomononuclear cells of different lymphoid compartments, suggests that the tonsils may be sites of prolonged infection, even without recent COVID-19 symptoms. In addition, the presence of non-structural SARS-CoV-2 protein NSP-16 provided evidence of viral replication in at least half of the lymphoid tissues that were positive for SARS-CoV-2. This supports the concept of persistent viral activity in these tissues.

The authors concluded that these findings support the hypothesis that SARS-CoV-2 reservoirs may exist in hypertrophic tonsils of COVID-19 asymptomatic children for an unknown time.

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