Saliva as an ideal milieu for emerging diagnostic approaches in primary Sjögren’s syndrome

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ABSTRACT
This review will summarise the state of the art of salivary diagnostics in primary Sjögren’s syndrome exploring the potential usefulness of both traditional and emerging biotechnologies for primary Sjögren’s syndrome non-invasive and early detection.

Introduction
Salivary glands are main target organs in primary Sjögren’s syndrome (pSS). Therefore, over the years saliva has been seen as an ideal biological fluid that closely reflects the underlying pSS glandular autoimmune exocrinopathy. Recently, a renewed interest has arisen for saliva as an attractive milieu for the diagnosis of pSS, especially due to the development of emerging high throughput biotechnologies that allow comprehensive efforts to unravel the whole and gland specific saliva composition (1, 2). More specifically, salivary miromics and proteomics are two novel and in many aspects complementary approaches for the discovery of sensitive and specific biomarkers for pSS (3, 4). In parallel, sialometry and sialochemistry which have been proposed as diagnostic tool for pSS since the 1970s (5, 6), as well as salivary immunological assays are being re-evaluated as useful instruments in clinical management of pSS patients (2, 7). In this review, we will summarise the state of the art of salivary diagnostics in pSS taking into consideration the potential usefulness of both traditional and emerging biotechnologies for pSS non-invasive and early detection.

Working with saliva in pSS: pros and cons
Saliva is widely recognised as an attractive biological fluid for study of pSS and the advantages of working with saliva in pSS are undisputable. In fact, saliva contains both salivary gland produced and serum-derived markers that can mirror local and systemic pathological changes related to pSS-related pathology. In addition, compared to blood, saliva can be repeatedly collected in a non-invasive manner, representing a cost-effective approach for the screening of large populations (2, 8-13). Despite these advantage the flow, the composition and the protein concentration of saliva are quite variable among individuals; even within repeated measures of the same individuals. Those changes are attributed to many parameters, such as time of sampling, flow rate or stress among others (1). Two further important issues in pSS salivary diagnostics have to be considered as they may generate considerably different biomarkers for the same disease: collection of whole saliva versus individual gland saliva, and unstimulated versus stimulated saliva (14). So far, the vast majority of the studies for pSS salivary biomarkers have been performed in unstimulated whole saliva probably due to the fact that whole saliva collection is easier to perform in clinical settings (14). Nonetheless, whole saliva is largely influenced by the oral micro-environment of each individual whereas collection of individual gland saliva might provide more specific information regarding diseases of particular salivary glands. Similarly, in some cases, stimulated saliva collection may be required to obtain optimal amounts of saliva samples. Thus, even if promising diagnostic markers for pSS have been identified among salivary proteins and peptides, yet translation of these findings to clinical practice will require a better standardisation of the collection and processing of saliva as well as further validation, refinement, and technical improvements. The available data however, represent a promising starting point for looking at saliva requirements.

Key words: Primary Sjögren’s syndrome, saliva, proteomics, miRNAs

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Sialometry and sialochemistry in pSS

Sialometry and sialochemistry are traditional instruments employed in the assessment of patients with pSS since several decades ago (6). Sialometry allows the volume measurement of saliva in standard conditions for whole and gland specific saliva and in unstimulated and stimulated conditions. A reduced rate of secretion of unstimulated whole saliva is currently considered to be of diagnostic value in pSS (15). The collection of glandular saliva may reveal preferential involvement of salivary glands, such as selective hypothesis of the submandibular/sublingual (SM/SL) salivary glands. This is particularly useful since SM/SL salivary glands involvement is often observed in pSS since the early stages of the disease (16-18). Sialochemistry, on the other hand, examines electrolytes and proteins saliva. This studies will reflect the qualitative changes of the saliva of the affected individuals with pSS (16). Consistent findings reported previously include increased concentrations of sodium and chloride (19), elevated levels of IgA, IgG, lactoferrin, b2 microglobulin, albumin, salivary kallikrein and inflammatory mediators (i.e. eicosanoids, PGE2, thromboxane B2) (6, 17, 18, 20-22). In addition, a decreased concentration of phosphate has been reported in saliva of patients with pSS (20, 23). Over the time, the diagnostic value of both sialometry and sialochemistry has been extensively criticised especially in terms of specificity (24). Another element to consider is the lack of standardisation for these studies which might lead to potential pitfalls. For example the changes in concentration of any of the elements analysed in saliva from pSS could have been just a consequence of the difference in volume. Thus, both sialometry and sialochemistry represent at the moment mainly complementary tests for the assessment of pSS in clinical practice. Recently, sialochemistry has been re-evaluated as a potential tool to explore the underlying pathogenetic mechanisms of pSS. This has been related to the fact that steroid hormones can be detected in saliva and demonstrated excellent correlation with electrolyte levels (25, 26).

