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Human Saliva: A Potential Diagnostic Medium

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Abstract:- Human saliva is incredibly a versatile organic fluid. Its collection is easy and non-invasive. The biomarkers present in the saliva prove themselves to be of diagnostic and prognostic importance for oral diseases like, periodontitis, oral lichen planus and oral leukoplakia. These biomarkers include growth factors, enzymes and interleukins. They also help in diagnosis of these diseases and for evaluation of risk of malignancy, monitoring of disease and the response to provided treatment. However, more research are required to discover biomarkers and verification of their diagnostic and prognostic role in context with oral diseases is needed. This article review a complete and thorough assessment of importance of saliva as a diagnostic device for the potential diagnosis of oral and systemic disease.

Keywords:- *Saliva, Salivary biomarkers, Systemic disease, Cytokines, Genes.*

I. INTRODUCTION

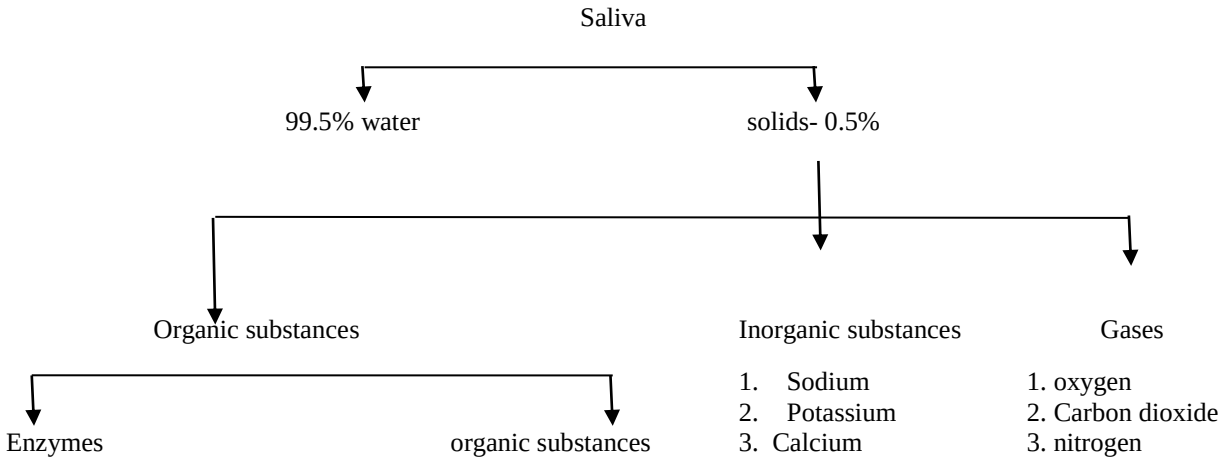
The salivary glands secretes saliva thatensures stability in the oral cavity environment. “Oralfluid” is composed of saliva, gingival cervical fluids, mucosal transudate, bacteria and food remains.[1] The sourceof saliva is interstitial fluid from blood capillaries whicharrives via the salivary gland ducts where it is modifiedfrom isotonic into hypotonic fluid.[2]

Strictly speaking, oral fluid is the mixed saliva and the constituents present in the mouth. So even a small amount of blood contamination can elevate salivary analyte level.

II. PHYSIOLOGY OF SALIVA

Saliva consist mainly 98 % water and remaining is formed from other important compounds, like electrolytes (Sodium, calcium, magnesium, potassium, hydrogen carbonates, and phosphates), glycoproteins, antiseptic substances (hydrogen peroxide, IgA), mucopolysaccharides and various enzymes (lingual lipase, α-amylase, lysozymes). The daily volume of saliva production is 500-1000 ml. The submandibular gland produces 70% of total saliva production, the parotid gland produces 25 %, and sublingual salivary gland produces to about 5 %. Saliva has a normal pH of 6.2-7.6 and has relative density of 1.004-1.009 . The composition of saliva is shown in Figure I.

Electrolytes and total protein concentration of saliva and plasma is given in table I. Figure II is showing functions of saliva. Table II explains the mechanism of action of various components of saliva on food and on teeth. Figure III is showing how saliva is used as a diagnostic tool. Table IV gives briefing about what salivary changes are seen in various diseases.



