Saliva as a mirror image of whole body: A review and our experience

***Balwant Rai, **Jasdeep Kaur**

*MS (Forensic Odontology), P.G (Std.Medical Imaging, Health and Safety Officer, Crew-78, MDRS, USA, School of Dentistry, Oral Pathology and Maxillofacial Surgery, ** BDS, MS (std Forensic Odontology School of Dentistry, Oral Pathology and Maxillofacial Surgery

Abstract

There have been increasing numbers of applications using saliva, as the target substrate for performing clinical diagnostic tests. These have focused primarily on point-of-care testing. These point of care testing approaches range from, for example, currently available, highly specialized screening tests for the presence of antibodies recognizing HIV to the potential development of "lab-on-achip" platforms. Sampling of saliva is advantageous, since non-invasive, stress free, easy and frequent collections are possible. Apart from acting as first time of defence against foreign pathogenic, it also constantly cleanse and lubricate the oral cavity. Number of biochemical, immunological, toxicological and microbiological analysis are possible in saliva and importance of oral tissues evaluation and neoplasm screening how long been established also, saliva is routinely referenced in forensic odontology and toxicology especially in drug abuse and alcoholism.This can become a non-invasive alternative to blood & urine test, for early disease detection and management in future.

Key words Saliva, clinical diagnostic test, lab on chip

Introduction

Whole saliva is a combination of gingival crevicular fluid, which has a composition similar to serum, and fluid released from salivary glands, of which the parotid, submandibular and sublingual are the three major sources.[1] The components of saliva are water, proteins, electrolytes, organic molecules secreted from salivary glands, blood, microbes, epithelial lining cells, extrensic factors (food, mouth rinse, tooth paste) and additional fluids (Fig. 1).

Mouth is one of the best laboratories the body to study issues in human biology that go beyond dental research.

I. Physiology & Biochemical Aspects

1. Physiology

Reprints Requests: Dr. Balwant Rai, MS
Forensic Odontology
P.G. Std. Medical Imaging, Health and Safety Officer, Crew-78, MDRS, USA
E-mail: drbalwantraissct@rediffmail.com

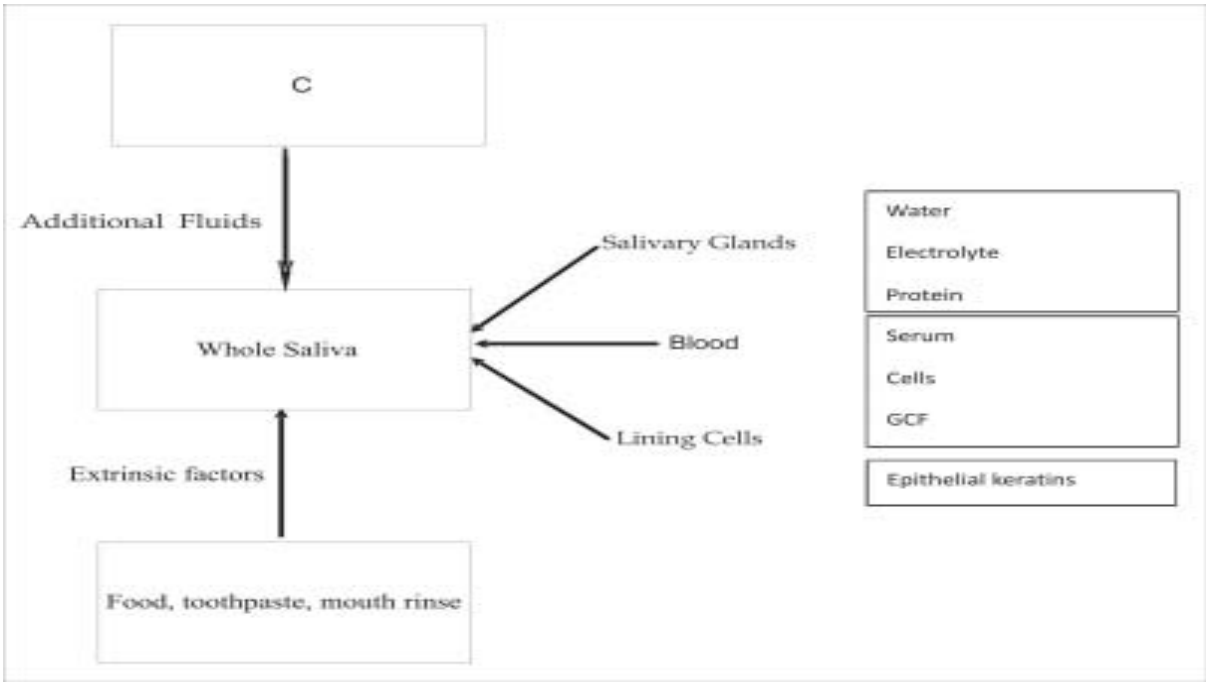
(a) Salivary secretions:

