Aplicabilidade da saliva no diagnóstico da COVID-19: uma revisão

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Aplicabilidad de la saliva em el diagnóstico de COVID-19: una revisión

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Resumo

Em 2020, a Organização Mundial da Saúde (OMS) classificou a COVID-19 como uma pandemia global. Desde então, existe a necessidade de novos métodos que facilitem o diagnóstico e controle dessa doença. Atualmente, a transcrição reversa seguida de reação em cadeia da polimerase em tempo real (rRT-PCR) de amostras respiratórias obtidas por swabs representa o padrão ouro na detecção qualitativa da infecção por Sars-CoV-2. Contudo, esse tipo de coleta apresenta diversas desvantagens, tornando a saliva uma potencial ferramenta para o diagnóstico da COVID-19. Diante disso, o objetivo desse estudo é avaliar, por meio de uma revisão sistematizada da literatura científica atual, a aplicabilidade da saliva para o diagnóstico da COVID-19 em comparação aos atuais métodos utilizados. Realizou-se uma busca nas bases PubMed, SciELO, Scopus e Web of Science, utilizando descritores, estratégias e critérios

preestabelecidos, por dois avaliadores, de forma independente, seguida de uma busca manual nas referências do artigos selecionados para leitura integral. As estratégias de pesquisa identificaram 476 estudos e 1 estudo foi adicionado por meio de busca manual. Após análise, 200 artigos foram excluídos por estarem duplicados entre os resultados encontrados nas bases. Com a finalização do processo de triagem, 12 artigos foram incluídos nesta revisão. Concluiu-se que é necessário a produção de novos estudos a fim de se obter dados ainda mais confiáveis e efetivos acerca do uso da saliva no diagnóstico da COVID-19. Contudo, as pesquisas têm demonstrado que esse material pode ser um excelente tipo de amostra alternativa para a detecção do SARS-CoV-2.

Palavras-chave: Coronavírus; Saliva; Controle de infecção; Doenças infecciosas.

Abstract

In 2020, the World Health Organization (WHO) classified COVID-19 as a global pandemic. Since then, there is a need for new methods to facilitate the diagnosis and control of this disease. Currently, reverse transcription followed by real-time polymerase chain reaction (rRT-PCR) of respiratory samples obtained by swabs represents the gold standard in the qualitative detection of Sars-CoV-2 infection. However, this type of collection has several disadvantages, making saliva a potential tool for the diagnosis of COVID-19. Thus, the aim of this study is to evaluate, through a systematic review of current scientific literature, the applicability of saliva for the diagnosis of COVID-19 in comparison to current methods. A search was carried out in MEDLINE, SciELO, Scopus and Web of Science databases, using descriptors, strategies and pre-established criteria by two independent evaluators, followed by a manual search in the references of articles selected for full reading. The research strategies identified 476 studies and 1 study was added through manual search. After analysis, 200 articles were excluded because they were duplicated among results found in databases. With the completion of the screening process, 12 articles were included in this review. It was concluded that it is necessary to produce new studies in order to obtain even more reliable and effective data about the use of saliva in the diagnosis of COVID-19. However, studies have shown that this material can be an excellent alternative sample for the detection of SARS-CoV-2.

Keywords: Coronavirus; Saliva; Infection control; Infectious diseases.

Resumen

En 2020, la Organización Mundial de la Salud (OMS) clasificó al COVID-19 como una pandemia mundial. Desde entonces, existe la necesidad de métodos que faciliten el diagnóstico

y control de esta enfermedad. Actualmente, la transcripción inversa seguida de la reacción en cadena de la polimerasa en tiempo real (rRT-PCR) de muestras respiratorias obtenidas mediante hisopos representa el estándar de oro en la detección cualitativa de la infección por Sars-CoV-2. Este tipo de recolección tiene desventajas, por lo que la saliva es una herramienta potencial para el diagnóstico de COVID-19. Por tanto, el objetivo de este estudio es evaluar, mediante una revisión sistemática de la literatura, la aplicabilidad de la saliva para el diagnóstico de COVID-19 en comparación con los métodos actuales utilizados. Se realizó una búsqueda en las bases PubMed, SciELO, Scopus y Web of Science, utilizando descriptores, estrategias y criterios preestablecidos, por dos evaluadores, seguida de una búsqueda manual en las referencias de los artículos seleccionados para lectura completa. Las estrategias identificaron 476 estudios y se agregó 1 estudio mediante búsqueda manual. Tras el análisis, se excluyeron 200 por estar duplicados entre los resultados encontrados en las bases. Con la finalización del proceso, se incluyeron 12 artículos en esta revisión. Se concluyó que es necesario producir estudios para obtener datos aún más confiables y efectivos sobre el uso de la saliva en el diagnóstico de COVID-19. Sin embargo, la investigación ha demostrado que este material puede ser un excelente tipo de muestra alternativa para la detección del SARS-CoV-2.
Palabras clave: Coronavirus; Saliva; Control de infecciones; Enfermidades infecciosas.

