SALIVA IN RESEARCH AND CLINICAL DIAGNOSIS – AN OVERVIEW

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Zubaidah Hj Abdul Rahim
Associate Professor
Oral Biology Department
Dental Faculty
University of Malaya
50602 Kuala Lumpur

ABSTRACT

Saliva collection is non-invasive and less stressful when compared with blood collection. Extensive studies on saliva has been carried out and the use of saliva as a biological sample in clinical diagnosis and for monitoring hormones, drugs and pollutants and viruses has been recommended. The complexities associated with saliva such as proper collection device and strict standardisation of a number of factors which include time of collection, types of saliva and storage made it less favourable to blood.

Keywords: Saliva, collection apparatus, storage conditions, clinical diagnosis.

INTRODUCTION

Saliva is the fluid produced by the salivary glands. About 0.5 to 1 liter of saliva is produced per day by the different salivary glands, of which 92 % of the total salivary volume is produced by the major salivary glands, the remaining by the numerous minor (accessory) salivary glands. Of the major salivary glands, parotid glands contribute 6065 % of the total salivary volume, submandibular glands contribute 20-30 % of the total salivary volume and sublingual glands produce 8% of the total salivary volume. Saliva from the parotid glands enters the oral cavity via Stensen's ducts, from the submandibular glands via Wharton's ducts and the sublingual saliva via the Bartholin's ducts.

Saliva collection has not been considered appealing because of the frothy and slimy look of the saliva collected and the unpleasant odour it may produce on storage. Despite this, research has repeatedly proven the importance of saliva, both as a fluid crucial to the oral health and as a useful substance in clinical diagnoses.

Saliva as a fluid crucial to the oral health

Saliva is made up water (99%) and organic and inorganic components which are responsible for the roles exhibited by saliva. Saliva is known to facilitate digestion with the presence of α-amylase (the activity may be minimal because food does not stay in the mouth long enough for it to be significant in digestion); the high content of water in saliva provides a medium for food to be dissolved and influences the perception of taste; and the mucous glycoprotein (mucin) in saliva helps to lubricate the food in the mouth, thus making it easier to form a food bolus for swallowing. The glycoprotein also facilitates mastication and speech.

Besides the roles already mentioned, some of the salivary proteins have defensive properties (antibacterial activity) which help to maintain the ecological balance. The oral cavity is protected against damage due to pH changes by the components of saliva which exhibit buffering capacity, such as bicarbonate, phosphate and proteins,. These components are responsible for maintaining the right environmental pH. Saliva also has anticaries activity as has been demonstrated by subjects exhibiting low salivary flow rate (1). Because it is supersaturated with respect to tooth mineral, saliva facilitate remineralisation of early caries lesions.

Saliva in clinical diagnoses

Saliva collection, unlike blood collection, is non-invasive. In recent years, it has been indicated in a number of reports that saliva may be used for the diagnosis and monitoring of disease. Changes in the levels of certain salivary constituents are reported to be associated with certain diseases (2).

Acute inflammation of salivary glands may affect the blood-saliva barrier, causing an increase in serum constituents, e.g. albumin, IgG and IgM (3,4). The level of sIgA and Iysozymes in saliva may be increased, as a consequence of the activation of the defence mechanism in the salivary gland.

Diseases such as cystic fibrosis and diabetes mellitus which are metabolic diseases, may affect the salivary glands (5). Changes in the levels of calcium and sodium (6), protein (7,8,9) and α-amylase (10) in saliva of these patients have been reported. The use of saliva as a biological sample in clinical diagnosis is possible when the composition of saliva is affected by the disease; for instance when the salivary gland is dysfunctional, it may lead to reduced secretion of saliva-specific substances. In Sjögren's syndrome, protein concentrations and the types of protein secreted are altered (11) and elevated salivary sodium, potassium and IgA concentrations are used for diagnosis (12). The levels of cortisol in whole saliva in human immunodeficiency virus (HIV) individuals have been reported to be elevated (13,14,15) though the use of cortisol levels in the diagnosis of HIV need to be further evaluated.

Sequential measurements of salivary estradiol-17β in saliva have been reported to be useful in the management of infertile patients. The concentrations of salivary estradiol 17-β correlate with levels in serum. The advantage in using saliva samples is that saliva collection is non-invasive and stress-free. Furthermore its collection can easily be done by the patient in their own home. Salivary phosphate correlates with serum estrogen and has been suggested for detection of ovulation (16).

Saliva is also recommended for monitoring hormones (17), drugs and pollutants (12,18) and viruses (12). Drugs like alcohol, theophylline, carbamazepine or lithium can be

monitored in saliva. Steroid hormones such as cortisol (19,20) or testosterone (21,22) correlate with serum levels. Salivary cortisol is useful for detection of stress (23). In the detection of digitalis toxicity, calcium and potassium concentration are useful (12). This is possible since salivary glands are target organs for several drugs and hormones.

