SALIVARY DIAGNOSIS: AN IMPORTANT NONINVASIVE INVESTIGATIVE MODALITY

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Abstract

In today's world everything is moving from analog to digital scale including diagnosis of diseases, in which there is dramatic shift towards noninvasive modality of investigations. Saliva being the natural body resource can be used as a tool to evaluate certain pathological conditions in the body even before they manifest in the general system.

Introduction

Disease of the salivary gland and particularly the parotid were recognized by Hippocrates and Littre in 1861 who reported suppurative and non-suppurative osteomyelitis. Of those associated with anatomy of the parotid gland include Thomas Wharton who described the submandibular gland and Neil Stenson who discovered the parotid duct. Wood in 1904 recorded the occurrence of pleomorphic adenoma in a 7-month-old child.

More and better tools for diagnosing human diseases are reaching doctors' offices, but many of the tests require a blood sample. Although most people are happy to endure a needle stick to rule out a dreaded diagnosis, the small costs involved add up when one considers screening entire populations. Furthermore, the need to draw blood makes such tests difficult to provide to people who have limited access to medical care. One solution to both problems is the use of saliva, which is inexpensive, noninvasive and easy to collect and preserve. Saliva is a "mirror of the body" and contains many of the same molecules that clinical scientists use to diagnose disease from a blood sample.

Neglected by the dentists and ignored by the physicians, saliva is the least known and the least appreciated of all the body fluids. Yet, this lowly secretion plays a vital role in the integrity of oral tissues, in the selection, ingestion and preparation of food for digestion, and in our ability to communicate with one another. The importance of saliva is best demonstrated by patients in whom virtually no salivary secretion reaches the oral cavity. Such a worse case scenario is seen in patients of Sjogren's syndrome, an autoimmune disease.

Saliva has been used for ages as a convenient source for licking postage stamps, sealing envelopes and even cleaning eyeglasses. From the dental professional perspective, it often is the fluid to be excluded from the operatory site during clinical procedures. Curiously, ancient cultures used the oral fluid as a crude lie detector test or the rice test. In this the accused was given a mouthful of dry rice, and if anxiety and presumably guilt so inhibited salivation so that the accused could not form a bolus and swallow the rice, he or she must be guilty and should suffer the consequences. In the early years of the 20th century, several attempts were made to identify and analyze ions, chemicals and molecules found in saliva. These efforts served as proof of principle for saliva to become a diagnostic fluid in the years and decades to follow, and indeed those efforts have found fruit as the century has gone on. The scientific biomedical research community has provided improved science and technology to identify, measure and monitor the levels of ions, chemicals and molecules not only in saliva, but also in other body fluids. Progress in microbiology, virology and immunology has provided technically feasible approaches to identifying, measuring and monitoring microbes, microbial antigens and host derived immunoglobins (IgA, IgG, IgM). And rapid advances in pharmacology has translated into precise methods of identifying, measuring and monitoring many drugs and abused substances introduced into the body. Saliva's use as a diagnostic tool has been tremendously affected by all of this scientific progress.

Independent of dental research community, veterinarians about that time also became interested in the diagnostic possibilities of saliva to detect drugs in a horse in violation of racing rules. The first documented use of saliva was in 1912, when a horse called Bourbon Rose won the World cup in France but was disqualified because it yielded the first positive drug test. Since the drug was given orally at that time, the saliva test was actually measuring the residual drug in the mouth. Currently most drugs are ingested and testing is conducted on urine or blood since the concentration in saliva is much lower. Percy R. Howe, a Boston dentist, was the first to demonstrate an actual excretion of medicaments into saliva, initially with capsules of iron, then iodine and several antiseptics, including salicylates, benzoyate and menthol.

Classification of Salivary Glands

There are 3 pairs of major salivary gland namely -

1. **The parotid gland:** They are situated in an irregularly shaped compartment between the facial and musculoskeletal boundaries of the craniomandibular and the craniocervical system.
2. **The submandibular gland:** They are associated with mandibulohyoid apparatus.
3. **Sublingual gland:** A complex of major and minor salivary glands, which lie in the submucosa of the floor of the mouth.

These multilobular glands are of variable size and shape and empty there secretory products into the oral cavity through compound duct systems. Traditionally these glands have been classified according to the histological and histochemical profiles of their secretory end pieces, parotid being a serous gland and submandibular being a mixed gland, predominantly serous, while sublingual is highly variable mixed gland, predominantly mucous.

Minor salivary glands are distinguished from major salivary glands on basis of: -

1. There reduced size.
2. Later embryonic development.
3. Paucity of there capsular tissue.
4. Abbreviated ductal systems.