Saliva is a complex oral fluid consisting of a mixture of secretions from both major salivary glands as well as minor glands of oral mucosa. Once saliva passes through the ducts and enters the oral cavity, it mixes with blood cells, microorganisms and microbial products, oral epithelial cells and cell products, food debries and upper airway secretions.

The human salivary glands produce about 600 ml/day of serious and mucinous saliva containing minerals, electrolytes, buffers, enzymes and enzyme inhibitors, growth factors and cytokines, immuno globulines mucins and other glycoproteins.[2-5] Proteins that are found in saliva such as lactoferrin, lysozyme, peroxidase, defensins and histatins, can destroy or inhibit the growth of microorganisms in oral cavity[6]. The components of saliva act as a "mirror of the body's health" and there is growing acceptability of saliva as diagnostic tool.

(b) Composition:

Fig. 1



Table

Organic components of saliva	
Amylase	
Lactoperoxidase	
Kallikrein	
Lactoferrin	
Growth factors:	Nerve growth factor
	Epidermal growth factor

pH 6.4 – 7.1

alkaline, viscous secretion

Water content 99.5%

Solid content 0.5%

Organic constituents -

Solids — 60% organic constituents; 40% inorganic constituents

* mucin

* enzymes Amylase, lysozyme

* albumin & globulin

* others

Urea, uric acid, cholesterol, vitamins, phospholipids

MVCIN

It gives viscosity to saliva, mucin is a glycoprotein, insoluble in water of dilute acid.

Microscopy of saliva reveals Epithelial cells Microbes Salivary corpuscles acteria

The average content of vitamins in saliva are

Vitamin	Average content (y/ml)
Thiamin	0.007
Riboflavin	0.05
Niacin	0.03
Pyridoxine	0.6
Pantothenic acid	0.08
Folic acid	0.0001
Biotin	0.0008
Ascorbic acid	2.4
Vitamin K	0.015

(WBCS) fungi mucus food debris protozoa

Salivary composition depends many factors: stimulation, diet, age, time of day, disease etc.

Ordinary saliva varies weakly alkaline to weakly acid, the pH ranging approximately 6.0-7.9 with optimum pH of 6.6. Lower pH values occur more frequently among caries susceptible individuals and dental erosion is often accompanied by greatly increased total salivary acidity.

Saliva is a dilute secretion with specific gravity of 10007 normal saliva contains glucose. Potassium, thiocyanate is present in saliva and cyanate may possibly come from ingested cyamides present in certain fruits, in tobacco smoke and from breaking down of proteinmaterial. Apoerythein, a protein fraction that protects vitamin B_{12} from digestive destruction is also present in saliva.

Secretion of saliva is governed by central nervous system along with sympathetic nervous system. No hormone mechanism in salivary secretion isknonts. Ordinarily the secretion of saliva is the result of reflex stimulation of secretary nerves the ough a centre in medulla oblongata, pschic stimuli, brought about by suchinfluences as thought of food, also stimulate its secretion.