Normally glucose is absent in saliva. But, it is found in diabetes mellitus

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	Plasma	Whole human resting saliva	Whole human stimulated saliva
Na+ (mmol/l)	145	5	20–80
K+ (mmol/l)	4	22	20
Ca2+ (mmol/l)	2.2	1–4	1–4
Cl_ (mmol/l)	120	15	30–100
HCO3_ (mmol/l)	25	5	15–80
Phosphate (mmol/l)	1.2	6	4
Mg2+ (mmol/l)	1.2	0.2	0.2
SCN_ (mmol/l)	<0.2	2.5	2
NH3 (mmol/l)	0.05	6	3
(NH2)2CO (mmol/l)	2–7	3.3	2–4
Protein (g/l)	70	3	3
Thiocyanate smoker	9	6-12	
Non smoker	2	1-3	
Calcium	5.8	2.2-11.3	6
Phosphate	16.8	6.1-71	12
Chloride	50	100	
Fluoride(ppm)	0.028	0.015-0.045	

Table 1: shows Electrolyte and total protein concentrations in whole human oralfluid and plasma [3]

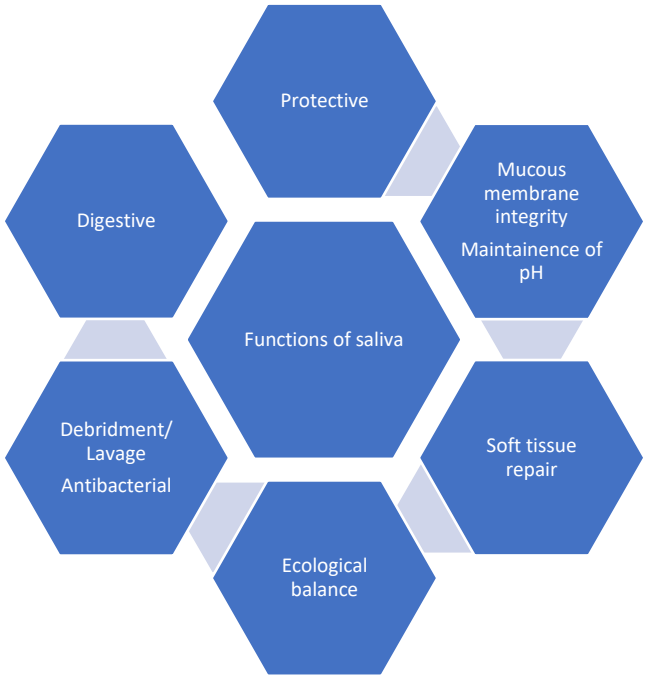

Fig. 2: Functions Of Saliva

Salivary component	**Mechanism of action**
PRP, Statherin, Calcium phosphate	Remineralization of teeth
Mucins	Reduces demineralization of teeth
PRP, Mucins	Lubrication
Bicarbonate and phosphate proteins	Buffering
Lipase, protease, amylase	Digestion of food
Zinc	Taste
Mucins	Bolus
Mucins, Ig cystatins	Antiviral
Ig mucins, histatins	Antifungal

Table 2: Shows the functions of various components of saliva acting on teeth and food. 4

III. WHY SALIVA AS DIAGNOSTIC TOOL?

Saliva can proved to be a better diagnostic medium than serum and tissue as it is

- Non-invasive Procedure
- Undemanding
- Easy collection of multiple samples
- Quick & Cost effective
- Samples easy to handle
- Minimum threat of Cross infections

IV. VARIOUS METHODS FOR SALIVA COLLECTION

There are various methods for saliva collection out of which Passive draining mthod is most commonly used. The advantages and process of various methods is explained in Table III.

