Saliva PCR as a simple detector of COVID-19 in patients with acute salivary sialadenitis

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ORIGINAL STUDY

Saliva PCR as a simple detector of COVID-19 in patients with acute salivary sialadenitis

Rasha F.A. El Dahab, Mariam A. Fouad

Abstract

Background: Coronavirus 2019 (COVID-19) is caused by the new, highly transmissible pandemic disease SARS-CoV-2. It was noticed that there were many cases of acute salivary sialadenitis in the era of COVID-19 and not enough studies were documented. This study aimed to assess if there is a correlation between COVID-19 and acute salivary sialadenitis and assessed saliva PCR as an easy diagnostic test for COVID-19 also compared it with the gold standard nasopharyngeal swab PCR.

Methods: A nonrandom observational research included 30 cases who had acute onset of salivary sialadenitis. They all underwent oropharyngeal, parotid and submandibular glands examination. Diagnosis of COVID-19 was done by nasopharyngeal swab & saliva PCR. Other lab investigations CRP, D. Dimer, S. Ferritin, also chest CT for COVID-19 detection were also done.

Results: The sample collected showed; 21 (70%) patients had unilateral acute submandibular sialadenitis, 4 (13.3%) of them had also unilateral acute parotitis on the same side, 20 (66.7%) of them had acute tonsillopharyngitis, 23 (76.6%) of them had smell disorders and 18 (60%) of them had taste disorders. In 66.7%; saliva PCR was positive for COVID-19 and in 53.3% nasopharyngeal swab PCR was positive for COVID-19. As regards D. Dimer, S. Ferritin and CRP; elevated values in 43.3%, 40% and 90% respectively.

Conclusion: Salivary gland inflammation is considered an extra-pulmonary manifestation of SARS-COV-2. Diagnosis of COVID-19 can be done with saliva PCR.

Keywords: Anosmia, Dysgeusia, rRT-PCR, SARS-COV-2, Sialadenitis

1. Background

Patients with COVID-19 often have a loss of taste sensation and a dysfunctional salivary gland. The mouth, along with the nose and the eyes, is thought to be a major entrance route for SARS-CoV-2. SARS-CoV-2 cell entry factors are expressed in the oral cavity, taste bud epithelium, and minor and major salivary gland epithelium [1–3].

Paramyxovirus (i.e., mumps), influenza A, parainfluenza virus, human immunodeficiency virus and coronavirus are among the viruses that cause salivary infections [4,5]. The diagnosis of Severe Acute Respiratory Syndrome Coronavirus2 (SARS-CoV-2) is dependent on the identification of viral RNA by real-time reverse transcription polymerase chain reaction (rRT-PCR) in respiratory specimens, particularly nasopharyngeal swabs (The gold standard test) [6]. These specimens demand medical personnel and Personal Protective Equipment is used to minimize the danger of nosocomial transmission [7]. In addition, nasopharyngeal swabs require facilities and time to report findings (many hours to one day) [8].

Some investigations have recommended substitution of another body fluid, such as saliva, for the identification of SARS-CoV-2 since the case gathers it themselves, does not need professional health care staff, and decreases the dangers for the operator [9]. Saliva might serve as a novel diagnostic...
sample source for cases, as it can be simply and non-invasively collected [10].

Saliva examined by conventional (rRT-PCR) or fast molecular biology assays (direct rRT-PCR without extraction) [11]. Fast Salivary Test; antigen testing using a lateral flow assay determines the existence of the virus in a matter of minutes by detecting the spike protein in saliva [12]. Consistently, high viral loads of SARS-CoV-2 were found in the saliva of COVID-19 patients [13].

This work assessed saliva PCR as an easy and safe test for COVID-19 detection.

2. Methods

2.1. Patients

This is a nonrandom observational research carried out on 30 patients who had acute salivary sialadenitis associated with other symptoms of COVID-19 collected from the ORL outpatient clinic in Shebin El Kom teaching hospital from January 2021 to May 2022.

Ethical approval was approved by GOTHI; Code NO. HSH00046; on 20 July 2022. informed consent was obtained from all participants included.

2.1.1. Inclusion criteria

(1) Patients with COVID-19 manifestations (General manifestations, smell, taste disorders and sore throat).

