Beyond dentistry: could prevention and screening for neurodegenerative diseases start in the dental office?

Francesca R. Buccellato*, Daniela Galimberti, Gianluca M. Tartaglia

The differential diagnosis of neurodegenerative diseases is complex and relies on clinical assessment, biomarker levels in cerebrospinal fluid, neuroimaging and neuropsychological assessment. The efforts of the scientific community are focused on two aspects: a) the discovery of minimally invasive biomarkers; b) the discovery of early biomarkers that can predict the progression to clinical disease in the pre-symptomatic stage of a disease. Considering the impact of the number of patients affected by chronic neurodegenerative diseases on public health expenditures, early diagnosis seems to be a primary need of our society.

Recent evidence suggests that saliva is a source of potential biomarkers for the early diagnosis of neurodegenerative diseases. Biomarkers are needed not only for diagnosis, but also for monitoring the efficacy of disease-modifying therapies during disease progression. Compared to other tests and imaging techniques used to diagnose neurological diseases, saliva is a biofluid that is easy to collect and manage. In addition, saliva is non-invasive and inexpensive compared to standard diagnostic tools, allowing screening of a broad group of healthy individuals.

The above intrinsic characteristics make saliva an ideal candidate biofluid for the study of neurodegenerative diseases. Saliva could be the standard fluid for early detection of biomarkers in asymptomatic or pre-symptomatic individuals for large population screening for Alzheimer's disease (AD) and other neurodegenerative diseases. In addition, following the coronavirus disease 2019 (COVID-19) pandemic, many diagnostic tests for a wide range of analytes are commercially available and suitable for the analysis of saliva samples, making the discovery of novel potential biomarkers more affordable. As a result of all these considerations, saliva has recently been proposed as a possible future gold standard sample for neurodegenerative diseases as an alternative to cerebrospinal fluid, neuroimaging, and blood (Ashton et al., 2019; Orive et al., 2022). Although Alzheimer's disease biomarkers, tau and amyloid-β, have been found in saliva, different authors have obtained conflicting and inconclusive results. In addition to these classic diagnostic biomarkers, new blood biomarkers of neurodegeneration, glial activation, and blood-brain barrier function have recently emerged. They may represent a future direction in the study of novel salivary biomarkers. Recently, salivary glial fibrillary acidic protein has been proposed as a biomarker to discriminate controls from mild cognitive impairment or AD patients (Katsipis et al., 2021). In this scenario, although new studies may lead to the discovery of an ideal salivary biomarker for the early diagnosis of neurodegenerative diseases in the future, there are no salivary tests that can replace the standard ones in the diagnosis of AD. Obviously, and at this stage of knowledge, there is no chance for saliva to completely replace the standard biofluid for neurodegenerative diseases. Furthermore, without claiming to do so, by using its intrinsic characteristics of easy collection and wide availability together with a low-cost approach, there is a strong opportunity to use it for large and early biomarker measures in the population.

The aim is to envision the creation of a forefront niche of "salivary bioscience diagnostics" that presents a high specificity. The contribution that dentists can give to this objective is that they are well distributed in the territory and they can see, at least twice a year, for oral health maintenance, healthy patients in a possible asymptomatic stage. The creation of this niche is expected to be a step forward, not only for the purpose of point-of-care diagnostics, but also for the preliminary standard for early detection of communicable and non-communicable diseases. It is easy to understand that only patients who are positive to a highly specific saliva test with a high negative predictive value will receive a second level test, which is now considered a standard diagnostic test.

Moreover, among the molecules that can be detected in saliva, microRNAs (miRNAs) represent promising biomarkers for the diagnosis, prognosis and therapies of different pathologies, including AD. miRNAs are involved in different pathways as they can target different genes and regulate, activate or inactivate their expression. Several miRNAs have been found to be dysregulated in plasma, serum or exosomes of patients with AD and other neurodegenerative diseases (Fenoglio et al., 2013; Galimberti et al., 2014). Recently, saliva has also been tested as a source of miRNA biomarkers in Parkinson's disease patients (Cressatti et al., 2020). Aiming to reproduce these findings in our laboratory, we have started to test whether miRNAs are detectable in saliva of healthy volunteers recruited at the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, using Taqman open array technology, capable of detecting 754 miRNAs simultaneously (Thermo Fisher, Waltham, MA, USA, Cat# 4470187). Approximately 100 miRNAs were detected. Also lncRNAs were detected with TaqMan single probe technology. Furthermore, changes in salivary metabolites have been associated with neurodegenerative diseases, opening a new perspective in salivary metabolomic studies (Hyvärinen et al., 2023; Song et al., 2023).