1. Introduction

In December 2019, a series of cases of pneumonia was observed in the city of Wuhan, specifically in the Chinese province of Hubei, with clinical presentations very similar to viral pneumonia. Subsequently, analysis of the genomic sequencing of samples from the lower respiratory tract indicated the appearance of Coronavirus Disease 2019 (COVID-19), caused by the severe acute respiratory syndrome virus 2 (Sars-CoV-2) (Huang et al., 2020; Randad et al., 2020).

On March 11, 2020, the World Health Organization (WHO) classified COVID-19 as a global pandemic (WHO, 2020 (a)). Since then, the infection has spread rapidly around the world, mainly due to the high contagion rate of the virus (Bulut & Kato, 2020). Until July 14, 2020, the COVID-19 pandemic was responsible for affecting approximately 13 million individuals and causing more than 570 thousand deaths (WHO, 2020 (b)).

According to current research, reverse transcription followed by real-time polymerase chain reaction (rRT-PCR) of respiratory samples represents the gold standard in the qualitative detection of SARS-CoV-2 infection (Azzi et al., 2020). The rRT-PCR method applied in the

diagnosis of COVID-19 is based on the amplification of viral RNA in several cycles until there is enough genetic material to be detected, with the purpose of effectively diagnosing individuals infected with SARS-CoV-2 (Mesa & Castillo, 2020).

However, although this monitoring standard is widely used in the world, it has a number of disadvantages, such as exposure of health professionals to a disease with high risk of nosocomial transmission during sample collection, excessive spending on personal protective equipment (PPE), discomfort for the patient — since swabs are inserted deep into the nose or mouth to collect naso and oropharyngeal samples, respectively, generation of aerosols by inducing coughing and sneezing, in addition to some situations in which collection is contraindicated, as in cases of coagulopathic patients (Tajima, Suda, & Yano, 2020; To et al., 2020; Ceron et al., 2020; Ng et al., 2020).

Saliva is a complex of multiglandular secretions mainly composed of peeled oral epithelial cells, gingival crevicular fluid, metabolites, hormones and electrolytes, in addition to large number of proteins, such as immunoglobulins. Since the current method has several disadvantages and is invasive, saliva can be an excellent alternative sample for the diagnosis of COVID-19, as it has been increasingly used for the purpose of assessing human health. Thus, with the development of appropriate methods of collecting and processing samples, saliva will provide useful clinical information about the disease, facilitating diagnosis, management and control of COVID-19 (Ceron et al., 2020; Ng et al., 2020; Woźniak, Paluszkiewicz, & Kwiatek, 2019).

Studies have shown that saliva can be a reliable tool in the diagnosis of COVID-19. For Pasomsub et al. (2020) and Azzi et al. (2020), the saliva RT-PCR test has high sensitivity and performance comparable to the current most used method, showing the importance of saliva and the need for further research to confirm its potential diagnostic value. Therefore, saliva could facilitate the diagnosis of the disease, given the simplicity of sample collection and good diagnostic performance (Martina et al., 2020).

Thus, the present study aims to evaluate, through a systematic review of the current scientific literature, the applicability of saliva for the diagnosis of COVID-19 compared to the current commonly used methods.

2. Methods

The electronic search for articles was carried out in July 2020 in MEDLINE (via PubMed), Scientific Electronic Library Online (SciELO), Scopus and Web of Science

databases, including studies published from 2015 to June 2020, without restriction of language and country. Descriptors used were "Saliva", "Salivary gland", "COVID-19" and "Coronavirus", obtained from the Medical Subject Headings (MeSH) directory, used in combination. The Table 1 expresses the results obtained in the survey carried out in the databases previously mentioned. In addition to restrict results, "articles published in the last 5 years" and "fully available free of charge" filters were selected.