1. Passive draining method	Most commonly used method. Preferred since it avoids potential differences generated by various reflex stimuli. Produced saliva drains into a calibrated vial,Volume is measured by nearest 0.1 ml or by the weighing the tube before and after test Devices are Saliva Collection Aid (SCA, polypropylene) and Proflow Sialometer™
2.Spitting method	Fluid produced into the mouth is spitted or ejected into a calibrated tube in each minute
3.Parotid Saliva collection method	Also known as Carlson Crittenden or Modified Lashley's cannula collectors. The collectors are placed over the Stensen duct orifices and are held in situ with gentle suction
4. Absorbent Method	For those who unwilling to passively drool into a cryovial
5. Dried saliva spot (DSS)	Identifies the amount of lidocaine in saliva
6.Paraffin chewing method	Collection of Stimulated Whole Saliva is done Patient puts a small piece of paraffin into the mouth and starts to chew •Before chewing oral saliva is completely swallowed •In 2 min periods saliva is drained into calibrated vial •Repeated 2 times
7.Citric acid application method	2% citric acid solution is exposed to the dorsolateral surface of the tongue in 30 sec periods for 2 min •Produced saliva is collected •Repeated twice •The saliva produced in 6 min gives the basis for salivary secretion per unit time

Table 3: Methods for saliva collection

V. PRECAUTIONS TO BE TAKEN DURING SALIVA SAMPLE COLLECTION

- **Pointer 1: pH of Saliva**
  Avoid alcohol for 12 hr and dairy products for 20 min
  Avoid high sugar food or acidity or high caffeine content (may lower pH or increase bacterial growth
  Avoid eating within 60 mins before sample collection
- **Pointer 2: Dilution of Salivary Biomarkers**
  Wait a minimum of 10 min after rinsing before collecting saliva to avoid sample dilution
  Check for the physical activity level of research participants
- **Pointer 3: Dental / medical treatment**
  Dental treatment should not be done within 48 hour before sample collection
  Check for the presence of oral disease
  Document recent medical procedures
- **Pointer 4: Patient Compliance**
  Can be hampered by increasing sample volume requirements.
- **Pointer 5: Blood Contamination**
  Avoid blood contamination of saliva.
- **Pointer 6: Sample Handling, Transport, and Storage**
  Keep it cold or frozen once collection is done.

VI. 360 DEGREE OF SALIVA DIAGNOSTICS

Saliva shows great potential as a diagnostic fluid used for monitoring of oral and systemic health.It act as potential biomarkers for diagnosis ,drug montoring, neurodegenerative conditions and inflammatory conditions.

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Fig. 3: shows 360 degree of Saliva diagnostics

VII. SALIVARY CHANGES IN SYSTEMIC DISEASES

The changes in salivary flow rate, changes in electrolyte balance and changes in S Ig A can be helpful to diagnose various systemic diseases like cystic fibrosis, Multiple Sclerosis, Alcholic liver disease,Graft versus Host disease, Diabetes Mellitus, Head and neck Cancer, Kidney dysfunction and Ovarian cancer. The changes in saliva in various systemic diseases are explained in Table IV.

1.Cystic Fibrosis	Increased visco-elasticity . Increased total salivary protein and calcium. Increased chloride ions (>60 mEq/ml) Increased concentrations of sodium and phosphate are also significantly increased .(Normal sodium - 8.7 to 24mEq/l) Higher salivary levels of Cathepsin-D, chloride, potassium and sodium ions with a lower salivary volume and pH compared to healthy individuals.(Normal potassium - 13-16 mEq/L) [5,6-10]
Multiple Sclerosis	Reduction in IgA production during rest, while in stimulated saliva, there is absence of a protein band of 140 kDa [11,12]
Graft versus Host disease	Hyposalivation increases 100 days after the transplantation. Increased Salivary sodium and lysozyme concentrations Decreased Phosphate's and S-IgA [6,13,14]
Diabetes Mellitus	Reduced salivary flow rate. Lower Albumin and IgG concentrations of non stimulated saliva (normal albumin level .09mg/ml normal Ig G level 2-3 mg/dl) [6,12,15]
Alcholic Liver Disease	Enlarged Glandula parotis resulting in a 50% reduction of the salivary flow rate Reduction of salivary sodium, bicarbonate and chlorine concentrations and total salivary protein concentration [6,12]
Head and neck Cancer	Presence of CD44 or telomerase [16,17] 6EEpidermal growth factor (EGF),Growth regulated protein -alpha (GRO alpha), (IL)- 1 alpha ,IL-Beta, IL-6 ,IL-8 ,TNFalpha and vascular endothelial growth factor (VEGF) [18]
Kidney Dysfunction	Hyposalivation, ammonium smelling breath, changes in taste and oral mucosal pain [19,20]
Ovarian Cancer	CA 125 determination in saliva [21]

