Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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'The Double-Edged Sword' – An hypothesis for Covid-19-induced salivary biomarkers

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ABSTRACT

Utilising biomarkers for COVID-19 diagnosis, prediction of treatment response and overall prognostication have been investigated recently. However, these ventures have only considered the use of blood-based molecular markers. Saliva is another biofluid that warrants being applied in similar fashion with major advantages that centres on its non-invasive and repeatable collection as well as cost-efficiency. To this end, this article presents a hypothesis for the sources of biomarkers useful clinically for COVID-19 disease outcome estimation and identify the likely implications of their detection in saliva.

Introduction

The Coronavirus disease 2019 (COVID-19) pandemic has characterised the turn of the 21st century's third decade with about 9,931,521 cases and 497,506 deaths recorded as of June 27th, 2020 [1]. Caused by the positive sense, single-stranded RNA, enveloped β-coronavirus with chiropteran origin and a high mutation rate – SARS-CoV-2, the surge in confirmed cases and the expansive nature of disease spread are as a result of airborne, direct surface contact, and possibly faecal-oral human-to-human transmission [2]. The principal method of determining COVID-19 case positivity involves reverse transcription PCR assays of viral-laden phlegm and oropharyngeal/nasopharyngeal swabs. Since swab collection methods are relatively invasive and associated with an increased risk of cross-infection, attention has now shifted to the use of biofluids for COVID-19 diagnosis.

A recent systematic review has highlighted the potential role of blood cell counts as well as serum electrolytes and protein biomarkers in the diagnosis and severity classification of COVID-19 patients [3]. Similarly, saliva – the prime biofluid of the head and neck, has been touted to serve as a preferred medium for non-invasive detection due to its specimen collection and handling advantages. Preliminary studies involving whole and gland-specific saliva for virus detection have also reported diagnostic efficiency values ranging from 30.7% to 100% with the many modalities employed [4–8]. However, these applications solely concern COVID-19 diagnosis and explorations into the quantification of salivary biomarkers for prognostication and surveillance of infected cases warrants additionally considerations. We have reason to believe that biomarkers that are intended for COVID-19 disease outcome estimation could likewise be detectable in saliva.

The hypothesis

We theorise that biomarkers for COVID-19 outcome measurements could be secreted in saliva via systemic- and local-based outflow mechanisms (Fig. 1).

Systemic-based mechanisms

- Overflow of serum peptides and proteins abnormal expression with concomitant secretion into saliva via the parotid, submandibular and sublingual glands. Abundant capillary beds in salivary glands transport blood-borne molecules through their walls into the interstitial fluid (ISF) from which secretion into the intraglandular ducts occur. Depending on the physical characteristics, the mechanism of biomolecule secretion involving specialised acinar or ductal cells of the salivary epithelium can involve intracellular or paracellular passive diffusion and active transport as well as transcytosis.
  - o Intracellular diffusion of COVID-19-induced biomarker starts at the basal membrane of the acinar or ductal cells, undergoes cytoplasmic transfer to the apical (luminal) cell membrane, and is emptied directly in ducts as part of the biofluid secretion.
  - o Paracellular diffusion of associated molecules involves ultrafiltration following ISF emptying through the tight junctions between the specialised salivary gland cells for subsequent secretion into ducts.
  - o ATP-induced transport of serum biomolecules against their

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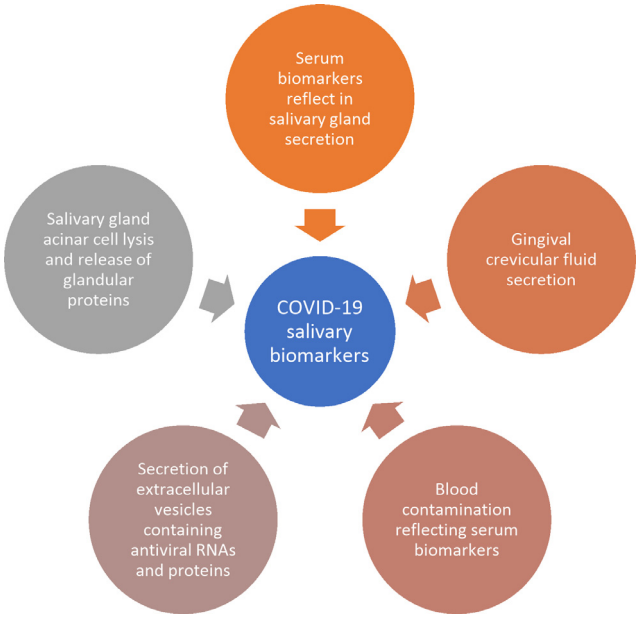

Fig. 1. Summary of pathways for secretion of COVID-19 related biomarkers in saliva.

concentration gradient with intracellular transport through protein pumps before ductal emptying.
  - o Vesicular-mediated transport of serum disease-preferential molecules contained within extracellular vesicles (EVs) from the basal to apical cell membranes of acinar cells for subsequent release into saliva.
- Secondary secretion of serum molecules through exudation of gingival crevicular fluid (GCF) into saliva. Increased hydrostatic pressure in microcirculation results in dilatation of gingival arteriolar vasculature, untightening of endothelial cell junctions, and ultimately, a deluge of blood-based disease outcome markers into GCF, and ultimately, saliva.
- Direct blood contamination of saliva representing an alternative pathway for spillage of serum biomarkers into saliva.

Local-based (Intraoral) mechanisms

- SARS-CoV-2 fusion, entry, and lysis of specialised salivary gland cells and oral epithelial cells in addition to local inflammation results in excess secretion of glandular proteins and influx of proinflammatory cytokine and chemokine proteins into saliva.
- Microvesicular buddings and/or exosomal releases containing antiviral microRNAs and proteins from salivary gland acinar and ductal cells directly into biofluid.

