SALIVARY DIAGNOSTICS - A REVIEW

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ABSTRACT

Saliva is a heterogeneous bio fluid that contains proteins, enzymes, hormones, antibodies and reflects the physiological status of the individual.

Detection of disease at their earliest stages has greater significance in affecting the patient discomfort, treatment modalities, prognosis, complications, recurrences and survival rate.

Salivary diagnostics is a non-invasive, quick, simple, safe, inexpensive method, ease to handle paediatric and geriatric patients.

KEYWORDS: *Salivary Diagnostics, Contains Proteins, Enzymes, Hormones, Antibodies and Reflects*

INTRODUCTION

Saliva is a clear bio fluid, hypotonic slightly acidic (PH 6-7), composed of 99.5% water, 0.5% inorganic ions (e.g..chloride, potassium, calcium, sodium, phosphate) and organic micro and macro molecules(e.g.. amino acids, histatins,lysozyme, proline rich proteins lactoferrin mucins and secretory immunoglobulins.

On an average, the normal salivation in an individual is approximately 0.3-0.7 ml of saliva per minute producing a range of 1-1.5 litre per day from 3 pairs of major salivary glands and numerous minor salivary glands of the oral cavity.

The purpose of saliva used in analysis is to detect disease at early stage, assess the progression of the disease and to determine the outcome of the treatment in a much simplified, easier and economical way.

Earlier diseases were detected by blood, serum, plasma, biopsy procedures which were invasive and created anxiety among individuals. Salivary analysis helps in receiving the relatively similar information with non-invasive procedure.

Properties of Saliva

Saliva possess antimicrobial property due to the presence of lysozyme, lactoferrin, defensin and secretory IgA. It has buffering capacity for plaque acids. The various bicarbonates, phosphates and proteins act as a buffering agent. Calcium, phosphate, statherin acts as mineralising agents for protection and repair of hard tissues. Mucins, proline rich glycoproteins and water act as potent lubricant and possess viscoelastic properties. Amylase, protease, nuclease enzyme in saliva possess digestive activity. Gustin and water helps in taste. Electrolytes help in cleansing property. Various growth factors like epidermal growth factor, fibroblast growth factor interleukin I and

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II act as protective and repairing fluid for mucosal surface of the oral cavity. Mucins also act in speech related functions.

Advantages of Salivary Diagnostics

Saliva collection is a simple procedure, can be conducted under direct supervision of the physician. It is a non-invasive procedure, hence simplifies the process in certain population in whom blood drawing procedure is difficult (paediatric, geriatric, bleeding disorders and individuals with compromised venous access). It is the safest method, as salivary secretions do not have infectivity or negligible route of transmission when compared to drawing blood from an infected individual. Thus saliva is easy to collect, and does not need highly trained personnel as in blood sampling, can be stored at room temperature, preservation of saliva DNA with high yield and integrity for a longer period. Saliva does not clot like blood and needs very less manipulation. It is easy to transport and economical. It also increases the willingness in patients to undergo health check-ups at regular intervals.

IgA LEVELS are normally 2.5-5 mg/ml in serum and 250-500 gm/ml in saliva.

IgG levels 5-30 mg/ml in serum and 5-30 mg/ml in saliva.

IgM levels 0.5-1 mg/ml in serum and 5-10 gm /ml several fold higher than those found in saliva.

Saliva Collection Methods and Devices

Saliva collection in pre-term new-borns, infants in neonatal ICU and healthy infants less than 3 months of age are challenging.

Infants of 5- 10 months of age do not allow for saliva collection due to irregular sleep wake cycles. Children of 1-2 years are very anxious and do not allow to collect saliva. In geriatric patients, it is difficult due to their use of drugs and decreased salivation.

Saliva is collected by various methods like spitting, draining swab absorbent and suction method.

The individual is asked to expectorate saliva in spitting method. The individual is allowed to drip off the saliva from the lower lip in draining method. Saliva can also be collected by placing the pre weighed swab at the orifices of the duct of major salivary glands or under the tongue for 2-3 minutes and removed for weighing in swab absorbent method. Saliva is continuously aspirated from the floor of the mouth into a graduated test tube and the amount of saliva is read from the tube in suction method.

Standardization of Salivary Collection

- Collection of saliva is usually done during day time and the patient is instructed not to eat or drink at least one and a half hours before the procedure.

- Patient is instructed to rinse the mouth with deionised water before saliva collection.

- Saliva is preferably collected for ten minutes for diagnostic purpose.

- If any patient is under medication for any systemic illness, then the drug is stopped with physician's advice. So that the medication does not affect or influence in saliva secretion.

Saliva as Biomarkers

Biological markers or biomarkers are the indicators present within our body and represent the physiological aspects in our body and deviates from their normal values in any pathologic alterations within our body. They exist as antibodies, proteins, and nucleic acids.

Blood, serum and saliva are the various biomarkers present in our body. As saliva collection and storage methods are easier and the results are reliable when compared to blood and serum samples, saliva is considered to be an ideal biomarker.

Oral diseases and systemic diseases like hereditary diseases, infectious diseases, auto immune diseases, and malignancy can be early detected using saliva as a biomarker.

Saliva also plays a major role in therapeutic drug monitoring and forensic applications.

Salivary biomarkers are oral micro biomes, proteomics and transcriptomics.

