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Editorial

Saliva is a Good Candidate to be the New Gold-Standard Sample for Neurodegenerative Diseases

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Although still not considered a traditional diagnostic sample type, saliva testing is a simple, non-invasive, sustainable, and affordable approach for the diagnosis of infectious and non-infectious diseases. In the last two years, diagnostic tests have been developed to fight the coronavirus disease 2019 (COVID-19) pandemic, and saliva specimens represent a highly specific and sensitive alternative diagnostic sample to detect SARS-CoV-2, even at the level of the nasopharyngeal swab [1]. Indeed, some countries are adopting saliva testing for SARS-CoV-2 detection, including South Korea, Germany, and Japan [2, 3]. This global and unusual situation support new point of view for the potential of saliva specimens in the diagnosis and management of diseases.

Saliva has emerged as a good source of samples for detection of disease biomarkers. The use of saliva as a diagnostic sample has several advantages. Saliva offers a new and easily accessible physiological fluid that can be collected in a non-invasive manner and assessed using different analytical assays. It is easy to collect and does not need specialized personnel. It may save time, is comfortable, and may be even more relevant as it is scalable and inexpensive, being able to extend its use to developing countries. In addition to its ease of collection, saliva is generally safer than blood and cerebrospinal fluid (CSF), and its collection does not expose the healthcare provider to needles, thus reducing the risk of pathogen transmission from patients suffering from chronic infection.

Several products based on saliva testing are already on the market. Commercially available kits can gauge the levels of a handful of hormones, including

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estrogen, testosterone, and cortisol, from a sample of saliva. Saliva has the potential to diagnose diseases with more complex origins, including cancer [4]. Accumulating evidence has demonstrated the diagnostic and prognostic value of saliva as promising novel and revolutionary liquid biopsy in cancer [5].

Recent progress suggest saliva testing could be a useful approach for the diagnosis of neurodegenerative diseases. Early disease detection is critical in assigning proper treatment therapy to affected patients with these diseases. Easily accessible, cost-effective, and accurate diagnostic biomarkers are need to improve the early diagnosis of these neurodegenerative diseases, especially in primary care [6]. Usually the tests performed for the diagnosis of neurological conditions are lumbar puncture or more recently blood tests. Their invasive nature, especially for the lumbar puncture, usually results in discomfort, pain, and disagreeable side effects for patients, which necessitates the search for accurate, more advanced, and less invasive testing methods. Positron emission tomography (PET) imaging is also a very valuable diagnostic tool, but very costly which also limits its use in clinical practice. Therefore, it is essential to establish a substitute that is less invasive but remains representative of the brain's pathological changes.

Saliva is being explored as an alternative to CSF and imaging biomarkers in the accurate detection of Alzheimer disease (AD) [7]. A collection of studies have shown that salivary amyloid-β $(Aβ)_{42}$ is detectable and increased in AD [8–10], whereas presence of tau species has been also explored in saliva but with inconclusive results to support these molecules as salivary biomarkers for AD [11–13]. However, salivary lactoferrin has shown very high accuracy and specificity to differentiate prodromal and dementia stages of AD from healthy subjects [14]. Current research continues to supply evidence indicating that salivary lactoferrin concentration is significantly reduced in AD patients when compared to healthy controls, patients suffering from frontotemporal dementia and Parkinson's disease (PD) [15], and even in memory impaired older subjects associated with brain Aβ burden [16]. Moreover, hypothalamic dysfunctions in AD may preclude immunity alterations, including reduction in salivary lactoferrin [17, 18], although other authors have not been able to replicate these findings [19]. Other recent and remarkable study showed that salivary GFAP levels decreased in mild cognitive impairment and AD patients and were proven a potential biomarker for AD [20].

Alternatively, to the use of saliva for the identification of circulating biological markers to help the diagnosis of early cognitive impairment associated with AD, saliva could also be useful to generate insights into the potential application of stem cells derived from salivary glands or saliva as therapeutics for the disease [21]. Previously, the presence of adult stem cells with mesenchymal characteristics in human parotid gland tissue was described [22]. New studies have reported the establishment of mesenchymal stem cell lines derived from mouse submandibular glands [23, 24]. As it is well known, mesenchymal stem cells have marked potential for use in cell therapy and regenerative medicine. In recent years, studies supporting the potential use of mesenchymal stem cell therapy for the treatment of autoimmune diseases have been published reporting their ability to modulate the immune response [25, 26]. Very recently, it has been observed that the treatment with salivary gland-derived mesenchymal stem cells via tail vein decreased the expression of interleukin 17 (IL-17), interferon gamma, and IL-6 levels and enhanced transforming growth factor beta and IL-10 secretion and restore salivary gland secretory function in the mouse models of Sjögren's syndrome [22].

