

# DEPARTMENT OF ORAL MEDICINE AND RADIOLOGY

## SALIVARY DIAGNOSTICS AIDS

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- INTRODUCTION
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# INTRODUCTION

- Saliva is a complex secretion. **93% by volume** is secreted by the **major salivary glands** and the remaining **7%** by the **minor glands**.
- Saliva is a promising option for diagnosing certain disorders and monitoring the evolution of certain pathologies or the dosage of medicines or drugs. Its advantages as a diagnostic tool include its being **easy to obtain** and the **positive correlation** between many parameters in **serum and saliva**.

## Cont'd...

- The earliest “sialochemical” studies on oral fluids were conducted by **Michaels and Kirk** in the 1900's, each of whom examined saliva for specific components that would be diagnostic for various systemic conditions, including gout and rheumatism.

# Salivary production

- Healthy adult subjects normally produce **500–1500** ml of saliva per day, at a rate of approximately **0.5** ml/min.
- Salivary output and composition depend on the activity of the **autonomic nervous system**.
- The **serous part** of the glands is under the control of the **sympathetic system** .
- The **mucous** part of both **parasympathetic and sympathetic systems**.

## Cont'd...

- **Parasympathetic** stimulation results in a **high flow** of saliva containing low levels of organic and inorganic compounds.
- **Sympathetic stimulation** produces a **low volume** of **protein-rich** and **K<sup>+</sup>-rich** saliva .

# CELLS OF SALIVARY GLAND

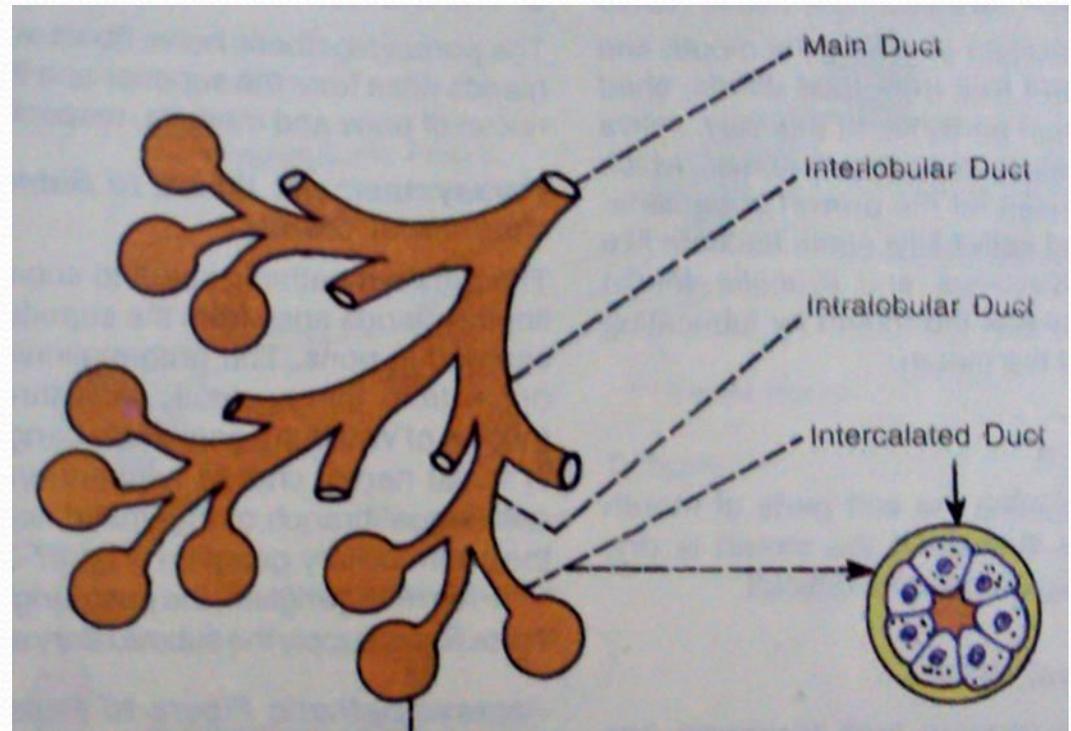
- Three major salivary gland
- PAROTID: Predominantly **serous** cells .
- SUBMANDIBULAR: Both **serous and mucous**.
- SUBLINGUAL: Predominantly **mucous** cells.