(2) Patients had acute salivary sialadenitis either unilateral or bilateral.

2.1.2. Exclusion criteria

Patients with chronic salivary disease.

Before their involvement, the goal and nature of the research were described to all participants, and their signed agreement was acquired. (GOTHI) granted approval for this study on 20 July 2022.

2.2. Methods

A complete history was taken focusing on general manifestations [Fever, anorexia, headache and malaise (FAHM)], nasal symptoms especially smell affection, pharyngeal manifestations (sore throat, dryness, mouth ulcers and taste affection), chest manifestation (cough, dyspnea and chest pain), past history of salivary gland disease and family history of COVID -19. All patients had complete ORL and H&N examinations focusing on oropharyngeal and salivary glands (Both parotid and submandibular), blood samples were collected and chest CT was done.

2.3. Blood sample analysis

Blood samples were divided into two parts; the first part was for D. Dimer and the second part was centrifuged at 3000 rpm for 10 min at 4 °C and Serum samples were quickly separated & then kept at −20 C until CRP and ferritin assays. Determination of ferritin and D. Dimer by electro-chemiluminescence immunoassay (ECLIA) using Cobas Roch 6000 instruments Roche's technology for immunoassay detection is based on usage of a ruthenium complex and tri-propylamine (TPA). Determination of CRP using Roche diagnostic kits by (Cobas Integra 400 plus instrument) [14].

2.4. Nasopharyngeal swap and saliva collection

All patients had a nasopharyngeal swab (the gold standard test) and a saliva PCR performed. In a sterile cup, 2 ml of saliva were collected. Detection of COVID-19 by ID NOW COVID-19 (Abbott Diagnostic, Scarborough, Maine 04074, USA), an automated test that employs isothermal nucleic acid amplification for qualitative identification of SARS-CoV-2 viral nucleic acids. It includes (1) a Sample receiver with elution/lysis buffer (2) A Test Base consisting of two sealed reaction tubes, each holding a lyophilized pellet; (3) A Transfer Cartridge for transferring the eluted sample to the Test Base; and The ID NOW Instrument. In addition to an internal control, the Test Base’s reaction tubes include the chemicals essential for the amplification of SARS-CoV-2. The templates (similar to primers) developed to target SARS-CoV-2 RNA extend a singular region of the RdRp section [15]. The results were either affirmative (COVID-19 was present) or negative (no COVID -19 detected).

2.5. Statistical analysis

After calculating the sample size, a statistical power analysis was conducted, based on findings obtained from this research 30 patients had acute salivary sialadenitis. The data collected were reviewed, coded and statistically analyzed using the SPSS program (statistical package of social science; SPSS Inc., Chicago, IL, USA) version 20 for Microsoft Windows. 2 types of statistics were used; (a) Descriptive: number (No.), percent (%), mean and standard deviation (X±SD). (b) Analytic statistics for a statistical hypothesis. An independent student's t for comparison between quantitative variables.
Fisher’s exact test for $2 \times 2$ tables. Qui square test ($x^2$) for comparison between qualitative data. The significance level was set as the $P$ value $\leq 0.05$.

### 3. Results

This research included 30 patients who had acute salivary sialadenitis their age range from 25 to 55 years with a mean of age $31.6 \pm 12.9$. 56.7% of them were male ($n = 17$) and 43.3% female ($n = 13$). As regards smell affection; 76.6% ($n = 23$) of patients had smell affection varies from hyposmia in 39.1% ($n = 8$) and total loss of smell (Anosmia) in 60.9% ($n = 18$). Only 10% ($n = 3$) with normal smell. 60% ($n = 18$) of patients had taste deterioration varies from hypogusia in 33.3% ($n = 5$) and ageusia in 66.7% ($n = 13$). Xerostomia was present in 93.3% ($n = 28$) also general manifestations (FAHM) were present in 96.6% ($n = 29$). Acute tonsillopharyngitis was present in 66.7% ($n = 20$). Acute unilateral submandibular saliadenitis was in 70% ($n = 21$) and bilateral in 30% ($n = 9$). It was noted that 4 (13.3%) patients presented with acute unilateral parotitis beside acute unilateral submandibular sialadenitis and only one patient (3.33%) had isolated unilateral parotitis (Table 1, Fig. 1). In this study; 3 (10%) post-COVID-19 vaccination cases and 5 (16.7%) post-COVID-19 cases were reported. COVID-19 severity levels were established by National Health Commission Of China [16]; where 53.3% ($n = 16$) and 46.7% ($n = 14$) were mild and moderate cases respectively (Table 1, Fig. 1). Saliva PCR was positive in 66.7% ($n = 20$), nasopharyngeal PCR was positive in 53.3% ($n = 16$) and chest CT findings suggestive of COVID-19 positive in 40% ($n = 12$) (Table 2, Fig. 2). As regards another lab. investigations; CRP, D. Dimer and S. Ferritin were elevated in 90% ($n = 27$),
43.3% (n = 13) and 60% (n = 18), respectively (Table 3, Fig. 3).