PD is the second most common neurodegenerative disorder after AD, and it is also subject the difficulty in accurately diagnosing. Since α-synuclein (α-syn) is both genetically and pathologically linked to PD, it has been proposed as promising candidate biomarker [27]. More recently, it has reported α-syn as a potential biomarker of PD diagnosis in peripheral tissues [28]. Postmortem submandibular gland biopsies are positive for Lewy-type α-syn in patients with PD but not in healthy subjects [29, 30], and this finding has made salivary α-syn one of the most investigated salivary biomarkers in neurodegeneration. A decade ago, Devic and colleagues showed that α-syn concentrations significantly decrease in the saliva of PD patients as compared to healthy controls [31]. These results observed in saliva are mirrored in CSF [32]. These findings were later replicated by Al-Nimer [33] and Vivacqua [34] teams as they detected a significant decrease in total α-syn (syn_{total}) in saliva of PD patients when compared to healthy controls, while α-syn oligomers (α-syn_{olig}) and α-syn $_{olig}$/α-syn_{total} ratio exhibited a significant increase in the saliva of PD patients as compared to healthy controls [34–36]. Moreover, salivary α-syn measurement revealed specific cut-off values able to differentiate PD patients from healthy subjects with high sensitivity and

specificity, being even higher for the α-syn olig/α-syntotal ratio (69.77% and 95.16%, respectively) [35].

Interestingly, dysregulation of microRNAs (miRNAs) has been implicated in various neurodegenerative conditions, including PD [37, 38]. Salivary miR-153 and miR-223 levels may serve as useful, noninvasive, and relatively inexpensive diagnostic biomarkers of idiopathic PD based on their sensitivity (81% and 72%, respectively) and specificity (71%) [39]. These miRNAs were found to regulate α-syn expression in brain [40], and salivary miR-153 and miR-223 down-modulation in PD could reflect primary changes in the central nervous system.

In Huntington's disease (HD), huntingtin protein was successfully detected in saliva of HD patients and healthy controls. There was a significant increase in total huntingtin (Htt) protein concentration in saliva samples obtained from HD patients when compared to controls [41]. Given that currently a non-invasive measure of Htt CNS concentration does not exist, salivary Htt might be proposed as an early detection biomarker for HD, although new studies measuring sensitivity and specificity of salivary Htt will be needed.

However, investigation on salivary biomarkers for neurodegenerative diseases remains questionable. Although saliva is a fluid not in contact directly or indirectly with CNS, it is secreted by salivary glands directly regulated by cholinergic parasympathetic nerves connected with the hypothalamus, a brain area seriously affected in AD [42–45]. In addition to the structural abnormalities, including amyloid plaques and neurofibrillary tangles, functional studies suggest that hypothalamic dysfunction is a common AD manifestation, often an early event in the course of disease [43]. We propose that AD-related hypothalamic alterations could result in dysregulation of salivary gland function [17], as recent studies reported [18, 46]. Previous evidence suggests that Aβ can induce cholinergic hypofunction [47] whereas activation of muscarinic receptors can inhibit the generation of amyloidogenic Aβ [48, 49]. In our recent study, a reduction in M3 muscarinic receptor levels was observed in human submandibular glands from AD patients and APP/PS1 mice [18]. This last down-regulation of muscarinic receptors in AD salivary glands could be behind the increase in salivary Aβ levels. However, the mechanisms leading these salivary alterations in AD related proteins are not completely understood.

Although much evidence over the last decade supports the use of blood-based biomarkers for investigating AD, as it has recently reviewed [50], blood drawing itself is also an invasive technique. Saliva collection is non-invasive, economical, safe, and simple and can be performed without the assistance of specialized health care personnel, even home collection, allowing for point-of-injury sampling.

While many diseases have confirmed salivary biomarkers [51], diseases affecting the nervous system have few confirmed markers available in saliva which are still being investigated. Important considerations must be taken in account when using saliva samples as diagnostic tools. Standardization and consensus in the saliva collection, processing and storage is needed in order to decrease biases and allow an accurate identification of salivary biomarkers, as we recently proposed [52]. Intrinsic and extrinsic factors affecting donors might have influenced the production of salivary proteins. Moreover, intra- or inter-laboratory variability of salivary analyses may influence the diagnostic classification. These variances may be attributable to differences between duplicate assays of the same sample within laboratories or associated with differences between laboratories. The need of reproducing research assay was also highlighted by Ashton and colleagues [53]. Thus, efforts on harmonization of procedures should be encouraged to optimize the accuracy of salivary biomarkers in AD. In any case, saliva-based methods may open new windows in disease diagnosis if more standardized, efficient, and broadly implementable kits are developed. Thought procedure, confounding variables, and protocols need to be optimized in the future, some of these testing products could become a glimmer of hope for many communities globally.

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