# CONT'D

- Secretion produced pass along a system of ducts
- INTERCALATED DUCT



- STRIATED DUCT
- EXCRETORY DUCT



# Saliva components and functions

- **Functions**
- **Lubrication :** Mucin, proline-rich glycoprotein, water
- **Antimicrobial action :** Lysozyme, lactoferrin, lactoperoxides, mucins, cystins, histatins, immunoglobulins, proline-rich glycoproteins, IgA

# Cont'd...

- **Maintaining mucosa integrity: Mucins, electrolytes, water**
- **Cleansing: Water**
- **Buffer capacity and remineralisation**  
Bicarbonate, phosphate, calcium, staterin, proline-rich anionic proteins, fluoride
- **Preparing food for swallowing: Water, mucins**

.

# Cont'd...

- **Digestion :** Amylase, lipase, ribonucleases, proteases, water, mucins .
- **Taste :** Water, gustin
- **Phonation:** Water, mucin

# EARLY MILESTONES IN SALIVARY DIAGNOSTICS

- The **ancient Greeks** were among the first to recognize the medicinal value of saliva over 2000 years ago when they placed **non-venomous snake** saliva on wounds to aid in **healing**.
- **Chittenden et al** conducted the study of the influence of **alcoholic drinks** upon digestion and secretion where the measurement of total organic constituents, salts and chlorine in saliva was assessed .

# Need for salivary diagnostics

- Oral fluid/Saliva, called the '**mirror of the body**', is a perfect medium to be explored for health and disease surveillance.
- While it is relatively **inexpensive**, the **non-invasive, simple and accurate screening** methods provide obvious patient benefits that consequently creates an ideal opportunity to bridge the state-of-the-art saliva-based biosensors and disease-discriminatory salivary biomarkers in diagnostic applications

# SIALOMETRY

- **Definition**
- The measurement of the rate of secretion of saliva, usually from the parotid and submandibular salivary gland, done for physiological reasons and for the investigation of salivary gland diseases.

**OR**

- A measurement of salivary secretion function, generally for a comparison of a denervated or diseased gland with its healthy counterpart.

# Cont'd...

- SALIVARY STIMULI
- SALIVARY FLOW RATE
- SALIVA COLLECTION
- SALIVA STORAGE

# SALIVARY STIMULI

- The parotid glands produce saliva with stimulation, such as eating, sucking on a lemon candy, chewing gum, or smelling a tasty meal.
- The submandibular glands produce saliva on a continuous basis and thus serve to keep your mouth moist even when you are not eating.

# SALIVARY FLOW RATE

- Salivary flow rate is given as ml/min/gland
- **Net' flow** - the resultant salivary volume secreted by an activated section of the gland minus the reabsorbed portion.
- 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**.

# Cont'd...

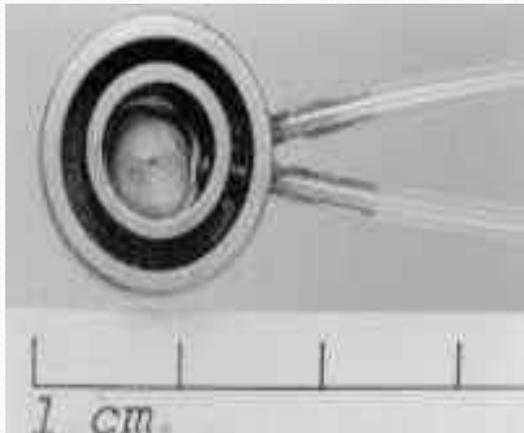
- Stimulated values **below 0.3 ml/min** are considered **pathological**.
- **Elevated flow rates seen under conditions**
  1. Gingivitis.
  2. Recent prosthesis
  3. Dominant cholinergic activity in parkinson's disease
  4. Intoxication

# Cont'd...

- **Low values are found during the use of**
  1. Antidepressants
  2. After duct disintegration caused by inflammation
  3. Irradiation
  4. After radical surgical treatment.

# COLLECTION OF SALIVA

- **SUCTION CUP**
- The inner chamber is placed over the duct orifice.



- **CATHETER**
- A polyethylene catheter (0.5-1.5) can be introduced through the duct orifice.



# STORAGE OF SALIVA

- Saliva specimens, after collection, should preferably be kept on **ice**, aliquoted and frozen as soon as possible to maintain the sample integrity.
- Snap-freezing of saliva in **liquid nitrogen**.
- Inhibition of the enzyme activity present in saliva ( **phenyl methyl sulfonyl fluoride, EDTA and thimerosal**).
- Addition of **sodium azide** ( $\text{NaN}_3$ ) to saliva specimens in attempt to **retard bacterial growth**.

# SALIVA AS A DIAGNOSTIC TOOL

- Recently there has been increasing interest in diagnosis based on saliva analysis.
- Saliva has a **simple** and **non-invasive** collection method.
- Oral fluid sampling is **safe** for the operator and the patient,
- **Easy** and **low-cost storage**.