Table 1 - Results of the study survey carried out in the databases.

Search strategy	PubMed	SciELO	Scopus	Web of Science
"Saliva" AND "COVID-19"	95	6	66	36
"Saliva" AND "Coronavirus"	85	8	78	42
"Salivary gland" AND "COVID-19"	13	0	15	2
"Salivary gland" AND "Coronavirus"	11	0	15	4
Total	**204**	**14**	**174**	**84**

Source: Authors.

For the inclusion of studies, the following criteria were considered: 1- having experimental character; 2- treating saliva as potential non-invasive diagnostic method for COVID-19; 3- presenting detailed clinical information about the research carried out. On the other hand, the following exclusion criteria were considered: 1- duplicated articles; 2- those that did not meet pre-established parameters; 3- those not considering saliva as potential sample for the diagnosis of COVID-19.

After identifying articles and eliminating duplicates, the first phase of the selection consisted of analyzing the title and summary of publications. Thus, the aim was to select, at this stage, those that had in their titles any of the keywords previously established or terms relevant to the theme. In addition, with regard to reading the abstracts, those that fit the theme and met the pre-established inclusion criteria were chosen.

Finally, studies proceeded to the full reading phase, completing screening with 12 articles to compose this review. In addition, manual search was performed on the references of selected studies during the full reading stage in order to identify those that included the established prerequisites, but that were not included in the electronic search.

All searches were carried out by 2 independent evaluators. Articles that raised doubts were sent to a third reviewer.

3. Results

The search strategies in databases identified 476 studies and 1 study was added through manual search. After analysis, 200 articles were excluded because they were duplicated among results found in databases. After screening based on the reading of titles and abstracts, 256 studies were excluded, leaving 21 publications to be evaluated through full reading. After this stage, 12 articles were included in this review. The Figure 1 presents the whole trial and selection process of the articles.

Figure 1 - Flowchart with identification of studies, inclusions, and deletions in the different steps.
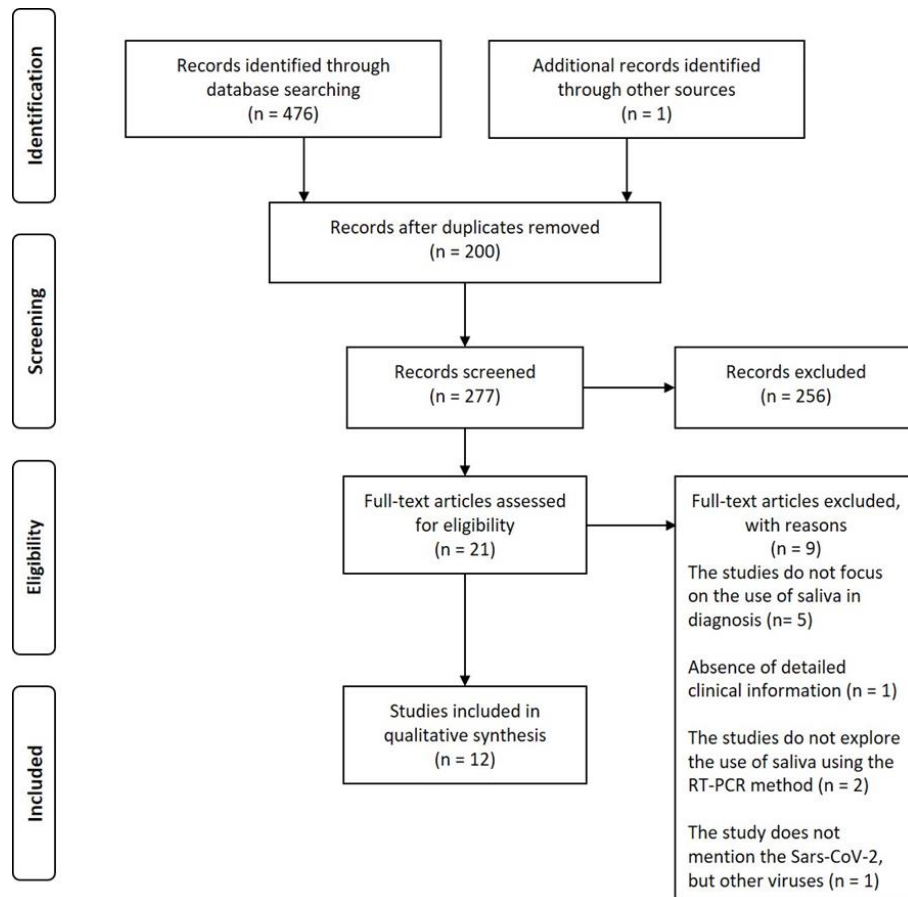
Source: Authors.

