# SIALOCHEMISTRY – A DIAGNOSTIC TOOL

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#### ABSTRACT

As a diagnostic fluid, saliva offers distinctive advantages over serum because individuals with modest training can collect it non-invasively. Analysis of saliva may be useful for the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases, and endocrine disorders, as well as in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use.

# **INTRODUCTION**

A large number of diagnostic analytes have been shown to be present in saliva, including steroid hormones1 and the HIV antibody.2 For the past two decades, oral health researchers have been developing salivary diagnostic tools to monitor oral diseases including periodontal diseases,<sup>3,4</sup> as well as for caries risk assessment.<sup>5</sup> These diagnostic advances range from genetic susceptibility analysis of interleukin-1 (IL-1) genetic alleles to the analysis of oral pathogens identified via lectin staining for caries risk assessment.<sup>6</sup> The current development of diagnostic biomarkers (via proteomic and genomic approaches) in conjunction with technological developments in salivary diagnostics will lead to the development of robust diagnostic tools for dentists to use in making clinical decisions and predicting treatment outcomes.<sup>7</sup>

#### METHODOLOGY

Whole saliva is a product of secretion of 3 major glands (parotid, submandibular, sublingual) and many minor glands (labial, buccal, palatal). Unstimulated saliva is usually obtained as the patient spits out every 60 sec or by forward bended head, the patient allows saliva to drip off the lower lip into a cylinder. By collection of saliva in the tube the flow rate per unit time can be measured. When volume measurement is not required the saliva can be collected on cotton rolls, gauze or filter paper. For evaluating salivary gland function or when large volumes of saliva are required for analytic purposes, stimulated whole saliva is used.

Method of collection is the same as for unstimulated saliva.8 The usual masticatory stimuli are paraffin wax or a washed rubber band. A standard gustatory stimulus is obtained by 2% citric acid applied directly to the tongue every 15 to 60 sec. Parotid saliva can be collected by aspiration from the duct opening with a micropipette. Parotid saliva is best collected with Lashley's vacuum.9 Submandibular and sublingual saliva can be collected by cannulation of the duct with micropipette, but in practice this is both uncomfortable for the patients and technically difficult since the duct orifice is mobile and has a strong sphincter. Because of that, alginate and silicone impression material is used for retention of the collecting tube. As alternative and simple technique is to block off secretion from the parotid glands with absorbent swabs and collect mixed submandibular and sublingual saliva by pipette from the floor of the mouth. Saliva from labial and palatal glands can be collected by filter paper disc or disc of other synthetic materials.<sup>10</sup> Salivary flow rate is given as ml/min/gland. Under 'resting' conditions the flow rate of the parotid gland amounts to 0-0.1 ml/min. After citric acid stimulation the range is 0.5-1.5 ml/min. Stimulated values below 0.3 ml/min are considered pathological. Elevated flow rates will be seen under conditions such as gingivitis, recent prosthesis and dominant cholinergic activity in Parkinson's disease, intoxication etc. Low values are found during the use

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of tricyclic antidepressants, after duct disintegration caused by inflammation or irradiation and after radical surgical treatment. The effects are more dramatic in resting saliva on account of intensified water resorption in the resting state. The choice of laboratory investigations should be based on presumed relationships with intra glandular transport processes (sodium), intra cellular synthesis (protein, amylase), and diffusion by plasma constituents (urea). Saliva also influences the oral environment in a number of ways. Measurements are given as concentrations c.g. mmol/ 1. This facilitates the assessment of ion/water shift and osmotic values. Secreted solutes, given as mmol/min or in mg/min (mmol/1 x ml/min), are useful in judging acinar destruction, as in irradiation and aging. Routine laboratory investigations include potassium calcium, sodium, chloride, bicarbonate, urea, and total protein, amylase, and osmolarity measurements.

# SIALOCHEMISTRY

The diagnostic use of saliva has attracted the attention of numerous investigators because of the noninvasive nature and relative simplicity of collection. Saliva collection also simplifies the diagnostic process in special populations in whom blood drawing is difficult, i.e. individuals with compromised venous access (e.g., injecting drug users), patients with hemophilia and children. The presence of six enzymes was established in parotid saliva: acid phosphatase, total esterases, cholinesterase, lipase, beta-glucuronidase, and lysozyme. Broth cultures used for this study with whole saliva indicated that all but sulfatase and lysozyme were produced by the oral flora.<sup>8</sup>