Oral Diseases

Dental Caries - streptococcus mutans and lactobacillus spp

Periodontal Diseases - actinomycetem comitans and porphyromonas gingivalis

Oral Carcinoma capnocytophaga gingivalis, streptococcus mitis, prevotella melaninogenica

Infectious Diseases

Peptic ulcer Helico bacter pyroli infection ncreased Ig G antibody in saliva

Shigella infection in children increased titre of anti-lipopolysaccharide and anti-shiga toxin antibody

Pigeon Breeder's Disease (interstitial lung disease) salivary IgG against Ag derived from pigeon.

Pneumococcal Pneumonia-detection of pneumococcal C polysaccharide in saliva

Lyme Disease –spirochete borrelia inf, transmitted from blood feeding tics-detection of anti-tick Ab in saliva.

Neurocysticercosis -Taenia solium larvae infection specific antibody to larvae.

Acute Hepatitis-A, B, Cigm Antibody is detected in Saliva

Measles, mumps, rubella

Rota virus infection in new-born-increased salivary IgA RESPONSE

Cytomegalovirus Infection

HIV antibody to HIV in whole saliva

Fever-salivary IgM and Ig G antibody specific to dengue virus are elevated.

Malaria Fever -IgG antibody directed against specific plasmodium falciparum antigen is directed in saliva

Hereditary Diseases

Cystic Fibrosis

Cystic fibrosis is an autosomal recessive disorder with the gene defect present in chromosome and codes for transmembrane regulating protein called cystic fibrosis Transmembrane conductance regulator.it is characterised by generalised exocrinopathy, affects the salivary gland function. There is increased calcium level in saliva, increased phosphate level, glycolipids and phospholipid protein level in sub mandibular saliva, elevated levels of electrolytes (like sodium, chloride), urea, uric acids, prostaglandin E2 in saliva.

Coeliac Disease

Coeliac disease is a congenital disease affecting small intestine leading to mal absorption of gluten. Serum and salivary IgA-Anti gliadin antibody is elevated.

21-Hydroxylase Deficiency

21-hydroxylase deficiency is an inherited autosomal recessive disorder characterised by congenital adrenal hyperplasia with impaired synthesis of cortisol from cholesterol by the adrenal cortex. Early morning salivary levels of 17 hydroxyl progesterone is an excellent screening test for the diagnosis of non-classic 21-hydroxylase deficiency.

Auto Immune Diseases

Sjogren's Syndrome

Sjogren'ssyndrome is an auto immune disease due to lymphocytic infiltration of CD4 Cells leading to decreased salivary secretions and lacrimation.it is manifested by xerostomia and keratoconjunctivitis sicca. The secondary form is also accompanied by rheumatoid arthritis. sialochemistry shows increased levels of sodium and chloride, elevated levels of IgA, IgG, lactoferrin,albumin and beta 2 microglobulin, inflammatory mediators like eicosanoids, PGE2, thromboxane B2, IL-6, alpha enolase, carbonic anhydrase I and II and salivary alpha amylase fragments.

SS is characterised by auto antibodies to La and Ro ribonucleoprotein Ag. IgA auto antibodies are also synthesised in salivary glands. There is decrease in acinar proteins like lysozyme c, polymeric Ig receptor and calgranulin.

Cushing's Syndrome

Steroid hormonal levels are increased with stress patients and Cushing's syndrome

Cardiovascular Diseases

C reactive protein is an acute phase reactant, a nonspecific inflammatory response factor, usually increased in patients with cardiac attack, restenosis of coronary arteries, stroke and peripheral vascular disease. Salivary Ig levels are increased in coronary artery disease.

In patients with myocardial infarction, elevated salivary biomarkers like C - reactive protein, myoglobin, myeloperoxidase coincides with electrocardiogram.

Renal Diseases

Biomarkers like cortisol, nitrite, uric acid, sodium chloride amylase and lactoferrin is associated with end stage renal failure. In patients who undergo haemodialysis, salivary nitrate and uric acid are checked before and after dialysis procedure. Salivary phosphate is a biomarker for hyperphosphatemia.

Malignancy

Salivary biomarkers help in early detection of malignancy. Inactivation of P53, which is a tumour suppressor protein, leads to the production of antibody.elevated levels of salivary defensin-1 is present in oral squamous cell carcinoma. Various tumour markers like cyfra21-1, tissue polypeptide Ag, cancer Ag (CA 125) are elevated in saliva.

M2BP, MRP14, profiling, CD59, and catalase are also seen in oral squamous cell carcinoma.

In female patients with breast cancer, there is elevated levels of tumour markers like C0erb, B-2 and Ca Ag 15-3.

In patients with epithelial ovarian carcinoma, tumour markers like Ca-125 IS ELEVATED.

Use of Saliva in Pain Research

Nature of pain is associated with neuropeptides like substance p, neurokinin and gene related peptide. Salivary testosterone is reported in increased aggressive behaviour and salivary serotonin is reported with patients with cognitive behaviour.

Use of Saliva in Forensic Medicine

Blood group antigens are present in saliva like A, B, H and LEWIS Ag. These biomarkers are used in identification in both criminal cases and paternity law suits.

Other Applications- Drug Abuse

Free levels of therapeutic drugs in saliva can be monitored.

Commercially available salivary biomarkers

OFNA Set

Oral fluid nano sensor test is a point of care, automated and easy to use integrated system which enables simultaneous and precise detection of multiple salivary proteins and nucleic acids. It is portable, hand held and quick method.

CONCLUSIONS

Thus saliva with its availability and properties, aids as a good diagnostic marker in early detection of oral and systemic diseases.

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