AVAILABLE DATA - HOW CONCLUSIVE IS IT IN COMPARING STUDIES

The composition of saliva is dependent on a number of factors, including flow rate and circadian rhythms (24), intake of drugs (25,26) and certain diseases. Before commencing saliva analyses, there are aspects to be considered and these include the total number of parameters to be determined and volume of saliva required, the types of salivary samples (stimulated, unstimulated, whole saliva or that from individual glands), storage conditions (no storage, 4°C,-20°C or below) and pretreatment of saliva (centrifugation, sonication, dividing into aliquots).

In saliva research which involves comparing studies, the types of salivary samples (2, 27,28), method of collection, time of day or year of saliva collection, storage conditions, saliva pretreatment, analytical methodologies and subject's medical status and medications are factors that must be considered. Accurate measures of salivary flow rate and composition are essential for many clinical, experimental and diagnostic protocols.

Types of salivary samples

Saliva consists of water, electrolytes, proteins, other organic compounds, microorganisms and cellular debris (being found in whole saliva). Saliva from individual glands and whole saliva are different in their properties. Submandibular and sublingual saliva are viscous and sticky, parotid saliva is watery, whereas whole saliva depends on the glandular contributions.

A problem with whole saliva is that it is contaminated with other non-salivary constituents. In the assessment of salivary gland dysfunction, whole saliva is superior and clinically more relevant than the individual gland saliva (29). Parotid saliva has been recommended for the routine monitoring of drug levels as well as exposures to toxic substances (30).

Saliva may be collected from resting salivary glands, which means that the glands are not stimulated. Saliva may also be collected from stimulated salivary glands and the stimulation can be gustatory, mechanical etc.

It has been recommended that when stimuli like chewing paraffin wax, parafilm, rubber bands, pieces of Teflon or chewing gum are used, the subjects should allow saliva to accumulate in the mouth until the desire to swallow occurs (31). At this time the subjects should expel the fluid smoothly into a vessel. The subjects are advised not to expectorate repeatedly as this will lead to errors in interpretation of the saliva/plasma concentration ratio because bubbles tend to be introduced. These types of stimuli will result in a flow rate of 1 to 3 ml/min (32).

Collection of saliva

Techniques used in saliva collection should be standardised

Different methods have been used for collection of saliva. Whatever method is used, the subject should rinse his mouth thoroughly with deionised water prior to the collection trial and void it. The subject should then sit comfortably with eyes open, head tilted forward slightly, rest for 5 minutes (before commencing unstimulated saliva collection) and minimise orofacial movements. Smoking, eating or drinking is not allowed for at least 3 hours prior to saliva collection.

The samples should be collected in chilled tubes, kept on ice and then frozen until analysis, the condition necessary for saliva to be used in compositional and enzyme studies (29). For steroid hormone analysis, it is advisable to include a bacteriostatic agent in the collection tube or otherwise the saliva should be collected via ductal cannulation (parotid saliva) (33).

Collection apparatus

The collection apparatus used in the saliva collection has to be standardised and should be reliable. Apparatus to be used for collecting whole saliva is different from those used for collection of individual gland saliva. The draining method using a proflow sialometer (Fig. 1), spitting method, suction method (29), swab or absorbent method (34) and salivette (35) have been used in the collection of whole saliva. Salivette (Fig. 2) absorbs a relatively large volume of saliva