Evaluation of the hypothesis

Incorporating saliva collection and analysis into the routine diagnosis and monitoring of systemic and oral conditions has become a promising research endeavour in recent times. The attractiveness of this biofluid stems from its inexpensive, easy, and repeatable collection that can be performed by untrained personnel with reduced risk of cross-infection [9]. Saliva is famed to comprise a hodgepodge of biological molecules that are secreted from major and minor salivary glands, Von Ebner's glands, GCF, oral epithelium as well as contributions from respiratory and nasal secretion. Secretions from major and minor salivary glands, Von Ebner's glands, and GCF in relation to the water composition and suspensory molecules are reflective of serum concentrations, thus, earning saliva the attribute of mirroring individuals' health and physical status [10]. Over time, the majority of serum proteins and other biomolecules posited to be emptied in whole saliva have also been isolated in gland-specific saliva without contamination from other input sources which bolster the hypothesis of direct proportionality between serum and saliva biomarker concentrations [11]. Likewise, congruency between plasma drug concentrations has been previously employed to explain simple and facilitation diffusion, active transport, and cytopemptic mechanisms that facilitate entry of biomolecules into saliva which supports our presented hypothesis [12–14].

Beyond the influx of analytes via salivary gland mirroring of plasma concentrations, exudation of GCF and blood contamination of saliva represents additionally proven mechanisms for correlations with blood solute concentrations. GCF is produced from passive filtration of plasma and bears identical proteins and enzymes which have been demonstrated using uncontaminated samples [15]. However, increases in GCF flow into saliva and the possibility of obtaining a blood-tinged saliva specimen increases with the occurrence of periodontal conditions such as chronic gingivitis and periodontitis as well as other inflammatory intraoral conditions.

Taken together, pre-validated serum COVID-19 biomarkers such as proinflammatory cytokines (such as IL-1β, IL-6, and TNF- α), lactate dehydrogenase (LDH), acute phase proteins (C-reactive protein), D-dimer and even ferritin could also be expressed and quantified in saliva with potential inputs from the salivary glands, GCF, blood contamination [3]. However, it is important to note that not all instances of increased serum biomarkers would result in concomitantly increased salivary expression. Williamson et al., [16] in an attempt to compare salivary and serum levels of 27 inflammatory cytokines in healthy individuals found significant weak positive correlations in 11.1% of biomarkers between both samples. Interestingly, IL-6 was amongst the proportionately increased proteins [16].

Local mechanisms have also been implicated in saliva serving as a rich source of biological markers. Molecules directly into saliva from in oral conditions like oral cancer, lichen planus, periodontitis, and oral manifestation of systemic diseases have proved useful for disease-related measures. As it relates to COVID-19, viral-mediated cell lysis of salivary gland cells have been suggested to result in acute and chronic sialadenitis associated with mild infection [17]. With ATP-induced entry of amino acids for saliva protein synthesis as well as abundant storage granules observed within the acinar cells, pathological cell lysis is bound to result in increased salivary protein levels [10]. Potential disproportionate levels of α-amylase, cystatin, and gustin levels would be expected with the increasing affectation of the salivary glands.

Viral conditions are also suggested to induce salivary protective mechanisms that include the increased expression of non-coding transcriptomic molecules within EVs that are of salivary gland origin and possess antiviral functions, notably the inhibition of target viral mRNAs [18]. Considering this, the level of these markers may increase as protective measures with active COVID-19 infection or its convalescence. Examples of previously suggested EV-miRNAs include let-7b and 7c, miR-17, miR-29b and miR-335, and miR-27b [18].

Consequences of the Hypothesis/Discussion

Based on mechanisms investigated to explain the presence of serum biomolecules within saliva, there is evidence to suggest that biomarkers being explored for the diagnosis, outcome stratification and prognostication of COVID-19 may be detected within saliva as well. If considered, utilisation of salivary biomarkers related to the disease would additionally provide a non-invasive and rapid means to predict treatment response and monitor disease progression with samples that can be collected repeatedly and handled excellently. Moreover, the effect of SARS-CoV-2 virus on asymptomatic or mildly symptomatic infected individuals can be studied using locally generated protein and miRNA biomarkers that may be indicative of salivary gland disease or institution of salivary protective mechanism against infection. Biomarkers indicative of immunity status against the disease can be

additionally quantified easily using saliva in recovered individuals or uninfected persons when a putative vaccine becomes available [19].

Outside the benefit of salivary biomarkers in COVID-19 disease outcome determination, their aberrant expression in infected individuals may obscure other 'pre-COVID' applications for oral and systemic conditions. For instance, SARS-CoV-2 infection has been suggested to result in increased salivary amylase levels which may falsely indicate stress-induced body changes due to the sympathetic nervous system in an asymptomatic individual [17,20]. Likewise, protein biomarkers like peroxidase, peroxiredoxin-2, LDH, IL-6, and TNFα as well as microRNAs like let-7c and miR-27b, which have been previously linked to oral cancer and oral potentially malignant disorders, may result in false-positive findings in non-cancer individuals that suffer from COVID-19 infection [3,9,18,21]. Therefore, clinicians seeking to utilise salivary biomarkers presently may wish to consider a higher likelihood of lower validity due to possible COVID-19 infection.

Conclusions

Whilst serum biomarkers have been described for COVID-19 disease outcomes, there is a likelihood of similar dysregulated expression within saliva from various sources including the salivary gland themselves, GCF, and blood contamination due to chronic oral diseases. Alternatively, useful biomarkers may as well be expressed intrinsically from the salivary glands either from COVID-induced acinar cell lysis or increased production of EVs containing antiviral microRNAs and proteins.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2020.110124.

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