Table 4

ISSN No:-2456-2165

VIII. SALIVARY CHANGES IN ORAL DISEASES

Saliva can be a good indicator for diagnosis of various oral diseases like caries, periodontal disease, lichen planus, oral squamous cell carcinoma, Primary Sjögren's Syndrome and per-implantitis. The changes in saliva is explained in Table V.

1. Caries	High caries activity of S. mutans contained >1 × 106 mL-1 oand/or 1 × 105 mL-1 of Lactobacillus.

Associated genes 24
CD14 - Immune responser mediator Caries determinants:associated genes and functions DEFB1 Antimicrobial peptide MUC7 Clears oral bacteria AQP5 Generates saliva

Determinant	Genes	Gene function	Caries outcome
Taste-diet	TAS2R38 TAS1R2	Controls ability to taste glucosinolates(bitter tasting compunds Controls ability to taste sweetness	Decreases caries risk Increases/decreases caries risk
Saliva immune system	CD14 AQP5	Immune response mediator Antimicrobial peptide	Absence increases caries risk Increase/decrease caries risk
Dental enamel	AMELX MMP20 ENAM	Important in enamel development and mineralization	Increases caries risk

Table 5: Showing various oral diseases that can be diagnosed by saliva

- **Periodontitis**

Mediators	Enzymes	Activated cells
IL1β, TNF-α and PGE2 [25,26]	Levels of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are elevated in periodontal diseases. Salivary AST[27-32]	Osteoclast

Table 6

- **Oral Squamous Cell Carcinoma (OSCC)**

Pro- inflammatory mediators	Prognostic Marker	Proteins
IL1 β, IL6, IL8, IL10, TNF-α, or COX2	TNF- alpha	CD44 (a cell surface glycoprotein involved in cell-tocell interaction), cancer antigen 125 (CA-125) Cyfra 21-1 (a fragment of cytokeratin 19), tissue polypeptide antigen (TPS)are increased in patients with OSCC [33]

Table 7

Lower expression
IL1β and IL10

Table 8

- **Oral lichen planus**

Proinflammatory mediators	Cytokines	Activated cells
IL1 and IL6 [34]	(TNF-α), IL1, IL-8, IL4, IL6(19)	B cells

Table 9

- **Primary Sjögren'sSyndrome**

Proteins	Auto-antibodies	Genes
S100A proteins [35]	Salivary protein-1 (SP-1), Parotid Secretory protein(PSP), And Carbonic anhydraseV	FcRL4 is expressed by epithelium-associated B cells in the salivary glands of pSS patients [36]

Table 10

- **Peri-implantitis**

Pro-inflammatory cytokines	Anti-inflammatory cytokines	Osteoclastogenic cytokines
IL1β, [37]IL6, IL12, IL17, and TNF-α .	IL4 and IL10 45,75[37,38]	RANK, RANKL OPG;

Table 11

- **Medication related Osteonecrosis of Jaw**

Cytokines mediators - IL1A,IL1β,L-1RA and IL6 [39,40]

Table 12

IX. DRUG MONITORING

With minimal discomfort to the patient, saliva can be obtained easily. so nowadays saliva can be used for drug monitoring. Drugs that causes reduction in salivary secretion are Analgesics, Anticonvulsants, Antihistamines, Antiarhythmics and Antipsychotics.

The drugs that are resonsible for salivary gland hypertrophy [are P]henylbutazone, Dobutamine, Cyclocitidine and Methotrexate. [22,23]

X. SALIVA BIOMARKERS[41]

[Deoxy] ribonucleic acid(DNA) , Ribonucleic acid (RNA) , Proteins, Glycoproteins, Immunoglobulins and metabolites present in saliva can act as salivary biomarkers to diagnose various oral and systemic diseases. Various possibilities of use of salivary biomarker is described in Table VI.