The sub mucosa of the oral cavity and the oropharynx is lined extensively by group of minor salivary glands. They contribute of about 8% of both stimulated and unstimulated saliva. Minor salivary glands are classified as-

1. **Labial salivary glands:** 100% of which line the upper and the lower lips.
2. **Buccal salivary glands:** Including aggregates of larger molar and the retromolar glands.
3. **Palatine salivary gland :** A complex of approximate 230 minor salivary gland which are situated in the sub mucosa of the soft palate and posterior part of the hard palate.
4. **Lingual salivary gland:** which include glands of Blandin and nuhn, gland of Weber and glands of von Ebner.

5. **Incisive salivary gland:** A small group situated in the floor of the oral cavity lingual to the mandibular anterior teeth.
6. **Glossopalatine salivary gland:** Located in the Glossopalatine fold.

Development

True Salivary gland is a distinctive feature of mammals, the only animal that perform purposeful masticatory movement. They are exocrine glands and discharge their secretions directly into the oral cavity. Salivary gland is usually regarded as arising from the ectoderm although a measure of uncertainty exists regarding the tissue of origin of submandibular and sublingual gland. Nevertheless they all develop in the same manner. The first sign of the gland is the appearance of epithelial bud which proliferates as a solid cord of cells into the underlying ectomesenchyme. This cord branches extensively but initially is not canalized. The end wigs of the solid cord shows the development of berry like swellings, in some glands the future acini. The cord canalize shortly by degeneration of there central cells to form ductal elements. Oral ectomesenchyme plays an essential role in differentiation of salivary glands. While the epithelial ingrowths ultimately forms the parenchyma of the gland, the ectomesenchyme differentiates to form the supporting connective tissue, namely the fibrous capsule and the septa which divides the gland into lobes and lobules and carry ducts, blood vessels, lymphatic and veins.

Components of The Salivary Gland

It includes-

1. **Connective tissue:** An important distinguishing feature between the major and the minor salivary gland is the presence of a distinct connective tissue capsule and a connective tissue septa arising from the capsule and extending into the gland.
2. **Secretary ducts:** The larger excretory ducts divide into ducts of decreasing size to form a complex system. So there are interlobar and intralobular ducts.
3. **Terminal secretary cells:** These cells are found in the micro lobules where they are arranged around a narrow lumen. They are separated from the adjacent connective tissue by a basal lamina.

Mechanism of Salivary Secretion

The salivary center consists of superior and inferior salivary nucleus in the medulla. Salivary glands receive double nerve supply both from the sympathetic and the parasympathetic. The parasympathetic to the submandibular and the sublingual gland arise from the superior salivary nucleus in the medulla as nervous intermidius and bypasses the Geneculate Ganglion, descends down through the facial nerve and then through the Corda Tymphani branch of the Facial nerve. The corda tymphani nerve descends downwards and reaching the cavity of the mouth meets the lingual nerve. Then the secretary fibers leave the lingual nerve and end in the submandibular ganglion. From the submandibular ganglion the postganglionic fibers arise and reach the submandibular and the sublingual glands.

The parasympathetic fibers to the parotid gland arise from inferior salivary nucleus in the medulla and descend downward through the Glossopalatine nerve, being separated from the tympanic branch, passes through the tympanic plexus and then through the lesser Superficial Petrosal nerve and ultimately ends in the Otic ganglion. From there the postganglionic fibers arise and reach the parotid gland as the Auriculo Temporal branch of the Facial nerve.

Sympathetic fibres to all the glands are derived from the first and second thoracic segment of the spinal cord. The postganglionic fibers arise from the superior cervical ganglion and pass along the walls of the arteries and supply the salivary glands.

Functions of Saliva

Saliva is a complex fluid, composition and properties of which varies according to the momentary balance of stimulation. The important functions are:-

1. **Cleansing:** The mucosa and dentition are coated by a layer of mucin which impedes the accumulation of food and other debris. The flow of saliva washes the mucin off.
2. **Lubrication:** There is considerable movement between the structures within the oral cavity in addition to the stretching of the mucosa during normal activities. Saliva by virtue of the rheological properties forms a film over the surface.
3. **Mucosal integrity:** Mucin are important in maintaining this protective covering for the epithelium along with electrolyte content of the saliva. Potential mucosal irritants are diluted in the saliva and the array of antimicrobial systems is involved in promoting a healthy commensal flora.
4. **Buffering:** The principle buffering agent in saliva is the bicarbonate, although phosphate and proteins also contribute to its buffering capacity protecting the dentition from demineralization by plaque and organic acids.
5. **Remineraliztion:** Saliva is supersaturated with calcium and phosphate. This results an equilibrium towards nondisolution of enamel.
6. **Antimicrobial:** The attachment of microorganisms to the mucosa and teeth is modulated by the surface antimicrobial agents which influence fungal, bacterial and viral growth and so maintaining a balanced commensal flora. These substances include immunoglobulins, lactoferin and lysosomes.
7. **Digestive:** Foods are dissolved and diluted in the saliva which allows them to be tasted and subsequently swallowed. Saliva also contains a number of enzymes capable of initiating digestion like salivary amylase and lipase.
8. **Blood Clotting & Wound Healing:** It has often been questioned whether blood clotting is quicker or slower in the mouth and what is the compariititive rate of healing in intra and extraoral wounds. It is shown that saliva could speed up clotting and later works have shown that proteins with activities similar to factor 7,8,9 and 12 have been found together with platelet activating factor.