4. Discussion

The severity of the SARS-CoV-2 is global. The first symptoms of COVID-19 were cough, rhinorrhea, sore throat, dyspnea & anosmia [17]. In addition, dysgeusia is one of the initial symptoms of COVID-19 [18].

By analysis of clinical presentation; the hyposmia/anosmia ratio was (64.3%) and the hypogeusia/ageusia ratio was (38.4%), in his study; Korkmaz M et al., 2021 reported that; hyposmia/anosmia ratio was (37.9%) and hypogeusia/ageusia ratio was (41.37%) [19]. Hopkins, C. et al., 2021 found that the incidence of impaired sense of smell or taste was 49.7% and 64%, respectively [20].

Acute tonsillopharyngitis was present in (66.7%) in this study and this agreed with Korkmaz M et al., 2021 who said that; sore throat is the most common oropharyngeal symptom [21].

Liu L et al., 2021 showed that SARS-CoV-2 can infect the salivary duct epithelium either bilateral or unilateral [22].

In studying the prevalence of acute salivary sialadenitis; 13.3% (n = 4) of patients had both submandibular and parotid sialadenitis and this finding agreed with D K C Wong et al. 2020 who reported non-suppurative submandibular sialadenitis in two COVID-19 patients [23] also Matuck B, 2021 documented an incidence of parotitis and submandibular gland sialadenitis [24]. In this study only 3.33% (n = 1) of patients had isolated parotitis and this agreed with Capaccio P et al., 2020 reported a case of parotitis in a case who was isolated with family members (all of whom tested positive for COVID-19 by RT-PCR) [25].

Clinical staging of SARS-CoV-2 infection into stages I (mild), II (moderate), III (severe), and IV (critical) is determined by the relationship among the virus and the immune system [26]. First-stage symptoms are nonspecific, hence PCR is the key diagnostic tool [27]. Hypoxic individuals may exhibit second stage symptoms, including increased lymphopenia and higher transaminase levels on blood tests [28]. The third and most severe stage is characterized by a drastic reduction in T lymphocyte numbers and a marked elevation in

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**Table 2. Nasopharyngeal swab PCR, saliva PCR and chest CT for COVID-19 diagnosis.**

<table>
<thead>
<tr>
<th>Test result</th>
<th>Nasopharyngeal swap PCR for COVID-19</th>
<th>Saliva PCR for COVID-19</th>
<th>Chest CT findings suggestive of COVID-19</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for COVID-19</td>
<td>n = 16 (53.3%)</td>
<td>n = 20 (66.7%)</td>
<td>n = 12 (40%)</td>
<td>3.8017</td>
<td>0.1494</td>
</tr>
<tr>
<td>Negative for COVID-19</td>
<td>n = 14 (46.7%)</td>
<td>n = 10 (33.3%)</td>
<td>n = 18 (60%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There is a non-significant difference between Nasopharyngeal swab PCR, Saliva PCR and Chest CT in the detection of COVID-19; P value = 0.149442 (>0.05).
inflammatory cytokines and biomarkers such as ferritin [29].

In this study, there was a substantial variance amongst saliva PCR and COVID-19 severity (P value = P value = 0.405 > 0.05) (Table 4, Fig. 4).

Among the biomarkers specifically investigated in COVID-19 were procalcitonin (PCT), C-reactive protein (CRP), ferritin (Fer), D-Dimer, interleukin –6 (IL-6), and lactate dehydrogenase (LDH) [30].