Saliva/oral fluid biomarkers	**Possibilities for use**
DNA	Standard genotyping Bacterial infection Diagnosing carcinomas of the head and neck Forensics
Mucins glycoproteins	Diagnosing carcinomas of the head and neck Detecting dental caries
Proteins	Diagnosing periodontitis Diagnosing carcinomas of the head and neck Detecting dental caries
RNA	Viral/bacterial identification Carcinomas of the head and neck
Viruses, bacteria	Epstein-Barr virus reactivation (mononucleosis)
Drugs and their metabolites	Monitoring drug abuse Detecting of drugs in the body
Cellular material	Diagnosing carcinomas of the head and neck

Table 6: showing various salivary biomarkers and its uses.

ISSN No:-2456-2165

XI. SALIVAOMICS [42]

A. Salivary Genomics

Salivaomics is the study of saliva and its functions, and related techniques (Ai et al. 2010, 2012;).The key components of salivaomics.Are as follows:

B. Salivary Genomics and Epigenomics

The study of the complete set of epigenetic modifications on the genetic material of a cell, known as the epigenome. Epigenetic modifications are reversible modifications on a cell's DNA or histones that affect gene expression without altering the DNA.

C. Salivary Transcriptomes

The transcriptome is the complete set of RNA molecules, including mRNA, microRNA, piwi-interacting RNA, and other small RNAs, such as rRNA and tRNA. Salivary transcriptomics has emerged as a powerful approach for salivary biomarker development (Park et al. 2006; Kaczor-Urbanowicz et al. 2018).

D. Salivary Proteomics

The first attempt at cancer diagnosis with salivary protein was made by Hoerman (1959), who showed that patients with prostate cancer exhibited elevated acid phosphatase enzymatic activity in parotid saliva.In comparison with serum proteins, salivary proteins appear to be more susceptible to degradation (Helmerhorst and Oppenheim 2007; Schulz et al. 2013). Esser et al. (2008) reported that salivary protein can be degraded rapidly even during saliva collection and handling, which may hamper the downstream experiments and application.

E. Metabolomics

Specific cellular process leaves unique chemical fingerprints; the study of their small molecule metabolite is called metabolomics.

As the end product of certain cellular processes in a cell, tissue or organ, metabolites are formed. Collection of these metabolites is represented by metabolome.

F. Secretory IgA

SIgA antibodies can help to maintain the integrity of the oral surfaces by limiting microbial adherence to epithelial and tooth surfaces; by neutralizing viruses,enzymes and toxins ss or by acting in synergy with other antibacterial factors such as lysozyme, lactoferrin, salivary peroxidase, and mucins.

G. Saliva Liquid Biopsy

With the help of Liquid biopsy we can detectmutations in biofluids. With the new paradigm shift in diagnostic era saliva is now being used to detect COVID, ICMR has now validated few test kits like COVID-19 antigen rapid card test (ORAL SALIVA), MERISCREEN COVID-19 Ag test kit-V.1, BIOCARD Pro COVID-19 Antigen Saliva Kit, BIOCARD Pro COVID-19 Antigen Saliva Kit , although these kits have not been approved yet but still it's a big step towards the future. [43]

Certain tests like Salistick are now being used to detect Beta-Human Chorionic Gonadotropin Hormone (B-HCG hormone) which is specific biomarker for pregnancy.[44]

H. New salivary gland:

A pair of new salivary gland have discovered by Dr Valstar in the posterior nasopharynx. Visualisation is done by emission tomography/computed tomography (PET/CT) with radio-labelled ligands for the prostate-specific membrane antigen (PSMA). [45]

XII. CONCLUSION

The use of Saliva as a diagnostic medium can improve considerably the diagnosis, prognosis, treatment, and post-therapy monitoring. Various components in this fluid can act as biomarkers for multiple diseases providing valuable information regarding the health status. The focus is on providing information about the important salivary constituents, the mechanism of using saliva as a diagnostic tool, and the clinical applications that can influence an early diagnosis.

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