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BRIEF REPORT

Saliva screening of health care workers for SARS-CoV-2 detection

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Abstract Health care workers (HCWs) are at high risk for SARS-CoV-2. In addition, pre-symptomatic or asymptomatic transmission accounts for around half of the cases. Saliva testing is an option to detect SARS-CoV-2 infection. To determine the performance of saliva samples for screening, HCWs were tested for SARS-CoV-2 by RT-PCR. Those with a positive result in saliva were tested by nasopharyngeal swabbing for viral RNA detection and blood collection to search for the presence of specific antibodies. In September–October 2020, 100 HCWs were enrolled and followed up. Six subjects (6%) tested positive in saliva. Of them, 5/6 were positive in a subsequent nasopharyngeal swab and 4/6 developed signs and symptoms compatible with COVID-19. Among the latter, 3 seroconverted while asymptomatic HCWs remained seronegative. Saliva screening was helpful for identifying SARS-CoV-2 infection in HCWs. This screening permitted rapid personnel isolation avoiding further transmission of the virus in the hospital setting.

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PALABRAS CLAVE
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Análisis del personal de salud a partir de saliva para detección de SARS-CoV-2

Resumen El personal de salud (PS) tiene un alto riesgo de contraer SARS-CoV-2. La transmisión presintomática/asintomática representa alrededor de la mitad de los casos y el análisis a partir de muestras de saliva puede ser una opción para detectar la infección. Para determinar el rendimiento de estas muestras, 100 voluntarios del PS se sometieron a la detección de SARS-CoV-2 por RT-PCR en muestras de saliva en el período septiembre-octubre de 2020. De aquellos con resultado positivo en saliva, se tomaron hisopados nasofaríngeos para detectar ARN viral y muestras de suero para evaluar anticuerpos específicos. Se detectó ARN viral en la saliva de seis individuos (6%). De ellos, 5/6 fueron SARS-CoV-2 positivos en hisopado nasofaríngeo y 4/6 desarrollaron signos y síntomas compatibles con COVID-19. Entre estos últimos, tres seroconvirtieron, en tanto que los voluntarios asintomáticos permanecieron seronegativos. La muestra de saliva fue útil para identificar la infección por SARS-CoV-2 en esta cohorte del personal de salud y así proceder al rápido aislamiento de los individuos infectados, lo que evitó una mayor transmisión del virus en el ámbito hospitalario.

Due to the frequent exposure to patients with COVID-19, health care workers (HCWs) are at high risk for SARS-CoV-2 infection. Pre-symptomatic or asymptomatic transmission account for around half of the cases. Therefore, increasing capacity and early diagnostic testing would help to reduce the transmission of the virus[5]. Screening approaches are mostly focused on symptomatic HCWs[16] and there are few studies detecting SARS-CoV-2 in pre-symptomatic or asymptomatic HCW[11,12].

The nasopharyngeal swab (NPS) is the most common respiratory sample utilized for SARS-CoV-2 diagnosis. However, NPS is associated with subjects' discomfort and further exposure to viral aerosols during sample collection[8]. Saliva testing is a non-invasive test to detect SARS-CoV-2 that avoids further HCW exposure. Saliva testing proved to be as sensitive as NPS in detecting SARS-CoV-2 in symptomatic patients[6,7]. However, the role of saliva testing among asymptomatic HCWs is still to be determined.

The objective of this study was to determine the performance of the saliva test to screen for SARS-CoV-2 in HCWs.

A descriptive prospective cohort study in HCWs was conducted at CEMIC University Hospital, Buenos Aires, Argentina. Voluntary SARS-CoV-2 testing in saliva was performed in both hospital sites: CEMIC Saavedra and CEMIC Pombo. HCWs were enrolled at 12 different hospital settings. These settings were classified as ''high'', ''middle'' or ''low'' in terms of exposure risk to SARS-CoV-2. High exposure risk settings included: emergency room, intensive care unit (ICU), infectious diseases, and personnel assisting COVID-19 positive inpatients or specimens (technicians, nurses, physicians, physical therapists, laboratory). Middle exposure risk settings included: personnel assisting COVID-19 negative inpatients (technicians, nurses, physicians, physical therapists). Low exposure risk areas included those without contact with patients or clinical specimens (pharmacy, administration).

An electronic form with personal data and information related to COVID-19 disease or exposure was collected at the time of sampling. Clinical follow-up for any signs or symptoms compatible with COVID-19 was obtained from participants for two weeks from the initial collection of saliva. A nasopharyngeal swab and a blood sample for serology were obtained from participants with positive RT-PCR results for SARS-CoV-2.

The study was approved by the CEMIC Ethics Committee (Protocol: 1298/20) and electronic informed consent was obtained from all participants.