The amount of saliva secreted by an adult in 24 hours varies between 1000 and 1500 ml[3]. In the absence of obvious external stimuli, the rate of salivary secretion in adult is between 0.1 ml and 0.25 ml per minute and values < 0.1 ml/min should be considered abnormal. The stimulate flow rate varies between 1-2 ml/min and values < 0.5 ml/min should be considered abnormal.[3]

Saliva is routinely categorized as resting (unstimulate) or stimulated.[3,8] The resting saliva reflects the basal flow rate and it is present in our mouths coating the oral tissues about 14 hours of the day. Stimulated saliva

Table: Saliva secretion rate

Secretion rate1	Normal values (ml/min)	Gland secreting
Resting saliva	0.1-0.25	Sub mandibular/sublingual glands (70%) Parotid (15-20%) Minor salivary glands (5-8%)
Stimulate saliva	1-3	Parotid (45-50%) Submandibular/sublingual (45-50%) Rest minorsalivary glands

is also protective and is present in our mouths for upto about 2 hours of the day.

Regulation of salivary gland secretions

The control of salivary secretion is exclusively neural. The flow rate of saliva during sleep is small. This spontaneous secretion keeps mucous membrane moist. Stimulated secretion occurs via nervous reflexes. Neural mechano receptors and chemo receptors in the oral cavity respond to dryness of mucosa, chewing chemicals in the food and texture of the food. Afferent impulses are integrated in medulla (salivary center) and salivary center receives inputs from cortex, amygdala and hypothalamus.

Salivary gland secretion may be inhibited temporarily with infections or drugs (anti cholinergic drugs). Permanent inhibition occurs in irradiation of head & neck, Sjogren's syndrome and is primarily associated with alimentary functions of saliva.

Causes of decreased salivary secretion

1. Water loss / dehydration

(a) non-renal loss:

reduced intake

increased loss: fever sweating, burns vomiting

blood loss

(b)renal los

diabetes insipidus (lack of ADH)

diabetes mellitus (osmotic diversies)

drugs (diveretics)

2. Salivary gland hypofunction

(a)Damage -irradiation

-age

-antoimmune disease: rheumatoid, S/oqren's, SLE

sarcoidosis

(b) neural dysfunction

-autonomic neuropath

-Alzheimer's

-psychoqenic

2. Biochemical aspects

Salivary Anti-oxidants (AOX)

AOX are present in all body fluids and tissues and protect against endogenously formed free radicals, usually produced by the electron transport system.[9] AOX enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) provide protection with in cells while low-molecular weight scavenging AOX are present in extracellular fluid. These include

Table 1: Composition of normal saliva

Constituent	Normal range
Ph	6.0 – 7.9
Ammonia	2 – 10 mg/dl
Calcium	4 – 8 mg/dl
Inorganic phosphate	10 – 25 mg/dl
Chloride	30 – 60 mg/dl
Carbonate	20 – 45 mg/dl
Protein	200 – 400 mg/dl
Cholesterol	2.5 – 9.0 mg/dl
Lipids	0.05 – 0.20 mg/dl

ascorbic acid, a-tocop herol[10-14] and spcarotene[15-18]. In addition, distary derived components such as uric acid, non-protein thiols and glutathione also act as AOX[19-21] as does albumin formed in plasma and saliva.[22]

Uric acid appears to be the dominant AOX present in saliva and displays a concentration similar to that of serum salivary. Ascorbic acid and albumin concentrations are lower than that of serum[11,22], stimulated saliva contains a lower concentration of AOX but when flow rates are taken into account, AOX capacity is higher than in unstimulated saliva.[11] Saliva an prove to be an excellent medium for monitoring AOX status and oxidative stress in future.

Saliva: Sample Collection, Preservation and Pretreatment for Analytical Techniques[1,11,21,23]

Saliva is ultra filtrate of plasma. In a clinic or lab, saliva is relatively easy to collect in sufficient quantities for analysis and the costs of storage and shipping tend to be lower than those for serum and urine.

Saliva is easy to obtain, with less invasion of privacy and ease of adulteration, compared with urine.

Salivary sampling protocols are advantageous as they make for frequent and easy collection of samples by non-invasive NEEDLE-FREE stress free techniques. This is especially useful in endocrinology because serial measurement of hormone levels and their diurnal variation is simplified with ease of collecting saliva.

Saliva can be used as an aid in diagnosis of a wide variety of oral and non oral diseases. But all things are not equal.

Advantages

1. Saliva measures free, bio available fraction of steroid hormones that have moved out of bloodstream and into the tissue. (Blood & urine measure the total levels)
2. Most reliable measurement of tissue uptake in case of topical hormone supplement.
3. Painless, non-invasive, needle free.