# Cont'd...

- Circumstances in which **blood and urine sampling is not available.**
- **Linkage with biochemical parameters** which appear in the circulation in various forms.

# SALIVA ANALYSIS IN DENTISTRY

- Studies in oral diseases to help assess the risk of **caries**, by measuring **saliva buffer capacity** and **bacterial contents**
- Oral fluid is mainly utilized for research and diagnostic purposes concerning systemic diseases that involve the salivary glands and oral cavity, such as **Sjogren syndrome** , benign and malignant **oral tumours**.

# DRUG MONITORING AND DETECTION OF ILLICIT DRUGS

- It is used to monitor a variety of drugs including **lithium, carbamazepine, benzodiazepines, phenytoin, theophylline , cyclosporine .**
- The presence of **thiocyanate** in the saliva is an excellent indicator of **active or passive smoking.**
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## Cont'd...

- Commonly **abused drugs** can be detected in human saliva including **alcohol, amphetamines, barbiturates, benzodiazepines, cocaine, opioids, phencyclidine** and **nicotine** for **tobacco smoke** .

# HORMONAL ANALYSIS

- **Steroids** can be accurately assessed in saliva:
- Cortisol,
- Dyhydroepiandrosterone
- Estra-diol
- Estriol
- Progesterone
- Testosterone

- Salivary levels of steroid hormones reflect the free and thus active form.
- **Salivary estriol test** predicted an increased risk of **spontaneous preterm labor** .
- **Night time salivary cortisol** sampling is a facile approach to screening for **Cushing's syndrome in children**.

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- Abnormal salivary **diurnal cortisol** rhythms have been shown to be predictive of disease progression in **metastatic breast cancer patients** .

# SYSTEMIC DISEASES

- **CVS**
- **Salivary endothelin concentrations are elevated in persons with chronic heart failure.**
- **Salivary TC, TG, LDL-C and VLDL-C concentrations were significantly high in patients with ischemic stroke.**

- Results have shown that **low salivary amylase** levels in preoperative patients with **ruptured aortic aneurysm**, associated with **increase in mortality**.
- Salivary **a-amylase** appears to be a more direct and simple end point of **catecholamine activity** than changes in heart rate when evaluating patients under a variety of **stressful conditions**.

# Cont'd...

- **EARLY DIABETES**
- Proteomic analysis of the human saliva in **type 2 diabetes** provides the potential mechanisms altered in diabetic saliva and their utility in detection and monitoring of diabetes.

# AUTOIMMUNE DISORDERS

- **Salivary IL-2 and IL-6** concentrations are significantly elevated among individuals suffering from **Sjogren's syndrome**.
- **IgA, IgG and IgM** autoantibodies can also be detected in the saliva

# INFECTIOUS DISEASE

- **VIRAL INFECTION**
- Saliva has been reliably used to detect **HIV-1 and 2**, and **viral hepatitis A, B and C**.
- **Salivary IgG** are suitable for use in the screening of **viral infections and immunization**.

# Cont'd...

- **Raised salivary IgG levels is seen in**
- HCV
- HAV (hepatitis A virus)
- EBV (Epstein Barr virus)
- CMV (Cytomegalo virus) and
- Rubella virus.

## Cont'd...

- **Salivary antibodies** have also been reported after **immunization against poliovirus, rotavirus and HAV**
- Important means to evaluate **systemic immunity** in diseases or to evaluate **immunity in response to vaccination.**

## Cont'd...

- Saliva has also been used to **detect antibodies** to the **rubella, parotitis and rubeola viruses** .
- In **neonates**, the presence of **IgA** is an excellent marker of **rotavirus infection**.
- **PCR detection** of the virus in the saliva would be a suitable method for early detection of **reactivations of herpes virus type 1**.

## Cont'd...

- When **testing saliva for HIV** using an enzyme-linked fluorescence technique in combination with Western blot assays, **saliva was superior to serum and urine with regard to both sensitivity and specificity.**

## Cont'd...

- Saliva can be used to measure **beta2 microglobulin** and/or **soluble tumor necrosis factor** and thus assess the disease activity in patients with **HIV infection** or other chronic **inflammatory disease states** .

## Cont'd...

- **PCR** is being widely used to measure many viruses, e.g. **human herpes virus 8** ,**rabies virus** levels in salivary secretions.
- **PCR** is also being used to measure the shedding of **cytomegalovirus** and **herpes viruses 6, 7, and 8** in the saliva of **HIV infected patients** .