Ferritin level serves as a severity risk factor [31]. In this study, there was a substantial variance among S. Ferritin level and saliva PCR in COVID-19 in diagnosis (P value = 0.03843 < 0.05) (Table 3, Fig. 3).

D-dimer is a fibrin breakdown product with a normal value of less than 0.5 g/mL and levels that rise with age and during pregnancy [33]. D-dimer has been discovered as a possible biomarker of COVID-19 patients’ prognosis [34]. Viremia and cytokine storm syndrome are the most prevalent causes of an elevated D-dimer level [35].

In this research; there was a non-substantial distinction amongst saliva PCR and D. Dimer in COVID-19 diagnosis. Table 3, Fig. 3 and this agreed with Nasiri K, 2021 who declared that there was no substantial variance among both specimen types for identification of COVID-19 [36]. Contrary to Li Y et al., 2020 who found that there was a substantial variance [37].

In this study, there was a substantial variance amongst saliva PCR and COVID-19 severity (P value = P value = 0.03843 < 0.05) (Table 4, Fig. 4).

Concerning COVID-19 diagnosis in both nasopharyngeal swab and saliva PCR, COVID-19 was detected in 53.3% (n = 16) and 66.7% (n = 20) of

<table>
<thead>
<tr>
<th>Test</th>
<th>Elevated Number (%)</th>
<th>Normal Number (%)</th>
<th>s±SD</th>
<th>X²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>27 (90%)</td>
<td>3 (10%)</td>
<td>53.43 ± 20.85</td>
<td>4.8118</td>
<td>0.028266</td>
</tr>
<tr>
<td>D. Dimer</td>
<td>17 (56.7%)</td>
<td>13 (43.3%)</td>
<td>1.16 ± 1.09</td>
<td>3.2997</td>
<td>0.069294</td>
</tr>
<tr>
<td>S. Ferritin</td>
<td>12 (40%)</td>
<td>18 (60%)</td>
<td>241.8 ± 119.7</td>
<td>4.2857</td>
<td>0.03843</td>
</tr>
</tbody>
</table>

**Saliva PCR is Positive for COVID-19 in 20 patients (66.7%) and negative in 10 patients (33.3%).**

**Normal CRP = 6, normal D. Dimer <0.5 μg/ml [32] and normal S. Ferritin level = adult male 30–400 ng ml-1, adult female 13–150 ng ml-1 [33].

**There is a significant difference between CRP and saliva PCR in COVID-19 detection; P value = 0.028266 (P value < 0.05).**

**There is a significant difference between saliva PCR and S. Ferritin in COVID-19 detection; P value = 0.009181 at (P < 0.05).**

**There is a significant difference between CRP and saliva PCR in COVID-19 detection; P value = 0.028266 (P value < 0.05).**

**Table 3. Relation between ordinary lab investigations in COVID-19 and Saliva PCR for COVID-19.**

**Table 4. Saliva PCR relation to case severity in COVID-19 patients.**

<table>
<thead>
<tr>
<th>Saliva PCR</th>
<th>Case severity (no. &amp; percent of cases)</th>
<th>X²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>11 (68.8%)</td>
<td>5.1167</td>
<td>0.02869</td>
</tr>
<tr>
<td>Negative</td>
<td>5 (31.2%)</td>
<td>5 (35.7%)</td>
<td>0.02869</td>
</tr>
</tbody>
</table>

There is a significant difference between saliva PCR and COVID-19 severity.
nasopharyngeal swab PCR and saliva PCR samples, respectively, and there was no substantial difference among nasopharyngeal swab PCR and saliva PCR in the detection of COVID (Tables 2 and 5, Fig. 2). In comparison to nasopharyngeal swap PCR, the sensitivity and specificity of saliva PCR for COVID-19 were 66.67% (95%CI: 40.99–86.66%) and 16.67% (95%CI: 2.09–48.41%), respectively (Tables 6 and 7, Fig. 5). Ibrahimi N et al., 2021 meta-analysis exhibited higher concordance, 92.5% (95%CI: 89.5–94.7), across researches and pooled sensitivities of 86.5% (95%CI: 83.4–89.1) and 92.0% (95%CI: 89.1–94.2) from saliva and nasopharyngeal/oropharyngeal swabs respectively and strongly recommended that saliva might be utilized for standardized tests of COVID-19 patients and “en masse” screening of populations [38]. To K et al. (2019) revealed that the overall sensitivity and specificity for saliva were 90.8% (81.9–96.2%) and 100% (97.3–100%), and 96.1% (88.9–99.2%) and 98.5% (94.7–99.8%) for NPA [39].