4. Private, convenient for both patient and doctor.
5. Hormones are stable at room temperature for weeks, so can be shipped worldwide easily.
6. Transport of saliva samples to laboratory requires no special handling.
7. Less expensive than conventional blood testing.
8. Ease of collection allows for routine monitoring and adjustment of hormone supplement if required.

Drawbacks of use of saliva as diagnostic fluid:

1. Most diseases are diagnosed in the blood and usually the concentration of substances tested are higher in blood than in saliva.
2. Physicians know little about saliva and are understandably reluctant to use it as a diagnostic fluid.
3. Dentists are not overly concerned about salivary findings in systemic diseases.
4. Clinical laboratories are now automated with settings on their machines for blood and urine. Setting them up for saliva will be required.

For health care professionals and scientists, saliva tests are safer than blood tests, which are more likely to result in exposure to HIV or hepatitis. For the patient, from invasive collection techniques for saliva can dramatically reduce anxiety and discomfort, thereby simplifying collection of serial samples for monitoring general health and disease states over time.

Different techniques have been devised for the collection of saliva.

Usually, an individual is asked to rinse out his mouth with water and then chew an inert material such as a piece of rubber or paraffin was from 30 seconds to several minutes. The first mouthful of saliva is discarded; there after the saliva is collected into a small glass bottle.

1. Also saliva can be absorbed onto a swab.
2. Non invasive home testing of saliva : saliva testing kits are available.
3. Or collection of specific gland saliva.
4. In case of edentulous patient 2% citric acid is employed to obtain stimulated saliva.

Saliva collection device available

Salivette, omnisal, orasure

Saliva is allowed to pool in the bottom of the mouth and collected into a plastic vial centrifuged at 3000 rpm for 510 minutes and supernatant fraction is stored at -20^0C or -80^0C until analyzed.,

Spit kit: Patient should contain :

1. Plastic disposable tray to collect saliva
2. Standardized price of paraffin wax or unflavoured sugarless chewing gum
3. pH paper
4. strip to assess buffer capacity of saliva
5. dipslide for microbiological test

Other requirements: Measuring device, incubator

Preservation for cortisol:

Quick freeze them on collection with tharving and centrifugation, glycoproteins in saliva ppt out, learning behind a pipetlable clear fluid.

Collection of saliva may be difficult from individuals who experience:

1. anti cholinergic symptomatology (tricyclic antidepressant overdosage.
2. lcoholics.

Salivary function tests:

They may vary from simple screening tests which may be performed easily in clinics to more complex ones to be done in central labs.

Screening tests:

Sialometry

pH measurement

Buffer capacity

Table: Saliva as a diagnostic fluid

Clinical condition	Analyte
Digitalis toxicity	Ca, K
Affective disorders	PG
Immuno deficiency	IgA
Stomatitis in chemotherapy	Albumin
Cigarette usage	Cotinine
Gastric cancer	Mitrate, mitrite
Forensic	Blood group antigens
Infections : subella,measles, mumps	Ab
Celiac disease	Anti IgA gliadin
Liver function	Caffeine clearance
Autoimmune diseases	Anti SS-A/RO Ab, anti SSB/ La Ab
HIV infection	Ab detection
Hepatitis A, B	Ab detection
Drug abuse	Drug testing

Table: TDM in Saliva

Antipyrine	Penicillin
Carbamazepine	Procanamide
Cortisol	Prilocaine
Digoxin	Progesterone
Sestriol	Testosterone
Ethanol	Theophylline
Lithium	
Methadone	
Metoprolol	

Microbiologic dipslide tests:

Lactobacilli

Strep mutans

yeast

Special tests:

Sialochemical

Microbiological,

Immunological

Table: Areas using saliva as a diagnostic tool

Virology

Immunology

Microbiology

Endocrinology

Epidemiology

Forensic

Biochemistry

Salivary assays currently available[23,24]

Assays

Ab to viruses, bacteria

*HIV

*Hepatitis A&B

Unconjugated steroid hormones:

Estrogen

Testosterine

Progesterone

Free cortisol

Environmental toxins:

Cd

pb

Hg

Tobacco: Cotinine

Drugs:

Ethanol

Theophylline

Lithium

PCR to detect viruses: herpes

Bacteria: H.pylori

Tumour marker:

c-erb B-2 in breast cancer (prognostic indicator)

Target population:

1. To identify hormone imbalances:

   Deficiency or excess: in case of, menopause and ropaese reproductive disorder chronic illness diseases of aging
2. To identify tumor markers
3. to diagnose and monitor disease progression:

   Alzheimer's disease

   Sjogren's syndrome

   Cystic fibroses

   Diabetes
4. Research possibilities beyond oral and systemic diseases in areas of:

· Genetic defects

· Mitritional status

· Age specific changes

Salivary markers of systemic disease –

1. Hereditary diseases:

Celiac disease : Celiac disease is a congenital disorder of small intestine involving malabsorption of gluten. Serum IgA antigiadin antibodies (AGA) are increased in the condition. Measurement of salivary IgA-AGA has been reported to be a sensitive and specific method for screening of disease and monitoring patient compliance of required glutin free diet.[25]

(b) Congenital renal hyperplasia: Due to 21a-hydroxylase deficiency. Early morning salivary levels of 17 hdroxyprogestenine (17-OHP) has been reported to be an excellent screening test for the diagnosis and accuratel reflect the serum levels.[26]

(c) Cystic fibrosis (CF): Elevated levels of calcium and proteins in submandibular saliva is observed in these patients. Elevated sodium levels in minor salivary glands are reported in CF patient.[27]

2. Autoimmune syndrome

Sjogren's syndrome:

Anti SS-A / Ro and anti SS-B/La antibodies are also present in saliva and a high correlation between presence of these antibodies in whole saliva and serum has been reported.[28]

3. Infection diseases

(a) Shigellosis – Evaluation of secretary immune response in saliva of children infected with Shigella revealed higher titres of antilipoly saccharide, anti-shiga toxin antibody[29]. Salivary levels of these immunoglobulins have been suggested to be of use in monitoring the disease.

(b) Pigeon breader's disease – Om interstitial disease induced by exposure to antigens derived from pigeons and measurement of salivary IgG against these antigens can help in evaluation of these patients.[30]

(c) H.pylori infection – It is associated with gastutis, peptic ulcers and possibly stomach cancer. Onset and severity of disease can be determined by monitoring IgG antibodies against this bacterium in saliva and oral cavity.[31] also, polymerase chain reaction (PCR) can aid in its detection.[32]

(d) Pneumococcal pheumonia – Detection of pneumococcal polysaccharide in saliva by ELISA offers or valuable complement to conventional diagnostic methods.[33]

(e) Lyme disease – Detection of anti-tick antibody in saliva has a potential as a biological marker of exposure to tick bites and a screening method.[34]

4. Malignancy

Salivary analysis may aid in early detection of certain malignant tumors.

5. Viral diseases

A number of studies have now shown that antibodies to viral infections can be detected in saliva eg. HIV, hepatitis A & B, subella virus, measles rota virus, and mumps.[35,36]

PCR based virus detection is a useful method for early detection of herpes simplex virus. Anti dengue IgM4IgG levels in saliva have demonstrated sensitivity of 92% and specificity of 100% in diagnosis.[37]

Table: Diagnostic tumor markers in saliva

Saliva assay	Diagnostic role in
p53 antibody	Oral squamous cell carcinoma
Defensins– I	Oral squamous cell carcinoma
c-erb B-2	Breast cancer early detection & follow up screening
CA15-3	Breast cancer early detection & follow up screening

6. Hormone levels

In endocrinology, the ease of collecting saliva is simplifying serial measurements of hormone levels and their diurnal variation eg. salivary free cortisol assay is a valid indicator of cortisol concentration in serum and is not dependent on salivary flow rate[38] is saliva estradiol – has been formed to predict preterm labour[39]. Home-estradiol kits are available for women at risk for premature, low-birth-weight babies in saliva insulin – to monitor insulin levels.[40]

7. Nutrition

Expectorated whole saliva might one day replace blood as a monitoring medium for

nutritional deficiencies and impaired immune response.

