



**SIALOCHEMISTRY- A NON-INVASIVE DIAGNOSTIC TOOL  
IN SYSTEMIC DISEASES.**

**SUKANYA. D<sup>1</sup> AND DR. SUBHA.M<sup>\*2</sup>**

*<sup>1</sup>Final Year BDS, Saveetha Dental College and Hospital, Chennai.*

*<sup>2</sup>Department of Oral Medicine and Radiology, Saveetha Dental College and Hospital, Chennai.*

**ABSTRACT**

Saliva, a non- invasive bio-fluid secreted by the salivary glands not only lubricates the oral cavity but also has antibacterial, antifungal and antiviral properties and also regulates pH. It contains mucin, protein, salt, starch and hormones. It can be easily collected and can be used as a diagnostic fluid in assessing most of the systemic disease. Also they play a major role in monitoring drug level and forensic odontology.

**KEYWORDS:** Saliva, Sialochemistry, Diagnosis, Systemic diseases.



**DR.SUBHA.M**

Senior Lectures, Department of Oral Medicine and Radiology,  
Saveetha Dental College and Hospital, Chennai.

## INTRODUCTION

Saliva is secreted by three types of major salivary glands and numerous minor salivary glands. Gland specific saliva is helpful in diagnosis of any disease in a specific gland. They differ from person to person in both quality and quantity depending on age, gender, disease states<sup>1</sup>. Saliva contains crevicular fluid, mucosal transduction, expectorated bronchial and nasal secretion and blood derivatives from oral wounds, bacteria and bacterial products, viruses and fungi epithelial cells and other component. Saliva has most components of serum hence useful in diagnosis of various systemic diseases in a non-invasive manner. Along with systemic diseases they help in

screening hormone levels and drug levels in the body. It is cost effective, easy to collect the sample, requires simple equipment and less training for the operator.

### SALIVARY SECRETION

Salivary secretion occurs in two stages, in stage acinar cells secrete a sodium chloride (NaCl) rich fluid called primary saliva and in stage 2 the primary saliva loses NaCl due to resorption as it passes through the ductal tree along with secretion of bicarbonates (KHCO<sub>3</sub>). Due to the poor permeability of ductal epithelium to water the final saliva is hypotonic

**Table 1**  
**MAJOR FUNCTIONS OF SALIVA<sup>2,5</sup>**

Functions	Components playing a major role.
<b>Protective function</b>	
Lubrication	Mucin proteins rich glycoproteins water.
Anti- microbial action	Lysozyme, lactoferrin , lactoperoxides, mucins, cystine, histatins, immunoglobulin , protein rich glycoproteins glycoproteins , I gA.
<b>Maintaining mucosa integrity</b>	Mucins, electrolyte, water.
Growth factor	Epidermal growth factor (EGF), transforming growth factor $\alpha$ , TGF- $\alpha$ , transforming growth factor $\beta$ , TGF $\beta$ , fibroblast growth factor (FGF), insulin like growth factor IGF-I& IGF-2, nerve growth factor (NGF).
Cleansing	Water
Buffer capacity and remineralisation	Bicarbonate, phosphate calcium staterin, proline rich aniovier proteins, fluoride.
<b>Food and Speech Related</b>	
Preparing food for swallowing	Water mucins .
Digestion	Amylase, lipase, ribonuclease , proteases, water, mucins.
Taste	Water, gustin.
Phonation	Water, mucin.

**Adapted from FDI Working Group 10, Core (1992) and Fox(1989).**

### TYPES OF SALIVARY SAMPLE<sup>3</sup>

Saliva is collected to provide a sample for various assays but more frequently to assess flow rates which is required for objective confirmation of xerostomia. It can be collected as unstimulated whole saliva, stimulated whole saliva, stimulated or unstimulated saliva from a specific major salivary gland. Whole saliva is a product of secretion of 3 major glands (parotid , mandibular , sublingual ) and many minor gland ( labial, buccal, palatial ). Unstimulated saliva is collected by asking the patient spits out every 60 seconds into a test tube by this method the flow rate per unit time can also be assessed, when volume measurement not required it can be collected in the cotton, gauze or filter paper. Stimulated saliva is collected when large volume is

required. It can be done by masticatory stimuli using paraffin wax or a washed rubber band or by gustatory stimulus in which 2% citric acid applied on the tongue 15- 20 sec. Then saliva is collected the same way as unstimulated salivais collected.