The main aspects of the 12 studies selected at the end of the last stage of the screening process are shown in Table 2. A number of advantages of the use of saliva in the diagnosis of COVID-19 were identified, in addition to the versatility regarding sample collection, configuring the potential use of this type of sampling for mass testing.

Table 2 - Summary of main characteristics of each selected study.

Study/ Year	Objective	Participants	Methods	Main results	Conclusions
Randad et al. (2020)*	To determine whether salivary Sars-CoV-2 specific antibody responses would identify prior Sars-CoV-2 infection with similar sensitivity and specificity as serum and whether salivary antibody testing would reflect the temporal profiles observed in serum.	167 saliva and 324 serum samples, including 134 and 118 negative saliva and serum samples, respectively, collected before the COVID-19 pandemic and 33 saliva and 206 serum samples from participants with RT-PCR-confirmed SARS-CoV-2 infection.	They evaluated the correlation of results obtained in saliva vs. serum and determined the sensitivity and specificity for each diagnostic media, stratified by antibody isotype, for detection of SARS-CoV-2 infection based on COVID-19 case designation for all specimens. Matched serum and saliva SARS-CoV-2 antigen-specific IgG responses were significantly correlated.	Within the 10-plex SARS-CoV-2 panel, the salivary anti-nucleocapsid (N) protein IgG response resulted in the highest sensitivity for detecting prior SARS-CoV-2 infection (100% sensitivity at ≥10 days post-SARS-CoV-2 symptom onset). The salivary anti-receptor binding domain (RBD) IgG response resulted in 100% specificity. Among individuals with SARS-CoV-2 infection confirmed with RT-PCR, the temporal kinetics of IgG, IgA, and IgM in saliva were consistent with those observed in serum.	SARS-CoV-2 seems to trigger a humoral immune response resulting in the almost simultaneous rise of IgG, IgM and IgA levels both in serum and in saliva, mirroring responses consistent with the stimulation.
Azzi et al. (2020)	To analyze salivary samples of COVID-19 patients and compared the results with their clinical	25 subjects with laboratory confirmed COVID-19 were recruited into this	Salivary samples of 25 COVID-19 patients were analyzed by rRT-PCR. The following data were collected: age, sex, comorbidities, drugs. Lactate dehydrogenase (LDH) and ultrasensitive reactive C protein (usRCP) values were registered on the same day when a salivary swab was collected. Prevalence of positivity in saliva and	All the samples tested positive for the presence of SARS-CoV-2, while there was an inverse association between LDH and Ct values. Moreover, two	Saliva is a reliable tool to detect SARS-CoV-2. The role of saliva in COVID-19 diagnosis could not be limited to a