Another area of research is the inflammatory response, which is involved in the development and progression of many pathological conditions, including neurodegenerative diseases. In this regard, it has been proposed that dysbiotic oral bacteria can lead to systemic inflammation and contribute to neuroinflammation. Oral bacteria primarily act by releasing proinflammatory cytokines into the bloodstream, which could cross the blood-brain barrier via the trigeminal nerve and reach the brain (Sansores-España et al., 2021). In addition, dysbiosis of the oral microbiota could trigger changes in the gut microbiota through the oral-gut-brain axis, contributing to the release of proinflammatory cytokines into the bloodstream. Once in the brain, microglia may respond to proinflammatory cytokines by regulating the immune response, while astrocytes may respond by increasing cytokine production and consequently neuroinflammation. Thus, the possibility of detecting miRNAs or inflammatory cytokines in the saliva of AD patients is a promising approach in the search for an alternative biomarker that opens new diagnostic and therapeutic perspectives (**Figure 1**).

Interestingly, oral biosensors and wearable bioelectronic platforms using nanomaterials have recently been investigated for the detection of salivary biomarkers in neurodegenerative and demyelinating diseases (Goldoni et al., 2022).

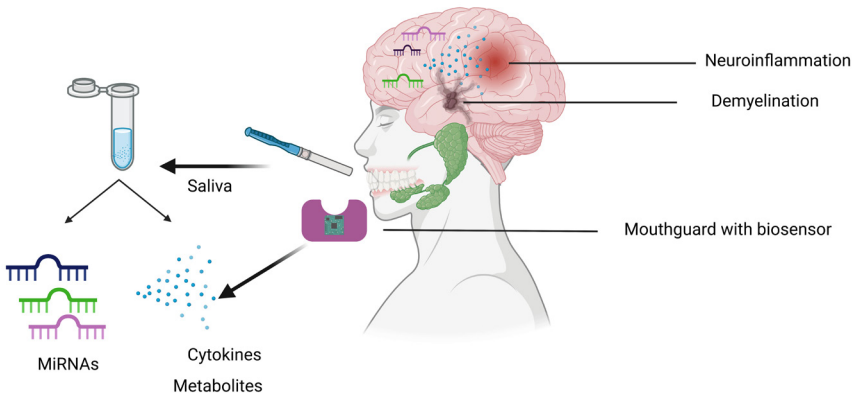

Figure 1 | **Schematic figure of different biomarkers detectable in saliva using Taqman Open array technology or oral biosensors.**
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To date, researchers can develop extremely sensitive and selective biosensors that can screen biomarkers present at low concentrations in complex biomatrices such as blood, urine, and saliva. The design of intelligent systems based on biosensors promises the realization of portable, flexible, multifunctional, and efficient operating devices that enable real-time, rapid, and *in vivo* detection of biomarkers of interest. The use of mouthguard-based devices for saliva testing is a new technology that has been proposed in recent years. However, only a few examples of multiplexed biosensing platforms can be found in the literature for applications in neuroinflammation and neurodegeneration, and none of these studies used saliva for the biosensing application. Nevertheless, this newly available technology could pave the way for the use of biosensing in early molecular diagnosis of neurodegenerative diseases as an alternative to current diagnostic methods. Furthermore, the group foresees that the development of such early diagnostics will empower clinicians and patients. Clinicians will save time that is usually spent on unnecessary routine patient visits and can thus use this time for other tasks. On the other hand, the patient will play a much more active role than in the past. In the near future, a patient could actively collect saliva and interact directly with his own medical data, as he will have exclusive ownership of his data, using a technological biosensing platform, and will be encouraged to share the data with his clinicians to make optimal use of it. Last but not least, it will generate tangible and extremely valuable economic and social benefits. For the time being, further research is needed to discover and validate accurate biomarkers in saliva and to develop biosensing platforms that will be able to detect a specific salivary signature for different neurodegenerative diseases.

In particular, the development of wearable biosensors may be useful not only in the dementia spectrum, but also in demyelinating diseases such as multiple sclerosis, to predict the occurrence of attacks in relapsing-remitting forms, thus allowing preventive treatment to minimize or ideally prevent the occurrence of neurological deficits.

Data from the literature show that oral health status is associated with cognitive performance and risk of Alzheimer's disease (Sureda et al., 2020). An association between Alzheimer's disease and several specific oral pathogens has already been established. In addition, the composition of the oral microbiota is altered in patients with multiple sclerosis, and these microbial changes have been postulated to be associated with multiple sclerosis disease activity and progression (Troci et al., 2022). These findings suggest that poor oral health could be defined as a risk factor for various neurological diseases. In this scenario, close collaboration between dentists and neurologists could provide access to saliva samples in both large healthy cohorts to define the normal ranges of a number of metabolites, and well-characterized patients.

The impact on national healthcare systems using point-of-care diagnostic technologies in saliva is potentially disruptive. Especially for the aging population in western countries and the increased demand for well-being in aging. Scientists and funding agencies are doing their best to reduce the burden of these non-communicable diseases through further advances in risk assessment, screening, diagnosis and treatment. However, the greatest challenge with these diseases is early detection at the earliest stage of disease. Although blood and other invasively collected fluids (e.g., cerebrospinal fluid) are excellent biofluids for the quantification of biomarkers, their collection is unfortunately inconvenient and painful for many patients. In this sense, there is a gap in which saliva and dentistry are emerging as a non-invasive and valuable source of information, as it contains many of the biomarkers found in blood.

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