### SALIVARY SAMPLE COLLECTION<sup>3</sup>

Navazesh (1993) revised salivary sample collection described by Mason and Chisholm(1975) as Spitting , drainage, suction, cotton-wool rolls. Spitting is a method of salivary collection in which patient is made to sit in a comfortable position with head inclined forward and encouraged to spit at one minute interval. Drainage is to allow saliva to drain out of the mouth into a funnel draining into a sterile collecting vessel. Suction requires the equipment associated with a dental chair. Patient is made to sit in similar position as

before to allow saliva to collect into anterior floor of the mouth. Then salivary ejector is placed behind the lower incisor teeth and the secretion is trapped in a bottle intervening between the ejector and the drainage system. Pre-weighed absorbent device like cotton wool rolls are put under the tongue for a duration of two minutes, then taken out and reweighed this allows quantitative only. Collection of saliva from major salivary gland is done by aspiration from the duct opening with a micro pipettes, parotid saliva is best collected with Lashley's vacuum<sup>4</sup>. While collecting saliva from submandibular and sublingual gland secretion

from the parotid gland should be blocked using filter paper disc and disc of other synthetic materials<sup>5</sup>. Normal Salivary flow rate is 1-1.5 ml/min<sup>5</sup>. When the rate increases and decreases it is called as sialorrhoea and xerostomia respectively. The flow rate also helps in diagnosis of various systemic diseases.

### **SIALOCHEMISTRY<sup>1,5,6,7</sup>**

Most systemic disease affect the composition of the saliva and the salivary gland thus the guide in the diagnosis of the disease (Table 2 and 3).

**Table 2**  
**Diseases showing alteration in inorganic ions in saliva<sup>2,4,5,6,7,17</sup>**

DISEASE	INORGANIC ION
Sialadenitis	Increased sodium ,potassium ,calcium ,phosphate (po <sub>4</sub> <sup>-</sup> ) levels.
Radiation damage	Increased sodium ,calcium ,magnesium and chlorine.
Sjogrens syndrome	Increased sodium ,chlorine (po <sub>4</sub> <sup>-</sup> ) in parotid saliva.
Cystic fibrosis	Increased sodium ,calcium (po <sub>4</sub> <sup>-</sup> ) and ca <sup>2+</sup> po <sub>4</sub> <sup>-</sup> concentration forms a diagnostic index.
Aldosteronism	Decreased sodium, increased potassium. N a <sup>+</sup> /K <sup>+</sup> ratio is of diagnostic value.
Hypertension	Decreased sodium.
Alcoholic cirrhosis	Increased potassium.
Hyper parathyroidism	Increased calcium levels .
Diabetes mellitus	Increased calcium levels.
Chronic pancreatitis	Decreased bicarbonate levels. (Hco <sub>3</sub> <sup>-</sup> ).
Psychiatric illness	Possibly increased sodium levels .
Digitalis intoxication	Raised Na <sup>+</sup> and K <sup>+</sup> product is of diagnostic value.

**Table 3**  
**Diseases showing alteration in organic substances in saliva<sup>2,4,5,6,7,17</sup>**

DISEASE	ORGANIC SUBSTANCES
Sjogrens syndrome	Increased lactoferrin ,kalikerin ,20 folds increase in phospholipid. Raised total protein , β <sub>2</sub> microglobulin in parotid saliva.
Cystic fibrosis	Raised total protein , amylase, lysozyme in submandibular saliva. Glycoprotein in parotid saliva.
Cirrhosis	Raised total protein & amylase in parotid saliva.
Hyperpara thyroidism	Raised total protein.
Diabetes mellitus	Raised total protein, IgA, IgG, IgM and increased glucose levels.
Sarcoidosis	Decreased amylase and lysozyme.

### **INFECTIONS**

Viral infections can be easily diagnosed by using salivary samples as they show the antibodies against the infective organism as seen in the blood. Various viral infections are diagnosed using antibody levels and immunoglobulin levels in saliva. Based on the levels of the IgM antibodies the acute hepatitis A (HAV) and hepatitis B can be diagnosed<sup>8,9</sup>. It is also used in screening for hepatitis B surface antigen (HBsAg). The antibody of HIV of saliva in infected individual is correlated to that of the serum antibody levels<sup>7</sup>. There is direct relationship in IgA levels of the saliva and prognosis of the HIV. Detection of infection and assessment of immunisation of measles,

mumps and rubella can be done<sup>10</sup>. Salivary IgA levels are better markers for rota virus in monitoring the immune response to vaccination and infection<sup>11</sup>. The reactivation of (HSV - 1) can be detected which plays a major role in the diagnosis of Bell's palsy<sup>12</sup>. Anti-dengue IgM and IgG can be detected which helps in diagnosis of dengue<sup>13</sup>. Bacterial infections like Helicobacter pylori infection, Pneumococcal pneumonia, Shigella ,Lyme disease can also be detected in the saliva<sup>7</sup>.