and laboratory data.	study, 17 males and 8 females.	association between clinical data and the cycle threshold as a semiquantitative indicator of viral load were considered.	patients showed positive salivary results on the same days when their pharyngeal or respiratory swabs showed conversion.	qualitative detection of the virus, but it may also provide information about the clinical evolution of the disease.
Tajima et al. (2020)	To establish an alternative and rapid diagnostic method using saliva specimens.	1 patient with laboratory confirmed COVID-19.	Determine the best time for obtaining the saliva specimens, both daytime saliva specimens (DSS) and early morning saliva specimens (EMSS) were collected in the period described. The patient was given a collection container marked with a 600-μL line the day before his submitting saliva specimens. The saliva specimen collections were carried out by himself, spitting saliva up to the marked line. The samples were pre-treated with sugar chain-immobilized magnetic gold nanoparticles (SMGNP) to concentrate and purify virus particles at a rate of 5 min for one specimen.	Saliva specimens collected during the day had a lower rate of positive concordance when compared to National Institute of Infectious Diseases NIID results. For DSS, the sensitivity was 25.0% (2/8) and the specificity was 100% (1/1) based on NIID results. In contrast, when the EMSS were used, the results came close to matching the NIID results of RT-PCR performed on the nasopharyngeal specimens. The number of the EMSS was small, but the sensitivity based on NIID results was 66.7% (4/6) and the specificity was 100% (4/4).	The results of the last four saliva specimens point towards an appropriate collection method. It can suggest that virus monitoring after definitive diagnosis should be performed with EMSS concentrated and purified using SMGNP, and then performed with a nasopharyngeal specimen after the EMSS produces negative results.
To et al. (b) (2020)	To report on the results of the saliva testing, given the benefits of this type of test.	12 patients with laboratory-confirmed 2019-nCoV infection in	The saliva was collected by asking the patient to cough out saliva from their deep throat into a sterile container, and 2 mL of viral transport medium was added. Then, these	The 2019-nCoV was detected in the initial saliva specimens of 11 patients (91.7%). The median viral load of the first available saliva	The results have demonstrated the potential for saliva to be a noninvasive specimen type for the

	Hong Kong.	specimens were subjected to total nucleic acid extraction by NucliSENS easyMAG (BioMerieux). The viral culture of 2019-nCoV was conducted in a biosafety level-3 facility, following the process described. The virus-induced cytopathic effect was examined daily for up to 7 days.	specimens was 3.3×10^6 copies/mL. Serial saliva specimens were available for 6 patients. The highest viral load was in the earliest available specimens for 5 patients (83.3%).	diagnosis and viral load monitoring of 2019-nCoV.
Faustini et al. (2020)*	To report on the use of an antibody assay to detect antibodies in subjects with lower levels of SARS-CoV-2 specific-antibody.	There were three groups of subjects analyzed: Hospitalized subjects (HS, N=18), non-hospitalized convalescent (NHC, N=39) subjects and asymptomatic non-hospitalized convalescent patients (AS, N=6).	An ELISA assay was systemically developed, optimizing different antigens and amplification steps, in serum and saliva from symptomatic and asymptomatic SARS-CoV-2-infected subjects.	Using trimeric spike glycoprotein, rather than nucleocapsid enabled detection of responses in individuals with low antibody responses. IgG1 and IgG3 predominate to both antigens, but more anti-spike IgG1 than IgG3 was detectable. All antigens were effective for detecting responses in hospitalized patients. Anti-spike, but not nucleocapsid, IgG, IgA and IgM antibody responses were readily detectable in saliva from non-hospitalized symptomatic and asymptomatic individuals. Antibody responses in saliva and serum were largely independent of each	etecting antibody responses in both saliva and serum is optimal for determining virus exposure and understanding immune responses after SARS-CoV-2 infection.

			other and symptom reporting.	
Pasomsub et al. (2020)	To investigate the potential use of saliva samples as a non-invasive tool for the diagnosis of COVID-19.	200 individuals under investigation who attended an acute respiratory infection clinic at Ramathibodi Hosptial, Bangkok, Thailand, between 27 March and 4 April 2020.	Saliva samples were prospectively collected and a standard nasopharyngeal and throat swab in persons seeking care at an acute respiratory infection clinic in a university hospital during the outbreak of COVID-19. Real-time polymerase chain reaction (RT-PCR) was performed, and the results of the two specimens were compared.	Using nasopharyngeal and throat swab RT-PCR as the reference standard, the prevalence of COVID-19 diagnosed by nasopharyngeal and throat swab RT-PCR was 9.5%. The sensitivity and specificity of the saliva sample RT-PCR were 84.2% and 98.9% respectively. An analysis of the agreement between the two specimens demonstrated 97.5% observed agreement.	Saliva might be an alternative specimen for the diagnosis of COVID-19. The collection is non-invasive, and non-aerosol generating. This method could facilitate the diagnosis of the disease, given the simplicity of specimen collection and good diagnostic performance.
To et al. (a) (2020)	To examine the serial respiratory viral load of SARS-CoV-2 in posterior oropharyngeal saliva samples from patients with COVID-19 and serum antibody responses.	23 patients with laboratory confirmed COVID-19.	The researchers obtained the samples of blood, urine, saliva and rectal swabs. The serial viral load was determined by reverse transcriptase quantitative PCR (RT-qPCR). Antibody levels against the SARS-CoV-2 internal nucleoprotein and surface spike protein receptor-binding domain were measured using EIA. The whole-genome sequencing was done to identify possible mutations arising during infection.	The average viral load in the posterior oropharyngeal saliva or from different respiratory samples at presentation was 5.2 log10 copies per ml. The salivary viral load was higher during the first week after the onset of symptoms and a decrease was observed over time. Advanced age correlated with a higher viral load. For 16 patients with serum samples available 14 days or more after the onset of symptoms, seropositivity rates were 94% for anti-	Posterior oropharyngeal saliva samples are a more acceptable non-invasive alternative for patients and healthcare professionals. In contrast to severe acute respiratory syndrome, patients with COVID-19 had the highest viral load near presentation, which could be responsible for the rapid