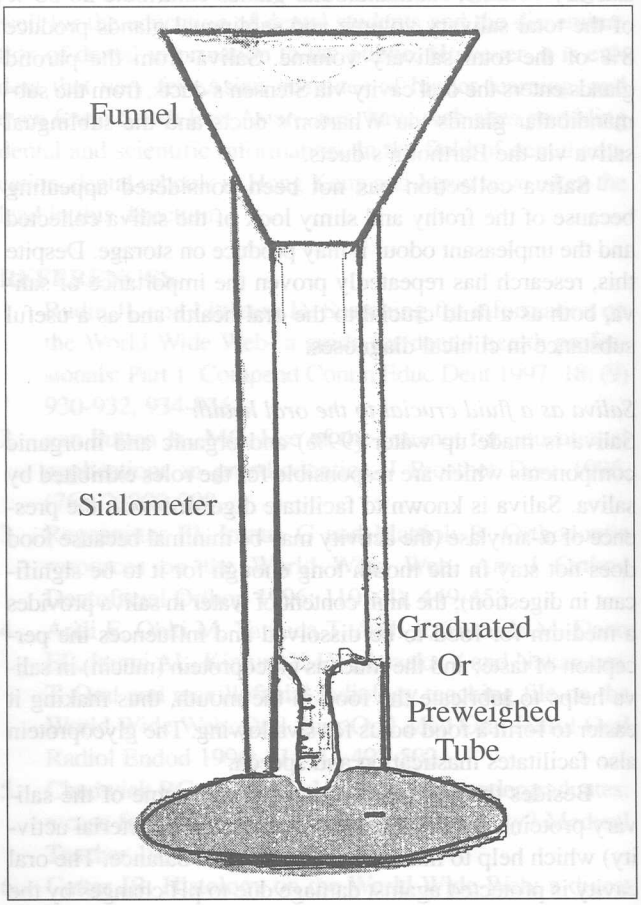

Figure 1. Proflow Sialometer

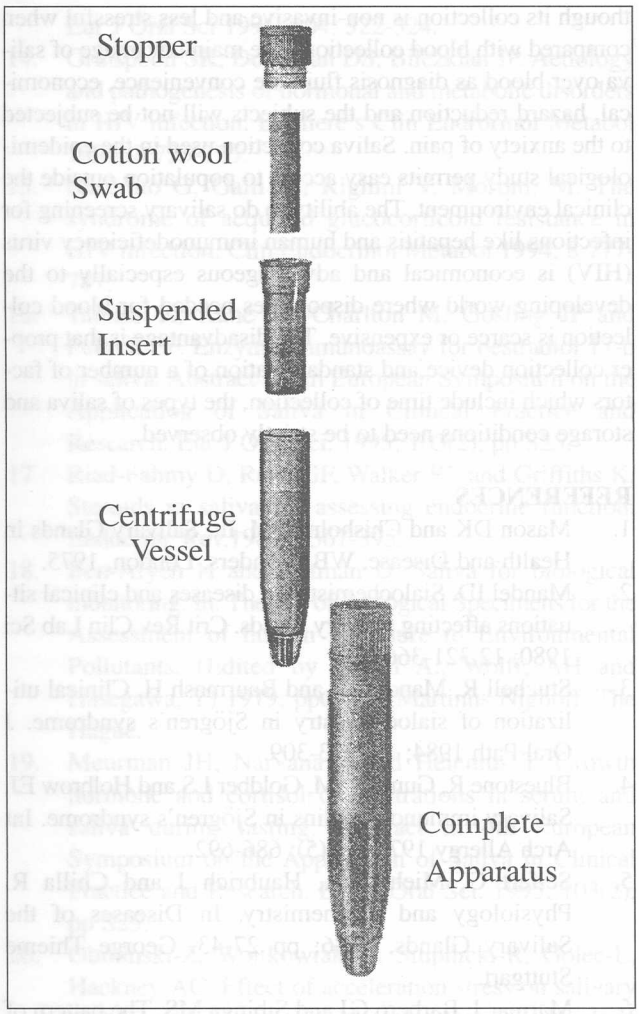

Figure 2. Salivette

(1.5 ml) in a short time (36) but it is not advisable to use it in the collection of saliva for the assay of several drugs and hormones especially testosterone (37).

The draining method has been recommended to be reliable and reproducible for the collection of unstimulated whole saliva. The spitting method has been recommended for unstimulated and stimulated whole saliva.

Collection of saliva from individual glands has been done by use of the Lashley cup (Fig. 3) (38), modified Carlson-Crittenden devise (39), micropipette (40) and SLURP collection cup (41). Using a Lashley cup requires trained personnel, supporting equipment and good light conditions whereas SLURP collection cup (Fig. 4) does not and both methods are said to be useful in the collection of pure parotid saliva and both have good correlation (41). Micropipettes, however, have been used in the collection of submandibular and sublingual saliva.

Time of day and year of collection

Flow rate is said to be highest in the afternoon (42). During winter, the parotid salivary flow rate is highest (this is important when long term study is necessary for research carried out in countries having four seasons) (43). The salivary composition is strongly affected by the flow rate. In general, the

