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## Perspective

# Salivary diagnostics in COVID-19: Future research implications

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Coronavirus disease 19 or COVID-19 was first reported and confirmed in Wuhan, China in December 2019.<sup>1,2</sup> Henceforth, it has rapidly spread to several countries, becoming a global pandemic, affecting 1,279,722 people world over and causing 72,614 deaths as on 7th April 2020, according to the reports of the WHO.<sup>3</sup> In this perspective, we briefly explain the possible role of saliva/salivary glands and gingival crevicular fluid, to investigate the novel Coronavirus 19 and we consider other ways in which such studies could be set in motion.

COVID-19, caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) which was provisionally named as 2019-nCoV, belongs to the genus Betacoronavirus and is the third animal coronavirus infection to affect humans.<sup>4</sup> The high pathogenic tendency of coronaviruses, to affect humans, were considered, only with the emergence of two life threatening epidemics, SARS (Severe Acute Respiratory Syndrome) in 2002–2003 in China and approximately ten years later MERS (Middle East Respiratory Syndrome) in the Middle Eastern Countries.<sup>5</sup> Both SARS coronavirus (SARS-CoV) and MERS coronavirus (MERS-CoV) are considered to have their origin from bats. The genetic sequence of SARS-CoV-2 has been shown to be 79.6% identical to that of SARS-CoV and 96% identical to a bat

coronavirus.<sup>6</sup> Despite the initial zoonotic nature of COVID-19, now the rapid spread is by human to human contact with the typical clinical features of fever, cough which is mostly nonproductive, malaise, dyspnoea and pneumonia.<sup>6,7</sup> Other infrequently presented symptoms include sputum production, hemoptysis, headache and gastrointestinal symptoms such as diarrhea, nausea and vomiting.<sup>8</sup> Therefore, besides the spread of the virus through oral and nasal secretions, a possibility of a fecal-oral transmission has been implicated. Few cases of acute myocardial injury and chronic cardiovascular damage have been reported in patients with COVID-19 as the disease progresses.<sup>9</sup> Transmission can also occur early in the disease process, even before the symptoms set in, highlighting the transmission potential of asymptomatic or mildly symptomatic patients.<sup>10</sup>

Coronaviruses are enveloped, single stranded RNA viruses with high rates of mutation and recombination.<sup>7</sup> The structural proteins include the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N).<sup>11</sup> It is the spike surface protein that plays a crucial role in binding of the virus to the host cell receptors. SARS-CoV primarily binds to the angiotensin converting enzyme 2 (ACE2) receptor on the host cell.<sup>11</sup> CD209L has also been implicated as a possible second receptor. MERS-CoV uses dipeptidyl peptidase 4 as the main receptor. In case of SARS-CoV-2, it has been confirmed that

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human ACE2 is the main receptor for viral entry into the host cell.<sup>12</sup> The entry of coronavirus into the host cell is a multi-step process using multiple distinct domains in the spike protein that facilitates attachment of the virus to the surface of the cell, engagement of the receptor, processing of proteases and membrane fusion. Genomic analysis of the SARS-CoV-2, also reveals the presence of an activation site on the spike protein, which is activated by furin, an enzyme which is found abundantly in many human tissues, the presence of which can be attributed to its rapid spread.<sup>13</sup> Following viral attachment to the host cell receptor, the entry of the virus into the cell, additionally requires the priming of the spike protein by cellular proteases, which is responsible for cleavage of the spike protein and subsequent fusion of the viral and cellular membranes.<sup>14</sup> As mentioned earlier, the SARS-CoV uses ACE2 as the receptor for viral entry and TMPRSS2 for priming of the spike protein.<sup>14</sup> The spike protein of MERS-CoV is activated by cellular proteases furin (a proprotein convertase present predominantly in golgi apparatus and cell surface), cathepsin L (an endosomal cysteine protease) and TMPRSS2 (a type II transmembrane serine protease).<sup>15</sup> The priming of the spike protein of SARS-CoV-19 also has been reported to be carried out by TMPRSS2.<sup>14</sup> The confirmed cases of COVID-19 and the mortality caused already surpass that of SARS and MERS.<sup>1</sup> This is a cause of great concern and warrants the need for prompt diagnosis, early intervention and adequate measures to contain the rapid pandemic spread.

Saliva plays an important role in the transmission of infection between persons by contact with the droplets expressed.<sup>16</sup> It also paves way for a convenient and non invasive mode of diagnosis. Additionally it can be collected by the patients themselves and thus reduces the risk of infection to the healthcare workers. Saliva samples also present with an adequate quantity of analyte, in patients who have insufficient or no sputum. Coronaviruses, including the SARS-CoV, have been detected previously in saliva, almost in par with the levels found in nasopharyngeal specimens.<sup>17</sup> SARS-CoV-2 has been detected in saliva of confirmed patients with COVID-19, even up to the 11th day after hospitalization, in one of the cases.<sup>17</sup> The presence of the COVID-19 virus in the saliva can have its source from either the salivary glands via the ducts or from the gingival crevicular fluid (from gingiva) or simply from secretions of the lower and upper respiratory tract that combines with the saliva.<sup>16</sup> The ACE2 epithelial cells of the salivary glands have been shown to be an initial target for the SARS-CoV, early in the disease process, in rhesus macaques.<sup>18</sup> The mRNA and protein levels of the cellular protease, furin, vary according to the cell type and high levels have been found in the salivary glands.<sup>19</sup> Similarly, expression of TMPRSS2 has also been seen in the salivary glands.<sup>20</sup> Thus, the possibility of the role of salivary gland cells in the initial entry, progress of the infection and as a source of the virus, should be considered and validated with further studies. This may also be the reason behind the transmission of infection between asymptomatic cases, since the organism is contained and proliferates within the salivary glands and has not progressed yet to the respiratory tracts. This may be further analyzed by collecting the ductal saliva secretions rather than whole saliva, using appropriate saliva collectors and collection procedures. A

modified Carlson–Crittenden/Lashley cup for collection of the parotid ductal secretions as well as the submandibular and sublingual saliva collectors, as described by Wolfe et al.<sup>21</sup> can be applied for better results, thus probably excluding the virus from the respiratory tract or the gingival crevicular fluid.

The possibility of the salivary glands as a reservoir, harboring latent infection, which may reactivate later, should also be considered and this warrants further research. Xu et al.<sup>22</sup> reported that ACE2 is abundantly expressed in the epithelial cells of the oral mucosa, with higher expression in the tongue, in comparison to the buccal and gingival tissues. These findings suggest that the oral cavity has high susceptibility to COVID-19 infection. Moreover, analysis of the gingival crevicular fluid (GCF) also provides a non-invasive diagnostic method. GCF can be collected by various techniques, but most studies use the absorption technique by employing paper strips or points.<sup>23</sup> GCF samples have been studied to isolate and assess Herpes Simplex Virus (HSV), Epstein Barr Virus (EBV) and human cytomegalovirus (CMV) earlier.<sup>24</sup> Therefore, assessing the presence of COVID-19 virus in the GCF, will be yet another convenient, non-invasive method, to isolate the virus and additionally, to confirm the pathway of entry into the oral cavity.

The beneficial role of saliva as a quick, non invasive diagnostic modality and the various possibilities it presents with, for investigation, during the course of the disease process, prognosis or presence of any antibodies to the novel COVID-19 virus, needs further exploration. Additionally, the involvement of any other receptors or cellular proteases which may throw more light on the pandemic disease pathogenesis may pave way to targeted drug therapies.

## Declaration of Competing Interest

The authors have no conflict of interests relevant to this article.

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