### **ENDOCRINE DISEASE**

Salivary hormone levels play a role in the diagnosis of the endocrine disorders. Elevation of salivary aldosterone identification of the

primary aldosteronism in hypertensive population(10%) in a non-invasive manner<sup>14</sup>. Testosterone can be identified in the saliva which is 1.5- 7.5% of the serum concentration<sup>6,15</sup>. There is fair correlation with the free serum levels and salivary progesterone levels. Preterm birth can be assessed in the elevated levels of estradiol<sup>16</sup>. 17- hydroxyprogesterone (17- OHP) in the early morning levels of the saliva plays a major role in the diagnosis of the hydroxylase deficiency<sup>6</sup>. Protein hormones are very large to reach the salivary glands so the levels in saliva is less evident and also they are contaminated by the gingival crevicular fluid. Other protein hormones that cannot be detected accurately are gonadotrophins, prolactin, and thyrotropin<sup>17</sup>. Studies to assess insulin levels in saliva showed misleading results between the salivary insulin levels and the serum levels of insulin<sup>18</sup>. Saliva also contains other components such as glucose, alpha - amylase and ghrelin<sup>18</sup>.

#### **ROLE IN ORAL CANCER**

Saliva shows high concentration of defensin is seen in patients with squamous cell carcinoma<sup>19</sup>. There is also increase in the peptides in the carcinoma patient which are found in the azurophil granules of PMNs which play a major role in cytotoxic and antimicrobial property. The expression of mRNA pattern is altered in the developing squamous cell carcinoma patients. Abberent gene promoter and methylation of DNA from oral mucosal cell which has been exfoliated is seen in squamous cell carcinoma. It also helps in diagnosis of breast carcinoma and ovarian carcinoma which shows an increase in c-erbB-2 (erb) and cancer antigen15-3 (CA 15-3).and CA125 respectively.<sup>19,20</sup>

#### **AUTOIMMUNE DISEASE**

Sjogren's syndrome is an auto immune disease which causes decreased oral and salivary secretions. Salivary investigation show increased levels of sodium ,chloride, elevated levels of IgG, IgA, lactoferrin ,albumin, inflammatory mediators like eicosanoids,PGE2, thromboxaneB2, and interleukin 6 there is also decreased levels of phosphates<sup>6</sup>.

#### **OTHER SYSTEMIC DISEASES**

Salivary amylase is increased in cardio vascular disease which has effect on catechol amine activity which in turn increases the heart rate under stressful condition<sup>21,22,23</sup>. Cystic fibrosis patient has shown elevated levels of epidermal growth factor and prostaglandins E2 (PGE2)<sup>1</sup>. It is also agreed that saliva of the patients cystic fibrosis contains increased levels of calcium resulted from the calcium protein aggregation which causes turbidity of the saliva and increases salivary concentrations. Salivary AChE activity helps in identifying Alzheimer disease related changes in central cholinergic activity and response to AChE inhibitors there showing the prognosis of the disease<sup>22</sup>. The presence of osteocalcin ( OC) and pyridinoline (PYD) in the saliva helps in the detection of the bone turn over in osteoporosis<sup>25</sup>.

#### **FORENSIC EVIDENCE**

It can be used in crime detection in which saliva is deposited during the biting process ,during cigarette smoking postage stamps envelopes and other objects in which ( DNA) can be detected<sup>26</sup>.

#### **DETECTION OF DRUG USE**

Saliva is useful in the detection of the psychiatric medication. They help in diagnosing antiepileptic drugs and anticancer drugs<sup>27</sup>. The numerous therapeutic drugs that can be monitored in saliva includes antipyrine, carbamazepine, cisplatin, cyclosporine, diazepam, digoxin, ethosuximide, metoprolol, paracetamol, phenytoin, quinine, primidone, sulphanilamide, theophylline, tolbutamine. Nicotine in saliva can be useful in the saliva cessation programme which can be detected by cotinine which is a metabolite of nicotine has a longer half-life, is an indicative of active or passive smoking<sup>28,29</sup>. Ethanol has a rapid diffusion into the saliva . Hence serves as an index in detecting blood ethanol concentration. Other abusive drugs that can be detected in saliva include amphetamine, cocaine, opioids, marijuana. Thus saliva plays a major role in assessing the drug levels in the body which would help in planning further therapeutics in a patient.

### ADVANTAGES OF SALIVARY DIAGNOSIS<sup>7</sup>

- 1) It can be collected in the non-invasive method.
- 2) Prevent cross infection.
- 3) In patients with haemophilia, children and in venous access compromised patients where collection of blood is difficult salivary sample play a major role in the diagnosis.

### CONCLUSION

Though blood is standard diagnostic tool saliva can be used as an alternative method in the

diagnosis of HIV which is no- invasive and reduces cross infection . Thus in this era it plays a important role in early detection of disease, monitoring disease, progression and evaluation treatment and life style. But the availability of laboratory facilities are minimal for salivary analysis and is not broadly accepted as the current literature is also minimal. Hence more studies are required to make best utility of this bio-fluid and make it a better screening, diagnostic and prognostic tool.

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