# BACTERIAL INFECTION

- **PCR assay** is available to detect **H. pylori DNA** in saliva in patients with **peptic ulcer**.
- **H. pylori antibodies in saliva** may be valuable for predicting risk for **gastric adenocarcinoma**.
- The presence of antibodies to other infectious organisms such as **Borrelia burdogferi**, **Shigella** or **Tenia Solium** can also be detected through the saliva..

## Cont'd...

- Use of saliva for the detection of **dental plaque-induced diseases, i.e. dental caries and gingivitis** has been emphasized to measure the two bacteria most frequently associated with dental caries (**Streptococcus mutans and Lactobacillus acidophilus**) and **porphyomonas gingivalis**, which is associated with **periodontal disease**.

## Cont'd...

- Criticized on the grounds of the following:
- Dental caries and periodontal diseases have **not been found to be always specific diseases** and they may be the outcome of infections by many individual microbial species.
- Both diseases are **chronic and progressive**.

## Cont'd...

- From the diagnostic point of view, it is **impossible to quantify the disease in an individual.**

# *ONCOLOGY*

- Biomarkers detected in the saliva, **protein p53 antibodies** in patients with **oral squamous cell carcinoma**.
- Scientists confirmed the validation of five biomarkers namely **M2BP, MRP14, CD59, profilin and catalase** for oral cancer detection.

## Cont'd...

- Researchers have found that samples of **salivary mRNA** as biomarkers for **oral cancer detection**.
- In recent study, the **salivary CEA and CA-50** levels were reported to be higher in salivary samples patients with **malignant salivary gland tumors**..

## Cont'd...

- **c-erbB-2 tumour marker and EGF** in the saliva is a promising tool for the early detection **breast cancer**.
- Saliva contained **CA 125**, a glycoprotein complex that is an often-used marker for **ovarian cancer**.
- Measurement of **salivary sialic acid** initially appeared aid for detecting **lung cancer**.

# Nephrology

- **Salivary creatinine** concentrations show a high sensitivity and specificity for determining the presence of **renal disease**.

# Psychiatry

- Saliva has been used to monitor therapeutic responses in the treatment of **anxiety** by measuring salivary levels of **3-methoxy-4-hydroxyphenylglycol (MHPG)**

# FORENSIC ANALYSIS

- Recently saliva has increasingly been investigated as a **source of DNA deriving from oral cells.**
- It provides a useful **source for biomarker profiling and forensic identification.**
- **DNA tests in saliva** could also be carried out for the detection of **HIV infection.**

# Sialochemistry analysis

- Sialochemistry for environmental heavy metals (**cadmium, lead, mercury**) may be useful in monitoring environmental, atmospheric and occupational pollutants.

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- In **mumps** the saliva shows a **sharp increase** in the **sodium** concentration and an exceptionally **low potassium concentration**.
- Sodium values rise to 90-120 mmol/l while potassium values fall below 10 mmol/l.
- During **inflammation of the salivary glands** , there is **decreased sodium reabsorption** and **potassium secretion in the striated duct**.

- **SJOGRENS SYNDROME:** High sodium concentrations ranging from 60 to 100 mmol/l are found .
- The potassium concentration lies between 10 and 20mmol/l.
- The total protein production in mg/min is lower during the disease while concentrations of protein persist at an elevated level.
- After **irradiation, rise in sodium level** is steep (80-120 mmol/l). Potassium values are stable.

# CONCLUSION

- Salivary diagnostics could dramatically change the clinical practice by introducing point-of-care testing and real-time disease surveillance.
- **Dentistry can help this vision become a reality, because many patients see their dentists more often than their physicians because of the concern for prevention focus and the need for regular visits to maintain oral health.**

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- Saliva-based tests offer the following **advantages** for patients and providers:
  - **Ease** of collection
  - Elimination of the common **fear of needlesticks**
  - **Lower costs** for sample collection and
  - **Reduced risks of percutaneous injury.**

- **Dentist** has the opportunity to be the primary health provider and help aid in the **early detection of disease** and provide quality health care.
- The saliva matrix is an **upcoming area of research** for basic and clinical application purposes.
- Saliva is a biological fluid that offers several opportunities in diagnosis, toxicology and in forensic science.

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- Furthermore, many **salivary proteins** offer great potential in **clinical and epidemiological research**, in **oral** as well as in **general health studies**.
  - **ONE DAY SOON PATIENTS MAY SPIT IN A CUP ,INSTEAD OF BRACING FOR A NEEDLE PRICK WHEN TESTED FOR CANCER OR DIABETES.**

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