Clinical signs of malnutrition and a compromised immune system frequently appear first in the oral cavity and sIgA (predominant Ig in saliva) acts as first line of defence in protecting against microbe invasive. Total sIgA, sIgA 1, sIgA2, total protein, cortisol may be significant markers for nutritional status.[41]

8. Drug monitoring

There has been an increase in interest and use of saliva to monitor drugs. Salivary levels of drugs have been determine d following the intake of therapeutic medications and in pharmacokinetic and metabolic studies.[42]

List of drugs for which salivary monitoring may be useful is given in table. A number of saliva drug-testing kits are now commercially available. Substances of abuse eg alcohol, cocaine, amphetamines, benzodiazepines, opiates can also be tested in saliva with great ease.[43]

Salivary level carbamazepine, phenobarbitone and phenytoin demonstrate excellent correlation with their serum levels.[44]

9. Oral diseases

Saliva can be used to detect oral and periodontal infections, to assess susceptibility to dental caries and to screen for oral neoplasma.

Increased concentration of albumin in whole saliva as a marker for stomatitis.[45-67] High levels of salivary nitrate, nitrite and nitro somine may be associated with development of oral and gastric cancer.[46-69]

Also, high MDA levels[47] and lower AOX status are observed in periodontal diseases.[47]

Vision & challenges for saliva testing

There is a great need for convenient, accurate and non-invasive point-of-care disease diagnostic tools. The challenges are to discover the diagnostic potential and optimize engineering technologies for biofluid saliva to position salivary diagnostics to be a novel, accurate, acceptable and feasible technology.

Possibilities available in salivary diagnostics[23,48-50]

To use saliva as a non-invasive bio-fluid for systemic disease diagnostics.

1.Nano-technology based salivary biosensors also known as NEMS biosensors (nano-electrical-mechanical systems):

To develop real time, ultra sensitive and ultra specific detection of salivary diagnostic analytes.

2. Lab-on-a-chip prototypes for saliva based disease diagnostics: micro electro mechanical system (MEMS) sensors.

3. Diagnostic molecular targets: salivary proteome

Discriminatory and diagnostic human mRNA are present in saliva of both normal and diseased individuals.

Salivary transcriptone

Normal salivary transcriptome (NSTC) consists of ~ 3000 mRNAs and 180 are common between different normal subjects. NTSC has been profiled and analyzed in saliva from head and neck cancer patients.

While the human salivary proteome is still several years away, the normal salivary proteome has been completed. RNA is as robust and as informative as any other analyte. Thus, salivary transcriptome offers the combined advantages of high-throughput marker discovery in a non-invasive biofluid with very high patient compliance.

Table: Technologies for salivary diagnostics

· NEMS bio sensor

· Lab on chip : oral fluid NEMS/NEMS chip

· Transcriptome

· Proteomics

· Conventional techniques – ELISA, immuno assay, chemilummiscence, RIA, PCR.

Table: Candidate systemic diseases for salivary diagnostics

Cardiovascular disease	
Cancer	Lung
	Prostate
	Ovarian
	Colon
Alzheimer's	
Osteoporosis	
Cerebrovascular diseases	
Nephritis	
Septicemia	
Chronic respiratory diseases	
Chronic liver disease	
Pneumonitis	

The vision of current research in oral fluid diagnostics is to provide a point-of-care portable diagnostic platform for definitive diagnostics for these 10 major human diseases, all from a drop of saliva.

With advancers in microbiology, immunology and biochemistry, salivary testing or clinical and research settings is rapidly providn to be a practical and reliable means of recognizing oral signs of systemic illness and exposure to risk factors. The post-genomic era provides opportunities for high through put approaches to genomics and proteomics. The novel technologies of miniaturization in conjunction with disease diagnostics via non invasive biofluid offers a revolutionary damage in medicine.

The components of saliva act as a "mirror of the body's health" and the widespread use and growing acceptability of saliva as diagnostic tool is helping individuals, researchers, health care professionals and community health programs to better detect and monitor disease and to improve the general health of the public.