			nuclearprotein IgG, 88% for anti-NP IgM, 100 % for anti-RBD IgG, and 94% for anti-RBD IgM. The levels of anti-SARS-CoV-2-NP or anti-SARS-CoV-2-RBD IgG correlated with the virus neutralization titer. No mutation of the genome was descried in serial samples.	spread of this epidemic.
Zheng et al. (2020)	To evaluate viral loads at different stages of disease progression in patients infected with the 2019 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) during the first four months of the epidemic in Zhejiang province, China.	96 consecutively admitted patients with laboratory confirmed SARS-CoV-2 infection: 22 with mild disease and 74 with severe disease.	The ribonucleic acid (RNA) viral load measured in respiratory, stool, serum, and urine samples. Cycle threshold values, a measure of nucleic acid concentration, were plotted onto the standard curve constructed based on the standard product. Epidemiological, clinical, and laboratory characteristics and treatment and outcomes data were obtained through data collection forms from electronic medical records, and the relation between clinical data and disease severity was analyzed.	Infection was confirmed in all patients by testing sputum and saliva samples. RNA was detected in the stool of 55 (59%) patients and in the serum of 39 (41%) patients. The urine sample from one patient was positive for SARS-CoV-2. The median duration of virus in stool was significantly longer than in respiratory and serum. The median duration of virus in the respiratory samples of patients with severe disease was significantly longer than in patients with mild disease. In the mild group, the viral loads peaked in respiratory samples in the second week from disease onset, whereas viral load continued to be high during the third week in the	The duration of SARS-CoV-2 is significantly longer in stool samples than in respiratory and serum samples, highlighting the need to strengthen the management of stool samples in the prevention and control of the epidemic, and the virus persists longer with higher load and peaks later in the respiratory tissue of patients with severe disease.

			severe group. Virus duration was longer in patients older than 60 years and in male patients.	
Yoon et al. (2020)	To evaluate the viral dynamics in various body fluid specimens, such as nasopharyngeal swab, oropharyngeal swab, saliva, sputum, and urine specimens.	2 patients with laboratory confirmed COVID-19.	Body fluid specimens were collected from the patients from hospital day 1 to 9, besides additional samples of the saliva were taken at 1 hour, 2 hours, and 4 hours after using a chlorhexidine mouthwash. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral load was determined by real-time reverse transcriptase polymerase chain reaction (rRT-PCR).	SARS-CoV-2 was detected from all the five specimens of both patients by rRT-PCR. The viral load was the highest in the nasopharynx, but it was also remarkably high in the saliva. SARS-CoV-2 was detected up to hospital day 6 (illness day 9 for patient 2) from the saliva of both patients. The viral load in the saliva decreased transiently for 2 hours after using the chlorhexidine mouthwash.	SARS-CoV-2 viral load was consistently high in the saliva; it was relatively higher than that in the oropharynx during the early stage of COVID-19. Chlorhexidine mouthwash was effective in reducing the SARS-CoV-2 viral load in the saliva for a short-term period.
Hung et al. (2020)	To investigate the ideal time for the collection of saliva, speculating that a sample in the early morning, before oral hygiene and breakfast, would increase the diagnostic yield.	18 patients with previously confirmed SARS-CoV-2 infection by molecular testing.	Posterior oropharyngeal saliva was collected at 5 different time points within the same day from 18 patients with previously confirmed SARS-CoV-2 infection by molecular testing. Cycle threshold (Ct) values were compared.	There was an overall trend of lower Ct values from specimens collected in the early morning, with a gradual decrease of viral load towards nighttime, but reaching statistical significance only when compared with the specimens collected at bedtime. Eight out of 13 subjects had a higher viral load in the early morning than the rest of the	The result suggests a diurnal variation of viral shedding from the upper respiratory tract with a trend showing higher viral load in the early morning. For community screening purposes, posterior oropharyngeal saliva could be taken throughout the day, but