We concur with Kelvin K. et al., who said that further molecular testing of saliva samples enhances the identification of respiratory virus [40].

Table 5. Nasopharyngeal swab PCR, and saliva PCR for COVID-19 diagnosis.

<table>
<thead>
<tr>
<th>Test result</th>
<th>Nasopharyngeal swab PCR for COVID-19</th>
<th>Saliva PCR for COVID-19</th>
<th>χ2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for COVID-19</td>
<td>16 (53.3%)</td>
<td>20 (66.7%)</td>
<td>1.111</td>
<td>0.29184</td>
</tr>
<tr>
<td>Negative for COVID-19</td>
<td>14 (46.7%)</td>
<td>10 (33.3%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There is a non-significant difference between Nasopharyngeal swab PCR, Saliva PCR and Chest CT in the detection of COVID-19; P value = 0.29184 (>0.05).

Table 6. Saliva and nasopharyngeal PCR validity.

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Value</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>75%</td>
<td>47.62%–92.73%</td>
</tr>
<tr>
<td>Specificity</td>
<td>71.43%</td>
<td>41.90%–91.61%</td>
</tr>
<tr>
<td>Positive likelihood ratio</td>
<td>2.62</td>
<td>1.09:6.30</td>
</tr>
<tr>
<td>Negative likelihood ratio</td>
<td>0.35</td>
<td>0.14:0.87</td>
</tr>
<tr>
<td>Disease prevalence*</td>
<td>53.33%</td>
<td>34.33%–71.66%</td>
</tr>
<tr>
<td>Positive predictive value*</td>
<td>75.00%</td>
<td>55.56%–87.80%</td>
</tr>
<tr>
<td>Negative predictive value*</td>
<td>71.43%</td>
<td>50.13%–86.14%</td>
</tr>
<tr>
<td>Accuracy*</td>
<td>73.33%</td>
<td>54.11%–87.72%</td>
</tr>
</tbody>
</table>

(*These values are dependent on disease prevalence.

Table 7. Difference between saliva and nasopharyngeal swap PCR in COVID-19 detection by using ROC curve.

<table>
<thead>
<tr>
<th>Area</th>
<th>Std Error</th>
<th>P. Value</th>
<th>Asymptotic 95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0589286</td>
<td>0.106068</td>
<td>0.405741</td>
<td>0.381396</td>
<td>0.797175</td>
</tr>
</tbody>
</table>

There is no significant difference between saliva and nasopharyngeal swab PCR in COVID-19 detection (P value = 0.405 > 0.05).

As regards the ROC curve; Lower Bound was 0.381396, the Upper Bound was 0.797175 and the area under the curve was 0.589286 with a standard error of 0.106068 (Table 7, Fig. 5).
Regarding another lab. investigations of COVID-19; there is a substantial variance amongst CRP and saliva PCR in COVID-19 detection; \( P \) value = 0.028266 < 0.05 (Table 3, Fig. 3).

4.1. Limitations of the study

This study was done only on mild and moderate cases not the severe one and also few numbers of patients with acute sialadenitis sought ORL advice and not all patients. Saliva PCR was not a good prognostic factor for COVID-19.

4.2. Conclusion

Saliva specimens can be regarded a viable test for screening SARS-CoV-2 infections and assist in avoiding false positives, even if nasopharyngeal PCR is the gold standard test for diagnosing COVID-19.

Saliva PCR is a good screening test because of its high sensitivity.

Saliva PCR has high sensitivity, specificity and accuracy.

4.3. Recommendations

Saliva PCR is indicated as a straightforward, safe, and noninvasive diagnostic tool for the identification of SARS-CoV-2.

Conflicts of interest

The authors declare that they have no competing interests.

References