References

1. Navazesh H. Methods for collecting saliva. Ann NY Acad Sci 1993; 694 : 72-7.
2. Watanabe S, Dawes C. The effects of different foods and concentrations of citric acid on the flow rate of whole saliva in man. Arch Oral Biol 1988; 33 (1) : 1-5.
3. Navazesh M, Mulligan RA, Kipnis V. Comparison of the whole saliva flow rats and mucin concentrations in healthy caucasion young and aged adults. J Dent Res 1992; 71 : 1275-1278.
4. Tabak L. In defense of the oral cavity; structure biosynthesis, and functions of salivary mucins. Ann Rev Physiol 1995; 57 : 547-64.
5. Lamkin MS, Oppentheim FG. Structural features of salivary function. Crit Rev Oral Biol Med 1993; 4 (3-4) : 251-9.
6 Xu T, Levitz SM, Diamond RD, Oppenhein FG. Anticandidal activity of major human salivary histatins. Infect Imun 1991; 59 (8) : 1549-54.
7. McLeod D. Saliva : A spitting image of body. AGD impact 1996; 24 (8) : 10-5.
8. Skopouli FN, Siouna-Fatourou HI, Ziciodis C. Evolution of unstimulated parostid whole flow rate and stimulated parotid flow rate as confirmatory tests for derestomia. Clin Exp Rheumtol 1989; 7 : 127-129.
9. Halliwell B. Reactive oxygen species in living systems: source, biochemistry, and role in human disease. Am J Med 1991; 91 (Suppl 3C) : 14-22.
10 Beutler E. Glutabione peroxidase. In red cell metabolism : a manual of biochemical methods, 2nd ed., pp. 71-3 [E Beutler, editor]. New York : Guine and Strattine.
11. Zoore S, Calder KAC, Millar NJ, Rice-Evans. A antioxidant activity of saliva and periodontal disease. Free Radic Res 1994; 21 : 417-25.
12. Halliwell B. Albumin – An important extracellular anti oxidant? Biochem Pharmacol 1988; 37: 569-71.
13. Kharb S, Gulati N, Singh V, Singh GP. Superoxide formation and glutathione level in patients with preclampsia. Gynecol Obstet Invest 2000; 49 : 28-30.
14. Kharb S. Vitamin E & C in preclampsia. Eur J Obstet Gynecol 2000; 93 : 37-9.
15. Frei B. Ascorbic acid protects lipids in human plasma and low density lipoputin against

oxidative damage. Am J Clin Nutr 1991; 54 : 1113S.