			4 time points.		preferably in the early morning to maximize the yield.
hen at al. (2020)	To assess the use of posterior oropharyngeal saliva as specimens for the detection of SARS-CoV-2 in an automated point-of-care molecular assay.	58 patients with laboratory confirmed COVID-19. .	The samples collected from the patients were tested with the Xpert® Xpress SARS-CoV-2 assay.	SARS-CoV-2 was detected in either NPS or saliva specimens of all patients. Among them, 84.5% (49/58) tested positive in both NPS and saliva, 10.3% (n=6) tested positive in NPS only, and 5.2% (n=3) tested positive in saliva only. No significant difference in the detection rate was observed between NPS and saliva. The detection rate was slightly higher for N2 (NPS 94.8% and Saliva 93.1%) than that of the E gene target (Saliva: 89.7% vs 82.8%) on both specimen types.	Posterior oropharyngeal saliva and NPS were found to have similar detection rates in the point-of-care test for SARS-CoV-2 detection.
Wyllie et al. (2020)*	To validate the use of saliva for SARS-CoV-2 detection.	44 COVID-19 inpatient study participants and 98 asymptomatic healthcare workers.	Total nucleic acid was extracted from 300 µl of whole saliva using the MagMAX Viral/Pathogen Nucleic acid isolation kit (ThermoFisher Scientific) following the manufacturer's protocol and eluted into 75 µl of evolution buffer. For SARS-CoV-2 RNA detection, 5 µl of RNA template was tested as	When the SARS-CoV-2 detection from patient-matched nasopharyngeal and saliva samples were compared, the researchers found out that saliva yielded greater detection sensitivity	Saliva is a viable and more sensitive alternative to nasopharyngeal swabs and can enable at-home-self-administered sample collection for accurate large-scale SARS-CoV-2 testing.

described, using the US CDC real time RT-PCR primer/probe sets for 2019-nCoV_N1 and 2019-nCoV_N2 and the human RNase P (RP) as an extraction control.	and consistency throughout the course of infection. Furthermore, they report less variability in self-sample collection of saliva.

*This article is a preprint. Source: Authors.

4. Discussion

Currently, reverse transcription followed by real-time polymerase chain reaction (rRT-PCR) of respiratory samples collected by swabs represents the gold standard in the qualitative detection of Sars-CoV-2 infection (Azzi et al., 2020). However, this type of collection has several disadvantages, making saliva a potential material for diagnosis due to its non-invasive nature. In this way, diagnosis, management and control of COVID-19 can be facilitated (To et al., 2020 (a); Ceron et al., 2020).

Saliva is an easily accessible fluid that can be self-collected through a non-invasive procedure and can be beneficial for mass-scale seroprevalence studies. In addition, virus entry is mainly via the upper respiratory tract and antibodies contained in saliva can provide a first barrier to the entry of Sars-CoV-2. As a result, saliva has been studied as a potential diagnostic tool and is expected to replace other materials, such as serum or naso and oropharyngeal smears for the diagnosis of COVID-19 (Faustini et al., 2020; Sri Santosh et al., 2020).

For these reasons, it is of fundamental importance to consider factors that may influence the analysis of viral load when using this specimen for the diagnosis of COVID-19. Assessing the best method and time for collection, in addition to the choice and purity of the antigen, are essential elements for a more accurate identification of the presence of Sars-CoV-2. In this sense, using saliva from the posterior oropharynx is considered a promising method due to the sensitivity and the viral load rate found in this type of sample, which is justified by the predilection of the virus by the respiratory tract (To et al., 2020 (a); To et al., 2020 (b); Zheng et al., 2020; Yoon et al., 2020; Hung et al., 2020; Chen et al., 2020).