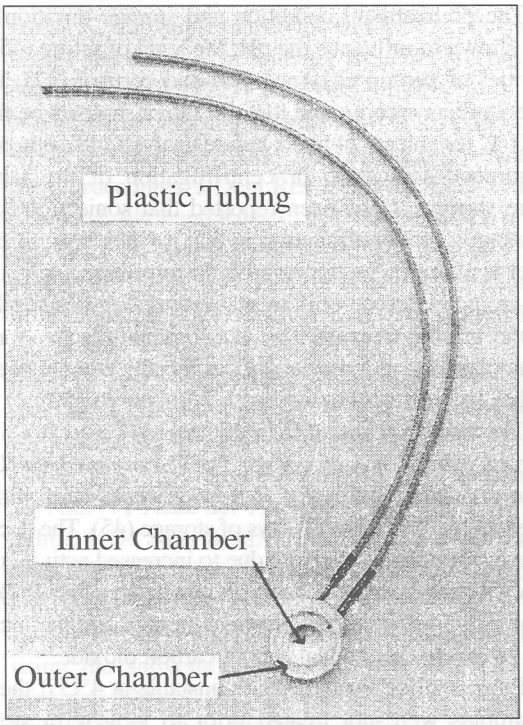

Figure 3. Lashley Cup

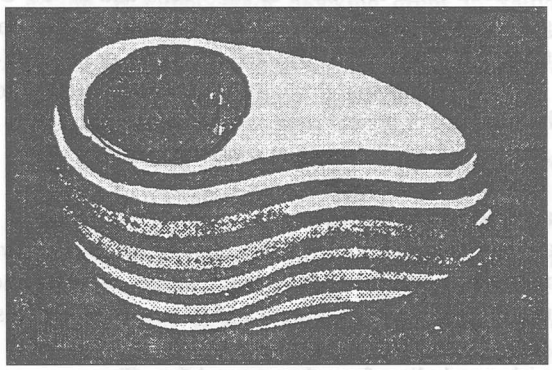

Figure 4. Slupr Colleciton Cuip

protein concentration is highest in the afternoon (42).

The levels of calcium, phosphate and protein in unstimulated whole saliva from an individual collected at different times of day for two consecutive days do vary (44). The levels of protein and phosphate varied from day to day and were usually lowest at 0600 h and generally increased to a maximum at 1800 h or 2400 h. The reverse pattern appeared for calcium concentrations which were maximum at 0600 h and minimum at 1800 h.

Pretreatment and storage

Research that involves compositional and enzyme studies may require samples that have to be treated prior to storage. The optimal storage condition and pretreatment of samples should be considered before any analysis is made on stored saliva samples. Storage condition may refer to storage at room temperature, 4°C, -20°C or below or no storage, whereas pretreatment conditions may refer to treatment like centrifugation or sonication or dividing into aliquots.

The pretreatment condition and storage duration have been shown to influence the pH, the level of nitrite (45), the activities of certain enzymes (46) and cortisol (47). While some workers recommend that the saliva samples be frozen at -40 °C for at least 24 hours before analysis (48), others may recommend sonication or centrifugation of the samples before storing. It has been reported that sonication has an advantage over centrifugation in that it yielded significantly higher levels of most steroids (49). In some cases, saliva samples are subjected to boiling water temperatures for 15-30 minutes prior to freezing. This is a common practice for saliva samples used in forensic work especially when it does not involve volatile and heat-unstable compounds (50).

The nitrite content of the saliva stored below 0°C is stable even after 24 days of storage. For saliva stored at 4°C, the nitrite content is stable for at least a week, after which it decreases by 80% upon 24 days of storage (45). The decrease in the nitrite content could be due to increased activity of the nitrite utilising microorganisms present in the saliva (51). The pH of saliva increases with days of storage and the increase may be due to the gradual loss of carbon dioxide.

Enzymes like a-amylase are unstable at 4°C if the saliva samples were not treated prior to long term storage; lysozyme when untreated is reported to be unstable at 4°C when stored for a few days or for weeks and at -20°C for longer storage period and glycosidases are shown to be unstable at 4°C and -20°C if stored for weeks or months (46).

It has been reported that cortisol in saliva samples stored in the presence of preservative such as citrate (l0g/l) was stable for as long as six weeks at room temperature (47).

Analytical Methodologies

Analytical methodologies used depend on the analyses. Immunological methods have been widely used for monitoring drugs (52) and hormones (53) in saliva. The disadvantage of using this method is the specificity required to distinguish metabolites from the parent drugs. Chromatographic methods like thin layer chromatography is not often used in drug monitoring of saliva because of the low drug levels in saliva (54). However gas chromatography - mass spectrometry has been the most popular analytical procedure for the measurement at the nanogram or picogram level. HPLC has also been used in the analysis but fluorescence detectors are favoured such as has been used to detect chlorhexidine at nanogram level (55).

Subjects' medications

Medications given to patients can interfere with the assays and the results obtained may not be so accurate. It is necessary to give allowance for this and research using saliva from subjects on medication should take into consideration the plasma: saliva ratio of the drug used in the medication and whether the drug used will interfere with the analytical procedures.

CONCLUSION

In conclusion, research on saliva and the use of saliva as an analytical tool in clinical diagnosis can be quite complex even though its collection is non-invasive and less stressful when compared with blood collection. The main advantage of saliva over blood as diagnosis fluid are convenience, economical, hazard reduction and the subjects will not be subjected to the anxiety of pain. Saliva collection used in the epidemiological study permits easy access to population outside the clinical environment. The ability to do salivary screening for infections like hepatitis and human immunodeficiency virus (HIV) is economical and advantageous especially to the developing world where disposables needed for blood collection is scarce or expensive. The disadvantage is that proper collection device and standardisation of a number of factors which include time of collection, the types of saliva and storage conditions need to be strictly observed.

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