Role of Saliva in Diagnosis

Salivary diagnostics is a late bloomer, coming into flower with he growing appreciation of saliva as a mirror of the body reflecting: -

1. Tissue fluid levels of natural substances and a large variety of molecules introduced for therapeutic, dependency or recreational purposes.
2. Emotional status from high anxiety, to low down blues, from mania to depression.
3. Hormonal status like cortisone and aldosterone.
4. Immunological status and responsiveness.
5. Neurological effects.
6. Nutritional and metabolic influences.

Collection of Oral Fluids

Non-invasive and non-painful techniques exist to collect whole saliva, as well as saliva from the individual major and minor salivary glands. Whole saliva is obtained easily and is

in most cases a good indicator of whole mouth dryness. Disease of the salivary gland can often be diagnosed from the secretions obtained directly from the glands. The quantification of salivary output is referred to as Sialometry.

When measuring the flow rate of saliva, the purpose and method of salivary collection procedures should be explained to the patient beforehand. Saliva should be collected about 1.5 to 2 hours after eating or after an overnight fast. Patient should be instructed to do nothing to stimulate the flow of saliva prior to the collection. This prohibition includes mastication of anything like food, chewing gum, candy and smoking, mouth washing, tooth brushing, drinking and so forth.

To obtain mean flow rates at least two tests should be performed, at about the same time of the day, on two different days. If the patient's baseline has been established earlier, the values obtained can be used as a comparative indicator of the patient's present salivary status. If the baseline is not known, as is virtually the case, the flow rates have to be compared with the pertinent population standards. As with any test, the results should be interpreted in the light of the patient's history, the presence of any signs of the disease and the results of other tests.

Assaying The Flow of Whole Saliva

Whole saliva may be collected and measured by a variety of volumetric and gravimetric techniques like drooling, spitting, suction and swabs. The volumetric methods to be described, especially the combination of drooling and spitting techniques, are easily performed in dental and medical office. Two measuring devise may be employed, a 'Sialometer' or any finely calibrated measuring cylinder. The Sialometer is a specially constructed, reusable devise, which enables the practitioner to collect both resting and stimulated saliva in a single vessel. Alternatively, two measuring cylinders of about 12 ml volume, calibrated to no less than 0.1 ml, and two funnels are needed.

The Collection of Resting (Unstimulated) Whole Saliva

The patient is seated, head slightly down, and is asked not to swallow or move his tongue or lips during the collection period. The saliva is allowed to accumulate in the mouth for 2 minutes, and the he or she is asked to spit the saliva into the receiving vessel. This procedure is performed twice more for a total of 6 minutes.

Collection of Stimulated Saliva

1. **Masticatory method (Paraffin method):** Ask the patient to hold a piece of paraffin in his or her mouth until it becomes soft (30 seconds) and then to swallow the saliva which has collected in his mouth. Now the patient is asked to chew the piece of wax in his usual manner of chewing for exactly 2 minutes, and then to expectorate the accumulated saliva into the receiving vessel. The procedure is repeated twice more.
2. **Gustatory method (Citric acid method):** A 2% solution of Citric acid is swabbed on to the latero - dorsal surface of the tongue every 30 seconds for a period of 2 minutes. The saliva is the expectorated into the receiving vessel. As in paraffin method the whole procedure is repeated for a total of 6 minutes.

Collection of Saliva from The Individual Major Gland

Parotid saliva is usually obtained using a modified; two chambered Carlson Crittenden collector. The inner chamber is placed over the orifice of Stenson's duct, the outer chamber is attached via a thin tubing to a rubber bulb, which when compressed, creates a slightly negative pressure and permits the devise to adhere to the surrounding mucosa. This devise makes it possible to collect pure parotid saliva in a non-invasive manner.

A simple method, which makes it easy for the dentist to collect submandibular / sublingual saliva, has been reported. The region of Wharton's duct is isolated with gauze and the orifice of Stenson's duct is covered up. The saliva resting or stimulated, which has collected during a known time is aspirated with a plastic micropipette.

Conclusion

The central challenge of dental education is to inspire young people to see the magnificence of science and its power to improve health, stop disease and prolong life. Witness how discovery of antibiotics ended the pestilence of bacterial infections which, over millennia, took such a high toll on young and old alike; how invention of anesthesia heralded the era of tolerable surgery; how perfection of dental restoration ended the age-old torment of caries. The scientists who spearheaded these developments profoundly changed human existence. The same change is required for shifting investigation to noninvasive.

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