Salivary immunological aspects in pSS: autoantibodies and cytokines

The presence of SSA/Ro and/or SSB/La autoantibodies as well as of rheumatoid factor has been described in whole and parotid saliva of patients with pSS (7, 27, 28). Controversial data have been reported regarding the sensitivity and the diagnostic value of salivary autoantibodies in pSS (7, 29). Some authors have identified the SSA/Ro and SSB/La autoantibodies in the saliva of pSS patients in the absence of circulating antibodies in the serum suggesting a local increased production of these autoantibodies specifically from the salivary glands of pSS patients (7, 30). Anti-spectrin antibody and IgA autoantibodies against mAChR have been detected as well, with potential implications in the pathophysiology of the development of pSS dry mouth (30-32). Nonetheless, the vast majority of the available data do not confirm a higher sensitivity of saliva with respect to serum in detecting SSA/Ro and SSB/La autoantibodies or other autoantibodies and the usefulness of their routine assessment in clinical practice remains limited (29). Similarly, there is little information concerning the expression of cytokines in saliva. In fact, several studies have investigated cytokine concentrations in the serum and salivary glandular tissues from pSS patients mainly describing pSS as a Th1 dominated disease (33). In particular, IFNγ consistently was found highly expressed in pSS patients as well as IL12, IL18, TNF-α, IL1α, IL6 and B-cell activating factor (BAFF) (34-36). For salivary cytokines measurement, only a few studies have been performed concordantly reporting elevated levels of IL-2 and IL-6 in whole saliva of pSS (37-39). In summary, the available literature data confirm that saliva might be able to reflect some immunological aspects of the pSS exocrinopathy and open new possibilities for the characterisation of pSS autoimmune alterations directly into the oral environment.

Salivary miRNAs in pSS

A great deal of interest is arising in the diagnostic and prognostic role that salivary mRNA (40) and microRNAs (41, 42) can play in pSS and other systemic diseases. Their expression patterns reflect the pathophysiological status of a tissue (42) and have been shown to be specific for particular disease states. This can be more important in case of pSS, where one of the major affected functions, is the function of salivary gland and production saliva itself. MiRNAs are endogenous, small (approximately 22 nucleotides in length) non-coding RNAs, that regulate gene expression post-translationally (43). Since their discovery in C. Elegans in 1993, they have become a “hot” area of interest and their biogenesis has been extensively studied (44). In mammalian cells the biogenesis of miRNA starts with the transcription of the pri-microRNAs (thousands of nucleotides long) by RNA polymerase II (44). In the nucleus, Drosha, an RNase III enzyme processes the pri-miRNAs to pre-miRNAs. Pre-miRNAs are then actively transported from the nucleus to the cytoplasm. In the cytoplasm, Dicer, a cytoplasmic endonuclease, and other RNA binding proteins processes the pre-microRNAs into double stranded RNAs, 19-24 nucleotides long (43). They can exert their effect by binding to the target mRNAs inducing either their degradation or blocking their translation. The target selectivity of the miRNAs depends on the complementarity of their sequences with the sequences of the mRNA and it has been shown since that binding can also occur on the 5’UTR and the coding sequence of the mRNA (45). Salivary miRNA analysis may help understand abnormalities in saliva production, the regulation of the peripheral inflammatory response in salivary glands (46), as well as the pathogenesis of salivary gland tumours (47). Lately, it has been hypothesised and demonstrated that miRNAs can be found encapsulated in separate structures like exosomes present in saliva (46). It has also been shown that the majority of the salivary microRNA is concentrated in exosomes rather than free in solution.
Salivary proteomics in pSS