Abnormal proteins are also produced under exceptional conditions, such as the development of tumors and nutritional deficiency. Low a-amylase concentrations are seen in cases of starvation and after destruction and degeneration of the acinar cells. Elevated aamylase is seen in abnormal ductal water loss. Furthermore, acute inflammation of the glands produces a rise in plasma and urine amylase due to gross glandular leakage. This will be seen in mumps as well as in the presence of a salivary calculus.<sup>11</sup> Salivary analysis can be valuable to discriminate and monitor swellings of major salivary glands such as chronic recurrent parotitis, where the blood-saliva barrier is violated in the inflammatory flare-ups and characterized by the leakage of serum components (albumin) and lactoferrin into saliva. Higher enzyme activities were found in the adult periodontitis patients compared to the healthy controls for alkaline phosphatase, esterase, β-glucu-

ronidase,  $\beta$ -glycosidase, and other amino peptidases. Saliva from patients with localized juvenile periodontitis contained the highest levels of butyrate esterase and cystiene amino peptidase.<sup>12</sup> Sialochemistry can be expected to reveal the differentiation between normal and abnormal function of the glands, information about gland dysfunction and its impact on the oral environment, clues to homeostatic fluctuations as a result of circulatory, innervatory, or hormonal adjustments. The development of microchips for salivary components offers great possibilities to use oral fluid for point-of-care testing.<sup>13</sup> Another fertile area of application for salivary analysis is in laboratory medicine, where determining and monitoring levels of various hormones (cortisol, progesterone, estriol, testosterone etc.) and drugs (diazepam, caffeine, lithium, theophylline, tolbutamide, methotrexate, antibiotics, anticonvulsants, etc.) is becoming a conventional procedure. Oral fluid can be used to detect recent use of illicit drugs. Saliva may be used for monitoring patient compliance with psychiatric medications.<sup>14</sup> A significant correlation exists between the salivary and serum lithium levels in patients receiving lithium therapy<sup>15</sup> Saliva is also useful for the monitoring of anti-epileptic drugs. Salivary carbamazepine levels showed positive correlation with serum levels. In another study, salivary levels of Phenobarbital and phenytoin demonstrated excellent correlations with serum levels of these medications. Salivary theophylline concentration demonstrated correlation with serum concentration of theophylline.<sup>17</sup> Saliva may also be used for monitoring levels of anti-cancer drugs. Saliva was found to be a reliable alternative to serum for the monitoring of irinotecan levels.<sup>18</sup> Salivary ethanol concentration may be used as an index of the blood ethanol concentration, provided that the salivary sample is obtained at least 20 min following ingestion. This will allow for absorption and distribution of alcohol, and prevent a falsely elevated reading due to the oral route of consumption.<sup>19</sup> Other recreational drugs that can be identified in saliva are amphetamines, barbiturates, benzodiazepines, cocaine, phencyclidine (PCP), and opioids.<sup>20</sup> Saliva can also be used to detect recent marijuana use by means of radioimmunoassay. Monitoring of salivary albumin can assist in the identification of stomatitis at a pre-clinical stage and enable the chemotherapy dosage to be adjusted or treatment for the stomatitis to be initiated at an early stage. Whole saliva is most frequently used for diagnosis of systemic diseases, since it is readily collected and contains serum constituents derived from local vasculature of salivary glands and reach oral cavity via the flow of gingival fluid. Saliva is increasingly being used as an investigational aid in the diagnosis of systemic diseases that affect the function of the salivary glands and the composition of the saliva, such as Jorgen's syndrome, alcoholic cirrhosis, cystic fibrosis, sarcoidosis, diabetes mellitus and diseases of the adrenal cortex. In the past 10 years researchers have demonstrated that saliva tests for anti-bodies to HIV represent a noninvasive alternative to quantification of antibodies in blood for monitoring the efficacy of antiretroviral therapies and disease progression to acquired immunodeficiency syndrome.<sup>21</sup> In disease diagnosis, however, saliva has many advantages over both serum and urine. For example, salivary assays for anti-bodies (to viruses and bacteria), unconjugated steroid hormones (e.g. estrogen, testosterone and progesterone), environmental toxins (e.g., cadmium, lead and mercury), tobacco (cotinine) and certain drugs (ethanol, theophylline and lithium) are sufficiently sensitive to accurately reflect the blood concentrations of these substances.<sup>22</sup>

Methods for determining salivary gland dysfunction include salivary flow rate measurements (sialometry) and analysis of salivary composition (sialochemistry) analyzed by using radioimmunoassay (RIA), for which whole saliva (oral fluid) is most frequently used. The concentration of IgA was somewhat elevated (commensurate with reduced flow rate); the levels of IgG, IgM, and albumin were normal. The major functional abnormality of the parotid gland in Jorgen's syndrome appears to be luminal transport in the ductal region; leakage of serum components is minimal. Sialochemistry can be helpful in differentiating Jorgen's disease from other diseases of the salivary gland and in assessing degree of pathologic change<sup>23</sup> p53 antibody is a tumor suppressor protein which is produced in cells exposed to various types of DNA-damaging stress, can also be detected in the saliva of patients diagnosed with oral squamous cell carcinoma (SCC), and can thus assist in the early detection and screening for this tumor. Higher concentrations of salivary defensin-1 were detected in patients with oral SCC in comparison to healthy controls.<sup>24</sup> The activity of CK (creatinine kinase), LDH, ACP (acidic phosphatase), GGT (gamma glutamyl transferase) in saliva from patients with periodontal disease, before and after treatment (experimental Group 30 samples), and in saliva from periodontally healthy patients (20 samples) were studied. The results showed the elevated levels of these enzymes before the treatment in periodontal patients when compared with healthy patients<sup>25</sup> in 273 workers, who were divided into non passive and active smokers based on salivary cotinine levels on 0, 1-7 and >8 mg/ml

