Saliva is not a useful diagnostic specimen in children with Coronavirus Disease 2019

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To the editor

Saliva specimens have shown promise for diagnosing COVID-19 in adults, with a sensitivity of 50.5-96% [1-8]. However, in children, the utility of saliva specimens is uncertain; thus, we compared saliva to nasopharyngeal (NP) swabs for diagnosing COVID-19 in children.

COVID-19-infected children admitted from 22 June-22 July 2020 had paired NP and saliva specimens tested using a real-time reverse transcription (rRT)-PCR assay for the E gene of SARS-CoV-2 [9]. After abstaining from food and drink for ≥ 0.5 hour, saliva (minimum 0.5 ml) was collected by patients spitting into a sterile container or by nurses who syringed saliva from inside the mouth of children who were unable to spit.

Cycle threshold (Ct) values of specimens were recorded according to the day of illness (onset of symptoms) for symptomatic patients or day of diagnosis for asymptomatic patients. We grouped days into day 1-3, 4-7, 8-10 and 11-15 for analysis. The Ct cut-off for undetectable virus was 45. Saliva PCR sensitivity was calculated by percent of patients with positive saliva results among those with positive NP results for a given time period.

Eighteen children were included; 12 (66.7%) asymptomatic and 8 (33.3%) symptomatic with mild upper respiratory tract infection. The mean age was 6.6 years (interquartile range [IQR] 1.8-11.1), and 10 (55.6 %) patients were male. Patients provided a mean of 3.1±1.4 paired samples (IQR 2-4, range 1-7).

Saliva and NP Ct trends are shown in Figure 1. In 5 (27.8%) patients, saliva PCR was persistently negative, including 1 asymptomatic child who only had samples tested on day 6 of admission (NP Ct 37.9, saliva negative). In another 5 (27.8%) patients, saliva that was initially negative on day 1-3 turned positive on day 4-7.

Saliva PCR had higher Ct compared to NP swabs. The Ct differences were statistically significant for all time periods except day 11-15. Saliva PCR sensitivity was highest at 52.9% on day 4-7 compared to the other time periods. Both paired samples were negative in 1 patient on day 8-10 and in 4 patients

on day 11-15. Saliva PCR became negative on mean day 9 ± 3.7 (IQR 6-11); the mean corresponding NP Ct was 31.0 ± 6.0 (IQR 28.8-33.3). Young children <5 years (n=5) had lower mean NP Ct when saliva was negative compared to older children ≥5 years old (n=13) (27 ± 6.8 vs 33.3 ± 4.2, p=0.037).

Males were more likely to be asymptomatic (90%, 9/10 vs 37.5%, 3/8, p=0.043) and had higher saliva Ct on day 1-3 (29.2 ± 5.6 vs 22.2 ± 6.4, p=0.045) compared to females. When saliva PCR was negative, males also had higher NP Ct compared to females (33.5 ± 4.8 vs 28.0 ± 6.1, p=0.048). There were no age or Ct differences between symptomatic and asymptomatic patients.

Our study's limitation was 7 out of 55 (12.7%) paired samples had a delayed (3-28 hours) first saliva collection while awaiting NP confirmation of COVID-19.

In conclusion, saliva is not a useful specimen for diagnosing COVID-19 in children. The peak saliva sensitivity was only 52.9% compared to NP swabs. Our previous buccal swab study had a peak sensitivity of 71.4% [10].

Note

References

1. To, K.K.-W, Tsang OT, Leung WS, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: An observational cohort study. Lancet Infect. Dis. 2020, 20, 565–574.

2. To, K.K.-W, Tsang OT, Yip CC, et al. Consistent detection of 2019 novel coronavirus in saliva. Clin. Infect. Dis. 2020; 71 (15): 841-843.

3. Azzi L, Carcano G, Gianfagna F, et al. Saliva is a reliable tool to detect SARS-CoV-2. J Infect. 2020;81(1): e45-e50. doi:10.1016/j.jinf.2020.04.005

4. Chen JH, Yip CC, Poon RW, et al. Evaluating the use of posterior oropharyngeal saliva in a point-of-care assay for the detection of SARS-CoV-2. Emerg Microbes Infect. 2020;9(1):1356-1359. doi:10.1080/22221751.2020.1775133

5. Pasomsub E, Watcharananan SP, Boonyawat K, et al. Saliva sample as a non-invasive specimen for the diagnosis of coronavirus disease 2019: a cross-sectional study [published online ahead of print, 2020 May 15]. Clin Microbiol Infect. 2020; S1198-743X(20)30278-0. doi:10.1016/j.cmi.2020.05.001

