LETTER TO EDITOR

USEFULNESS OF SALIVA SAMPLES FOR DETECTING SARS-CoV-2 RNA AMONG LIVER DISEASE PATIENTS

Dear Editor,

In a recent article in the *Journal*, Azzi and colleagues (1) evaluated saliva samples of 25 COVID-19 patients by real time RT-PCR. In this study, all individuals had severe or very severe infection. All of them had SARS CoV-2 detected in saliva samples and there is no information regarding the presence of liver diseases.

Diagnostic testing for COVID-19 is made through nasopharyngeal (NP) and oropharyngeal (OP) swabs. Saliva samples could be useful specimens since collection is less invasive, safer and allows the option of self-collection. Saliva samples have been evaluated for detecting viral hepatitis markers (2,3), however there is a lack of studies about usefulness of these samples for detecting SARS CoV-2 in hepatitis infected individuals and non-severe cases of COVID-19. The saliva collection can be safer than NPS samples, especially for those patients that presenting decompensated cirrhosis or other severe sequels, like hepatocarcinoma. This study aims to evaluate the usefulness of saliva for detecting SARS-CoV-2 RNA according the presence of liver disease patients.

Nowadays, Brazil has the second number of confirmed cases of COVID-19 in the world and no information is available regarding the number of cases in liver disease patients. The study protocol was approved by the Brazilian National research ethics committee under the number n° 4.014.273 and complied with the clinical research guidelines of the Declaration of Helsinki.

First, we evaluated extraction method and limit of detection of artificially spiked SARS-CoV-2 saliva samples (estimated viral load: 10^3, 10^2, 10^1, 10^0 copies/mL). Saliva were collected using Salivette Device as previous described (3). These samples were tested in triplicate using two extraction methods (M1: PureLink RNA Mini Kit, Thermo Fisher Scientific, Waltham, USA and M2: QIAamp Viral RNA Mini Kit, QIAGEN, Germany) following manufacturer's recommendations with some modifications (low elution volume) along to real time PCR that amplifies N1 and N2 regions (2019-nCoV CDC EUA Kit, Integrated DNA Technologies, Coralville, USA) (4). M1 used $200\mu L$ of samples to extraction and RNA was eluted in 100 μL, M2 used 140 μL of sample volumes and was eluted in 50 μL.

Both methods were feasible to extract SARS-CoV-2 RNA saliva, however using M1 the detection limit was 10 copies/mL and M2 the limit of detection was 1 copy/mL. M2 was applied to extract RNA from saliva and NPS from 13 volunteers (5 hepatitis cases and 8 non hepatitis cases).

Volunteers gave saliva samples using Salivette device after signing informed consent. A total of four individuals (two hepatitis cases and two without liver disease) were negative to SARS CoV-2 in NPS and saliva (100% of specificity). The overall positivity was 9/13 (69.2%) lower than observed in saliva from ambulatory pa-
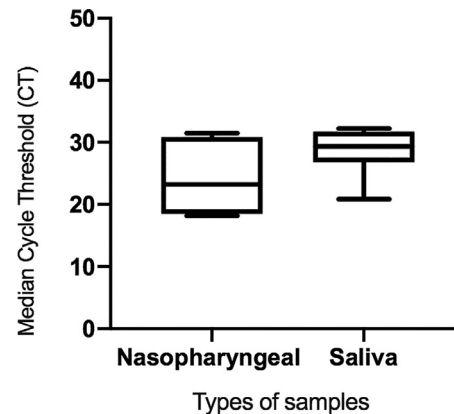
Figure 1. Box Plot Graph of cycle threshold (Ct) values in nasopharyngeal swabs and saliva specimens of positive samples for SARS CoV-2. Vertical lines indicate range of values, and the median Ct value is represented as black horizontal line within the box plot. The box indicates the 25th and 75th percentiles. Abbreviations: NPS, nasopharyngeal swab.

tients without liver disease (84.6%) (5). A total of 11/13 (84.6%) had concordant results in saliva and NPS samples what is lower than observed by Azzi and coleagues (1) and probably is the reflex of severity of disease among both studies. Positive concordant results in NPS and saliva were observed in seven individuals (two hepatitis cases and 5 without liver disease) until 7 days after onset of symptoms (100% of sensitivity). After 7 days of onset of symptoms, RNA was detected in NPS but it was not observed in paired saliva samples.

Figure 1 shows the comparison of median, maximum and minimum of cycle threshold (CT) values. Positive NPS and saliva samples presented median CT of 23.2 and 29.3, respectively.

This is the first report of SARS CoV-2 detection in saliva samples among liver disease patients showing best results until 7 days of beginning of symptoms. There is an urgency for alternative methods for SARS-CoV-2 RNA detection to overcome swab availability and increase the access of diagnosis. Saliva samples have been evaluated for SARS CoV-2 RNA detection in severe cases or hospitalized patients, but there is a lack of data about theses samples in mild cases or a standard protocol for sample collection and viral detection. In addition, there is no information regarding the usefulness of saliva for detecting SARS CoV-2 RNA in individuals presenting comorbidities, such as liver disease. The present study gives new information regarding the presence of SARS CoV-2 in saliva of liver disease patients. Since saliva can be collected easily, SARS CoV-2 RNA detection in saliva can be useful strategy to increase the access of sample collection for the diagnosis of COVID-19 in patients with liver disease.

References

1. Azzi L, Carcano G, Gianfagna F, Grossi P, Gasperina DD, Genoni A, Fasano M, Sessa F, Tettamanti L, Carinci F, Maurino V, Rossi A, Tagliabue A, Baj A. Saliva is a reliable tool to detect SARS-CoV-2. *J Infect* 2020;**81**(1):e45–50. doi:10.1016/j.jinf.2020.04.005.
2. Cruz HM, de Paula VS, da Silva EF, do Ó KMR, Milagres FAP, Cruz MS, Bastos FI, da Mota JC, Pollo-Flores P, Leal E, Motta-Castro ARC, Lewis-Ximenez LL, Lampe E, Villar LM. Utility of oral fluid samples for hepatitis B antibody detection in real life conditions. *BMC Infect Dis* 2019 Jul 17;**19**(1):632 PMID:31315573PMCID:PMC6637497. doi:10.1186/s12879-019-4183-0.
3. Portilho MM, Mendonça A, Marques VA, Nabuco LC, Villela-Nogueira CA, Ivantes C, Lewis-Ximenez LL, Lampe E, Villar LM. Comparison of oral fluid collection methods for the molecular detection of hepatitis B virus. *Oral Dis* 2017;**23**(8):1072–9. doi:10.1111/odi.12692.
4. Centers for Disease Control and Prevention (CDC). CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. Available on: https://www.fda.gov/media/134922/download. Accessed: June 26th 2020.
5. Williams E, Bond K, Zhang B, Putland M, Williamson DA. Saliva as a non-invasive specimen for detection of SARS-CoV-2. *J Clin Microbiol* 2020 JCM.00776-20. doi:10.1128/JCM.00776-20.

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