Over the last few years, another area of research that has made saliva a first-line diagnostic sample of choice for pSS is proteomic research. Concomitantly with the progressive unraveling of the human salivary proteome (50, 51), the possibility of using saliva as a diagnostic medium to detect and predict pSS disease progression has gained a growing attention (3, 14, 52). As a diagnostic fluid, saliva offers advantages over serum for proteomic research because compared to blood, saliva possesses a smaller amount of proteins with a minor risk of non-specific interference and hydrostatic interactions (53). More specifically, in pSS the analysis of salivary proteins allows mirroring locally the concomitant pathological involvement of major salivary glands which are one of the major target organs in the disease. So far, eight studies have been performed applying a proteomic approach to the study of saliva (see Table I) (9, 54-60). The vast majority of the studies have analysed the proteome of unstimulated whole saliva with the analysis protocols including both pool samples and individual samples. Parotid saliva has been characterised in two of the eight studies (57, 59). Different complementary techniques have been employed including 2DE and MALDI-TOF-MS, SELDI-TOF-MS and DIGE, ESI and HPLC (9, 54-61). Despite being diverse and preliminary, the focus of these studies has been the search for pSS diagnostic biomarkers with the ultimate goal of identify and validate a sort of "proteomic panel" for the early non-invasive detection of pSS. Proteins such as β2-microglobulin, Immunoglobulin κ light chain, Immunoglobulin γ light chain, α-enolase, cathepsin D, S-nanopeptide, cathepsin D, S, caspase 1, and caspase 3, have been identified as potential biomarkers for pSS. However, further studies are needed to confirm the validity of these biomarkers in a larger population of patients.
in pSS patients, while inflammatory phase proteins are increased when compared to normal subjects. The increase of inflammatory proteins seems to correlate to both the chronic inflammation of the salivary glands and to the persistent damage of the oral environment. Similarly, the increased expression of β2-microglobulin, Immunoglobulin K light chain and Immunoglobulin γ light chain in pSS saliva was thought to reflect both a systemic B-cells activation and an increased intra-glandular immunoglobulin synthesis (9, 59). Finally, the decrease of secretory proteins was attributed to both a potential damage/dysfunction of the acinar cells and to the presence of fragmentation processes in the saliva of pSS patients and a significant reduction in the density of the main spot of α-amylases. Cathepsin D, α-enolase and β2-microglobulin have been preclinically validated against Systemic Lupus Erythematosus (56), non-SS syndrome (9, 59) and secondary-SS (sSS) (9). As far as the comparison between pSS and sSS is concerned, patients with sSS showed an overall proteomic profile that was intermediate between that of the pSS patients and the healthy subjects confirming the validity of salivary proteomics in reflecting the autoimmune involvement of the salivary glands. Intriguingly, preliminary data from our group on a limited number of salivary samples from patients with systemic sclerosis (61) and rheumatoid arthritis (62) and no sicca symptoms also suggested that proteomic analysis of whole saliva might be able to mirror systemic autoimmune disorders even in the absence of a clearly manifest involvement of the salivary glands bringing new lights on the potential applications of salivary proteomics in rheumatology. This is not surprising considering that salivary proteomics has been shown to be informative for the detection of systemic diseases, like lung (63) or breast (64) cancers independently from the direct involvement of salivary glands. At the state of the art, nonetheless, despite these encouraging results, the possibility of translating salivary proteomics in a clinical setting is still challenging, especially due to the inter-and intra-subject variability; thus, large validation trials are required in order to obtain reliable proteomic biomarkers for the diagnosis of pSS and sSS. It is becoming clear, however, that in addition to the potential diagnostic applications, salivary proteomics might generate new and intriguing scenarios also for studying the complex pathogenetic mechanisms underlying pSS and for the identification of novel therapeutic targets for the disease. This aspect is of particular interest considering that many of the molecular pathways of pSS are obscure, and that no specific treatments are known to be effective. (65). Moreover, the early identification of pSS patients at higher risk for lymphoproliferative complications is still difficult due to the lack of prediction biomarkers (66-68). From this point of view, Hu et al. (69) performed a transcriptomic and proteomic analysis of human parotid glands from patients with pSS and patients with pSS and MALT lymphoma showing that panel of 8 candidate genes (GRB2, ARHGDB, CD40, proteasome subunit, aldolase A, peroxiredoxin 5, PARC, and cyclophilin A) that could distinguish the two groups of patients. Proteomic analysis showed that 70 proteins were up-regulated in pSS MALT lymphoma compared to normal subjects and pSS, and 45% of these proteins had mRNA transcript concordantly expressed (69). Isolated reports have also outlined a correlation between salivary proteomic profile and clinical course in patients with pSS syndrome and Non-Hodgkin’s lymphoma (NHLs) (54, 70). Reasonably, once validated and confirmed, these candidates could be translated into early prognostic biomarkers for lymphoma susceptible pSS patients.

In summary, salivary proteomics studies has led to stimulating results in the discovery of preliminary diagnostic, prognostic and therapeutic biomarkers for pSS. Encouraging additional more comprehensive studies aimed to enhance the potential of saliva for the discovery of reliable biomarkers for pSS. Larger sample size and standardisation of sample collection/treatment protocols may improve future studies.

Future developments

In the last few years, the discovery of novel biomarkers for pSS has become a necessity especially considering the emergence of novel biological therapeutic agents for the disease. Saliva has appeared as an obvious source for non-invasive biomarkers in pSS since it is a direct product of the affected target organs. Genomic and proteomic technologies have reinforced the feasibility of saliva as an attractive biological fluid for the search of biomarkers in pSS. At present nonetheless, individual efforts looking at single diagnostic parameters have not led to findings that can be translated in a clinical setting, partly because complex diseases like pSS might require a more systemic multidimensional approach that can efficiently capture the most relevant factors associated with this syndrome. In the near future, in order to pragmatically translate basic research into clinical practice some critical factors need to be overcome including a standardisation of the conditions for saliva collection and analysis protocols. It is undoubtedly however, that the application of emerging technologies to saliva has opened a new scenario for pSS diagnosis and prognostic stratification. Likely, in the long term the identification and validation of novel transcriptomic, proteomic, and molecular salivary biomarkers for pSS as well as the optimisation of functional studies could also help to shed new lights on the pathogenetic pathways underlying the different subsets of pSS leading to the development of new concepts for treatment modalities.

References

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