respectively. Levels of salivary markers, including IL-1b, lactoferrin, albumin were elevated in passive smokers compared to non smokers, were independently associated with cotinine level. They concluded that passive smoke exposure leads to elevation of IL-1b, albumin and aspartate aminotransferase (AST) levels in saliva.<sup>26</sup>

Patients with heart diseases were divided into 2 groups: Warfarin user and Warfarin non-user, and six components of saliva: salivary total protein (TP), albumin(ALB), C-reactive protein (CRP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK) were determined to assess intra oral pathologic conditions. As a result there were many cases showing a high ALB, CRP, AST and ALT level in Warfarin user and significant correlation found between clinical parameters and salivary components.<sup>12</sup> Increased levels of circulating and salivary cortisol (the stress hormone) result from the activation of the hypothalamic-pituitary-adrenal axis brought on by psychological stress and extensive physical activity. Salivary proteins can also maintain an ecological balance among the diverse bacteria that affect oral and general health. Human saliva contains proteins that can be informative for disease detection and surveillance of oral health.<sup>27</sup> Recent studies have shown that several species of oral streptococci, such as Streptococcus gordonii, bind to salivary alpha-amylase immobilized onto hydroxyapatite surfaces. Thus amylase, as a receptor for streptococcal adhesion to the tooth, contributes to the formation of dental plaque.<sup>16</sup> Several salivary and oral fluid tests have been developed for HIV diagnosis. Saliva may also be used for determining immunization and detecting infection with measles, mumps, and rubella. Saliva can be used for the detection of oral candidiasis, and salivary fungal counts may reflect mucosal colonization. Saliva may also be used for the monitoring of oral bacteria. Detection of certain bacterial species in saliva can reflect their presence in dental plaque and periodontal pockets. High-impact diseases, including cancer, cardiovascular disease, and neurological disease, are challenging to diagnose without supplementing clinical evaluation with laboratory testing. Even with laboratory tools, definitive diagnosis often remains elusive. The Oral Fluid Nano Sensor Test (OFNASET) technology platform combines cutting-edge technologies, such as self-assembled monolayers (SAM), bio nanotechnology, cyclic enzymatic amplification, and microfluidics, with several wellestablished techniques including microinjection molding, hybridization-based detection, and molecular purification. The use of the OFNASET helps multiplex detection of salivary biomarkers for oral cancer.<sup>28</sup> The UCLA laboratory recently discovered that discriminatory and diagnostic human mRNAs are present in the saliva of healthy people and people with disease. The salivary transcriptome offers an additional valuable resource for disease diagnostics. The behavior of these salivary transcriptome biomarkers is consistent i.e. their levels are significantly higher in the saliva of patients with oral cancer than in the saliva of matched control subjects<sup>25</sup> however, before a salivary diagnostic test can replace a more conventional one, the diagnostic value of a new salivary test has to be compared with accepted diagnostic methods. The usefulness of a new test has to be determined in terms of sensitivity, specificity, correlation with established disease diagnostic criteria, and reproducibility.

# CONCLUSION

It is becoming increasingly apparent to investigators and clinicians in a variety of disciplines that saliva has many diagnostic uses and is especially valuable in the young, the old and in large scale screening and epidemiologic studies. The saliva has found use as a diagnostic aid in an increasing number of systemic diseases that can affect salivary gland function and composition Changes in salivary flow rate may affect the concentration of salivary markers and also their availability due to changes in salivary pH. In addition, many serum markers can reach whole saliva in an unpredictable way (i.e., GCF flow and through oral wounds). These parameters will affect the diagnostic usefulness of many salivary constituents. Whole saliva also contains proteolytic enzymes derived from the host and from oral micro-organisms). These enzymes can affect the stability of certain diagnostic markers. Some molecules are also degraded during intracellular diffusion into saliva.

Different diseases that have inflammatory processes in common will show the same changes in their sialochemical patterns and are very sensitive. At the same time, the specificity regarding classified diseases is low. Therefore a correct diagnosis will always require a full clinical and laboratory investigation. However, sialochemistry is a useful means of chronologically, monitoring qualitative and quantitative changes.

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