At study entry, a self-collected saliva sample was obtained for SARS-CoV-2 diagnosis. Participants were instructed on how to collect the sample in a sterile plastic container without viral transport medium. Samples were conserved at 4 °C until processed in a biosafety cabinet within 12 h of arrival. Viscous saliva samples were mechanically disrupted by adding 500 μl viral transport medium [Minimun Essential Medium (Gibco); L-Glutamin 200 mM; HEPES 1 N; bovine serum albumin 5% (Sigma) sodium bicarbonate 7.5%; penicillin, streptomycin and amphotericin (pH = 7.2)]. Participants in whom SARS-CoV-2 was detected by PCR in the initial sample of saliva were requested to provide additional saliva samples and NPS. NPS were obtained and placed in a sterile tube containing 2 ml viral transport media.

Nucleic acid was extracted from 100 μl saliva sample and eluted in 15 μl using manual columns (Quick-RNA™ Viral Kit. Zymo Research CORP.) following the manufacturer's recommendation. One-step real-time multiplex RT-PCR laboratory-optimized was performed, targeting the SARS-CoV-2 E gene and the human RNAse P gene as quality control[4,6]. A positive result was considered when the cycle threshold (Ct) value for the SARS-CoV-2 E gene was lower than 40 and the human RNAse gene was positive. Analytical sensitivity was 1 copy/μl. Discrepant PCR results were tested using a commercial RT-PCR kit that amplifies the SARS-CoV-2 E gene and the S gene and includes an internal amplification control (Real Star® SARS-CoV-2 RT-PCR Kit 1.0. Altona Diagnostics Argentina S.R.L.). Real time assays

were performed in a CFX 96 Deep Well™ Real Time System (BioRad).

Serum samples were analyzed for the presence of SARS-CoV-2 specific IgM and IgG antibodies using ELISA kit COVIDAR IgM/IgG. The assay uses a trimer stabilized spike protein and the RBD (FIL-CONICET-Laboratorio Lemos, Argentina)[9].

Ct values of saliva vs. those observed in NPS were compared with a paired *Student's t*-test. A two-sided *p*-value of <0.05 was considered significant (GraphPad Prism 8.0.2, San Diego California, USA).

From September 9th to October 13th 2020, 100 asymptomatic/presymptomatic HCWs were enrolled in the study. Demographic characteristics were described as appropriate. Almost two thirds were female (67%) and the median age was 37 years old (IQR = 31—46). Around half of the participants (53%) described having been in contact with COVID-19 patients at some point and 3.8% of these participants reported previous SARS-CoV-2 infection, but were negative in saliva or NPS sample at the time of this screening.

Of 100 HCWs, 6 asymptomatic subjects (6%) were positive in saliva (2 physicians, 2 nurses, and 2 administrative personnel, respectively). All positive HCWs belonged to high exposure settings. Upon the initial results in saliva, all these HCWs were separated and licensed from work on the same day of sample collection.

NPS from positive patients were mostly obtained within 24–48 h from saliva testing. Five of six HCWs with positive salivawere positive in subsequent NPS (Table 1), no significant differences between Ct values (saliva vs. NPS) were observed ($p = 0.3173$). A patient with negative NSP showed a positive saliva test with a high Ct value. This result was confirmed in duplicate and also using a different RT-PCR assay (home-brew and commercial kit Altona Diagnostic). A follow-up saliva sample obtained 48 h later was also positive but with a higher Ct value. Among HCWs with positive saliva, 4/6 developed signs and symptoms compatible with COVID-19 within 2–4 days after the initial collection of saliva. The other 2 HCWs remained asymptomatic. Median Ct values in saliva were lower in pre-symptomatic (mean = 28.36; range: 15.26–36.80) versus asymptomatic (mean = 34.63; range: 33.71–35.53) HCW ($p = 0.4033$). Of 4 pre-symptomatic HCWs with positive saliva, 3 seroconverted. None of the two HCWs who remained asymptomatic seroconverted (Table 1). A follow-up saliva sample obtained from the asymptomatic HCW (ID #4) confirmed positivity although with a higher Ct value (Ct = 37). None of 94 HCWs who tested negative for SARS-CoV-2 in saliva developed symptoms.