Other authors also consider the use of saliva obtained by spitting into a container for analyzing the presence of the virus (Tajima et al., 2020; Pasomsub et al., 2019; Zheng et al., 2020; Yoon et al., 2020; Wyllie et al., 2020). Considering the methods above, it is worth

mentioning that saliva is an extremely valuable material for the diagnosis of the disease, especially due to the easy collection by patients themselves, making it a potential method for obtaining more real data regarding the pandemic, avoiding underreporting by promoting mass testing of the population.

However, other methods were also considered for the acquisition of diagnostic material from the same fluid. In studies carried out by Azzi et al. (2020) and Faustini et al. (2020), samples collected from the 25 and 63 included patients, respectively, were obtained using techniques called drooling technique and passive dribble. This method enables collecting only oral fluids, excluding secretions from the lower respiratory tract or from the oropharynx mucosa (Golatowski et al., 2013). In addition, Randad et al., (2020) evaluated samples obtained from saliva collected from the space between the gum and the tooth, which is enriched with gingival crevicular fluid (GCF). For this reason, the composition of this material is very similar to serum due to the presence of elements of the immune system in the GCF (Taylor & Preshaw, 2016; Brandtzaeg, 2013).

Furthermore, with regard to the comparison between the main collection methods - spitting and sputum - the results obtained by Yoon et al. (2020) suggest greater sensitivity in tests that analyzed sputum samples. However, this result cannot be affirmed with total certainty due to the limited number of patients included in the study, and further studies are necessary in order to concretely establish this information.

Currently, some studies have shown the existence of better times for the collection of saliva, but little is known about the most advantageous time for this collection. In studies carried out by Tajima et al. (2020) and Hung et al. (2020), morning saliva samples showed higher viral load in the early morning compared to other times of the day, corroborating the hypothesis that morning saliva has greater sensitivity for the diagnosis of COVID-19. Although several factors affect the viral load rate, the advantage of collecting saliva in this period is justified because, during sleep, the ciliary movement of the trachea and the descent of nasopharynx fluids cause this sample to be contaminated with sputum, thus increasing the amount of viruses. In addition, the authors agree that collection should be carried out before breakfast and tooth brushing in order to obtain the greatest efficiency. Although the study sample is very small, its results are very promising and must be taken into account, but further studies should be carried out to investigate the best time for saliva collection.

In addition, some authors highlight the high sensitivity and the comparable or even superior performance of saliva in relation to the current method that uses swabs (Pasomsub et al., 2019). According to studies by Wyllie et al. (2020), the excellent performance of saliva

occurs mainly when there is early hospitalization and becomes more consistent during hospitalization and prolonged recovery. In the same study, it was observed that 2 asymptomatic health professionals showed negative swabs results; however, the detection of SARS-CoV-2 was found when they performed tests with saliva, which suggests, according to them, that saliva may be an appropriate and even more sensitive alternative in the process of identifying asymptomatic and pre-symptomatic patients.

Similar results were obtained in studies by Azzie et al. (2020), performed with sample collected from individuals previously tested positive for SARS-CoV-2 using rRT-PCR with saliva samples. Of the group composed of 25 patients, 2 of them had negative results for swabs in tests performed on the same day, although saliva confirmed the infection. This suggests that patients discharged from hospital, after negative results for respiratory swabs, can transmit the virus through saliva as a result of inconsistent results.

There are several advantages of using saliva for the diagnosis of COVID-19. As samples are easily supplied by patients, the contact between contaminated individuals and health professionals is reduced, which consequently reduces the risks of nasocomial transmission. For the same reason, the use of PPE by professionals during collection is not necessary, thus reducing excessive spending on public health. In addition, unlike saliva and due to the use of deep swabs, the current method not only generates discomfort, but can also induce coughing and / or sneezing, responsible for the production of aerosols (Tajima et al., 2020; To et al., 2020 (a); Ceron et al., 2020; Ng et al., 2020).

5. Conclusions

When considering that most studies do not have high number of patients, it is not possible to state, in fact, that saliva can be used as the main diagnostic material. However, studies have shown that saliva can be excellent alternative sample for the detection of SARS-CoV-2, facilitating the diagnosis and control of COVID-19. From this perspective, further studies should be developed in order to obtain even more reliable and effective data on tests performed and sample collection methods.

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