6. Wyllie AL, Fournier J, Casanovas-Massana A, et al. Saliva is more sensitive for SARS-Cov-2 detection in COVID-19 patients than nasopharyngeal swabs. MedRxiv 2020.04.16.20067835. https://doi.org/10.1101/2020.04.16.20067835

7. Nagura-Ikeda M, Imai K, Tabata S, et al. Clinical evaluation of self-collected saliva by RT-qPCR, direct RT-qPCR, RT-LAMP, and a rapid antigen test to diagnose COVID-19 [published online ahead of print, 2020 Jul 7]. J Clin Microbiol. 2020; JCM.01438-20. doi:10.1128/JCM.01438-20

8. McCormick-Baw C, Morgan K, Gaffney D, et al. Saliva as an alternate specimen source for detection of SARS-CoV-2 in symptomatic patients using Cepheid Xpert Xpress SARS-CoV-2. J Clin Microbiol. 2020; 58(8): e01109-20. Published 2020 Jul 23. doi:10.1128/JCM.01109-20

9. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCovV) by real-time RT-PCR. Euro Surveill 2020 Jan; 25. Doi: 10.2807/1560-7917.ES.2020.25.3.2000045

10. Kam KQ, Yung CF, Maiwald M et al. Clinical utility of buccal swabs for Severe Acute Respiratory Syndrome Coronavirus 2 detection in Coronavirus Disease 2019–infected children. J Pediatr Infect Dis Soc 2020; 9(3):370–2.
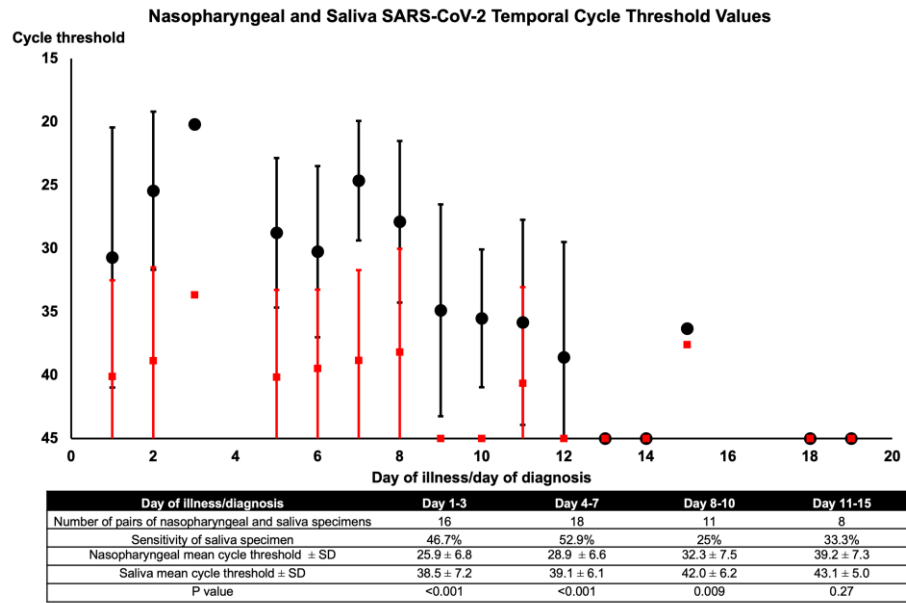
Figure legend:

⬤Nasopharyngeal SARS-Cov-2

🟥Saliva SARS-Cov-2

Data points denote the mean; error bars indicate SD. The annotations below the graph show the no. of specimens, sensitivity and Ct grouped by time period.

Figure 1

Nasopharyngeal and Saliva SARS-CoV-2 Temporal Cycle Threshold Values

Day of illness/diagnosis	Day 1-3	Day 4-7	Day 8-10	Day 11-15
Number of pairs of nasopharyngeal and saliva specimens	16	18	11	8
Sensitivity of saliva specimen	46.7%	52.9%	25%	33.3%
Nasopharyngeal mean cycle threshold $\pm$ SD	25.9 ± 6.8	28.9 ± 6.6	32.3 ± 7.5	39.2 ± 7.3
Saliva mean cycle threshold $\pm$ SD	38.5 ± 7.2	39.1 ± 6.1	42.0 ± 6.2	43.1 ± 5.0
P value	<0.001	<0.001	0.009	0.27