Expanding screening protocols for detecting pre-symptomatic or asymptomatic carriers among HCWs is critical to reduce the spread of SARS-CoV-2 in the hospital setting. Some of the challenges to establish these protocols are related to logistical issues, turnaround times and further exposure to HCW during sampling. Self-collected saliva provides an option to facilitate sample collection, reduce discomfort and minimize HCW exposure. There are few reports utilizing saliva samples in the routine testing of asymptomatic or pre-symptomatic patients, including HCWs[15]. Generally, saliva tests were utilized for follow-up after a positive NPS but not as a primary screening method[13]. In this study, we have used saliva samples as a primary screening method to detect SARS-CoV-2 among HCWs. The incidence of SARS-CoV-2 in pre-symptomatic/asymptomatic HCWs reaching 6% was higher than expected. Most of our positive cases corresponded to HCWs performing work activities in high or middle risk areas. Some authors reported negative results for saliva testing among HCWs[1] while others found percentages ranging from 1.5 to 12.5% using NPS in those asymptomatic[12,14,16]. Our screening results in saliva are comparable with studies using NPS. A high correlation between saliva and NPS was previously demonstrated in symptomatic patients using a highly sensitive home brew RT-PCR[6]. In this study, saliva was a useful, valuable and easy tool for SARS-CoV-2 screening and for identifying pre-symptomatic and asymptomatic individuals. Importantly, rapid identification of positive HCWs permitted early licensing avoiding potential SARS-CoV-2 spreaders within the hospital setting. Furthermore, saliva positivity anticipated clinical disease up to 3 days before symptom onset. Long-term SARS-CoV-2 RNA shedding can occur in saliva. In our study, the only patient who gave a discrepant result between NPS and saliva and remained asymptomatic showed a follow-up saliva with a higher Ct value, probably representing prolonged shedding in this sample type[17].

Of 6 positive HCWs, 2 remained asymptomatic. Studies evaluating nasopharyngeal swabs in HCWs demonstrated that 15% to 57% remained asymptomatic[2,11]. Seroconversion was demonstrated in most of our symptomatic patients. The patient who did not seroconvert even 90 days after symptom onset had a very mild disease. This finding is not unexpected since IgG titers have been associated with disease severity[9]. In addition, lack of seroconversion was previously described in 17% of HCWs infected with SARS-CoV-2[3].

Saliva is an easy to obtain sample that can be self-collected. It is especially convenient for screening in individuals without respiratory symptoms. Furthermore, special attention should be given to asymptomatic individuals who may spread the virus more easily due to higher human interaction than symptomatic patients[10]. Interestingly, previous studies with saliva testing have shown discrepant results. We believe the reason for such discrepancy is related to sample processing and the PCR techniques employed. We have systematically conducted and applied an optimized PCR (home brew). Our technique includes mechanical disruption of the saliva, no addition of any stabilizer or buffer and an increased concentration of magnesium (3.8 mM) in the PCR mix as well as modifications in cycling conditions[6]. Therefore, the optimization of our PCR resulted in an increased analytical and clinical sensitivity. Furthermore, this home brew assay incurred less costs than those related to commercial PCR kits (data not shown).

This study has several limitations. Our investigation was based on voluntary participation and this may have introduced some voluntary bias. In addition, other populations need to be tested to define the role of saliva to detect SARS-CoV-2 in symptomatic, pre-symptomatic or asymptomatic subjects. Finally, our sample size was not large. However, the number was deemed to be epidemiologically meaningful. Supporting this concept, we were able to detect 6% of HCWs infected with SARS-CoV-2 while they were pre-symptomatic/asymptomatic.

The screening protocol with the saliva sample test proposed in this work permitted rapid personnel isolation avoiding further transmission of the virus in the hospital setting.

Table 1 Health care workers with a positive SARS-CoV-2 PCR in saliva. Clinical follow-up and NPS correlation.

ID	Work area exposure	Age	Sex	Saliva		NPS		Signs and symptoms (days after saliva test)	Antibodies (days after saliva test)
				PCR (Ct)	Date	Days after saliva test	PCR (Ct)		
1	High (emergency)	48	F	POS (36.80)	10/09/2020	+1	POS (29.32)	Myalgias (+4)	IgM: Neg IgG: Neg (+90)
2	High (emergency)	45	F	POS (32.20)	11/09/2020	+3	POS (25.60)	Headache, dyspnea, fever and retroocular pain (+4)	IgM: Pos IgG: Pos (+20)
3	High (emergency)	46	M	POS (15.26)	14/09/2020	+1	POS (16.96)	Fever, myalgias and headache (+2)	IgM: Pos IgG: Pos (+30)
4	High (emergency)	46	F	POS (35.53)	15/09/2020	+1	NEG	Asymptomatic	IgM: Neg IgG: Neg (+34)
5	High (COVID hospitalization area)	42	F	POS (28.19)	17/09/2020	+7	POS (21.45)	Anosmia, dysgeusia, nasal congestion and myalgias (+4)	IgM: Neg IgG: Pos (+33)
6	High (COVID hospitalization area)	49	F	POS (33.71)	23/09/2020	+2	POS (26.81)	Asymptomatic	IgM: Neg IgG: Neg (+26)

NPS: nasopharyngeal swab; ICU: intensive care unit; Neg: negative; Pos: positive.

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Conflict of interest

MS is a consultant to Basilea, a speaker for Pfizer and the principal investigator in Argentina for NIH grant UM1AI104681. The rest of the authors have no conflict of interest.

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