16. Kharb S. Altered thiol status in preclampsia. Gynecol Obstet Invest 2000; 50 : 36-8.
17. Kharb S. Low whole blood glutathione levels in pregnancies complicated by preclampsia and diabetes. Clin Chim Acta 2000; 294 : 179-83.
18. Kharb S. Total free radical trapping antioxidant potential in preclampsia. Int J Gynecol Obstet 2000; 69 : 23-6.
19. Kharb S, Singh GP. Hyperuricemia, oxidative stress in preclampsia. Clin Chem Acta 2001; 305 : 201-3.
20. Kharb S, Singh GP. Role of extracellular super oxide dismutase in normal pregnancy. I Obst Gyn Ind 2001; 51: 63-4.
21. Kharb S, Singh V, Ghalant PS, Sharma A, Singh GP. Glutathcine levels in health and sickness. Ind J Med Sci 2000; 54: 52-4.
22. Kharb S, Ghalaut VS, Ghalaut PS. A comparison of antioxidant status and free radical peroxidatase in healthy persons. Med J Indones 1998; 17: 196-7.
23. Mandel ID. Salivary diagnosis : promises, saliva as a diagnostic fluid. Ann NY Acad Sci 1993; 694: 1-10.
24. Mandel ID. The diagnostic uses of saliva. J Oral Pathol Med 1990; 19 (3): 119-25.
25. Hakeem V, Fifield R, al-Bayaty HF, Aldred MJ, Walker DM, Williams J. Salivary IgA antigladin antibody as marker for coeliac disease. Arch Dis Child 1992; 67 : 724-7.
26. Carlson AD, Obeid JS, Kanellopoulou N, Wilson RC, New MI. Congenital adrenal hyperplasia : update on prenatal diagnosis and treatment. J Steriod Biochem Mol Biol 1999; 69 : 19-29.
27. Wiesman UN, Boat TF, Disant A gnese PA. Flow rates and electrolytes in minor salivary gland saliva in normal subjects and patients with cystic fibrosis. Cancet 1972; 2 : 510-2.
28. Fox RI, Kang HF. Pathogensis of Sjogren's syndrome. Rheum Dis Clin North Am 1992; 18 : 517-38.
29. Schultsz C, Qudri F, Hossain SA, Ahmed F, Ciznar I. Shigella specific IgA in saliva of children with bacillary dysentery. FEMS Microbiol Immunol 1992; 4 : 65-72.
30. Mcsharry C, Mac Leod K, McGregor S, Speekenbrink AB, Sriram S, Boyd F. Mucosal immunity in extrisic allegric alveolitis : salivary immunoglobins and antibody against inhaled avian antigens among pigeon breeders. Clin Exp Allergy 199; 29 : 957-64.
31. Loeb MB, Riddell RH, James C, Hunt R, Smaill FM. Evaluation of salivary antibodies to detect infection with helicobactor pylori. Can J Gastroenterol 1997; 11 : 437-40.
32. Li C, Ha T, Fergnson DA Jr, Chi DS, Zhao R, Patel NR. A newly developed PCR assay of H. Pylori in gastric biopsy, saliva, and feces. Evidence of high prevalence of H. Pylori in saliva subports oral transmission. Dig Dis Sci 1996; 41 : 2142-9.
33. Krook A, Freedlund H, Holmberg H. Diagnosis of pneamococcal pneumonia by detection of antigen in saliva. Eur J Clin Microbiol 1986; 5 : 639-42.
34. Schwartz BS, Ford DP, Childs JE, Rothman N, Thomas RJ. Anti-ticksaliva antibody : abiolosic marker of tick exposure that is risk factor for lyme disease seropositivity. Am J Epidemiol 1991; 134 : 86-95.
35. Perry JU, Perry KR, Pandey S, Mortimer PP. Diagnosis of hepatitis A and B by testing saliva. J Med Virol 1989; 28 : 255-60.
36. Parry KR, Brown DW, Parry JV, Panday S, Pipkin C, Richards A. Detection of measles, mumps, and mbella antibodies in saliva using antibody capture radio-immuno assay. J Med Virol 1993; 40 : 235-40.
37. Cuzzubbo AJ, Vaughn DW, Nisalak A, Suntayakorn S, Aaskov J, Devine PL. Detection of specific antibodies in saliva during denque infection. J Clin Microbiol 1998; 36 : 3737-9.
38. Vining RF, Mc Ginley RA, Maksvytis JJ, Ho KY. Salivary cortisol : a better measure of adrenal cortical function than serum cortisol. Ann Clin Biochem 1989 (9) : 20 : 329-35.
39. Lechner W, Heim K, Zech J, Daxenbichler G, Marth C. The relation between saliva estriol levels in pregnancy and infant birth weight. Arch Gynecol Obstet 1987; 241 : 9-12.
40. Fekete Z, Korec R, Feketeova E, Murty VL, Piotrowski J, Slomiany A, et al. Salivary and plasma insulin levels in man. Biochem Mol Biol Int 1993; 30 : 623-9.

41. Lawrence HP, Fillery E, Matear DW, Paterson L, Hawkins RJ Locker D. The association between salivary sIg'A, nutritional status, and functional dependence in the elderly. J Dent Res 2001; 80 : 43.

42. Drobitch RK, Svensson CK. Therapeutic drug monitoring in saliva. An update. Clin Pharmacokinet 1992; 23 : 365-79.

43. Kidwell DA, Holland JC, Athanaselis S. Testing for drugs of abuse in saliva and sweat. J Chromatogr B Biomed Sci Appl 1998; 713: 111-35.

44. Ben-Aryeh H, Laor R, Szargel R, Gutman D, Naon H, Paascal M, et al. Saliva for monitoring of patients with primary affective disorders. Isr J Med Sci 1984; 20 : 197-201.

45. Izutsu KT, Truelove EL, Bleyer WA, Anderson WM, Schubert MM, Rice JC. Whole saliva albumin as an indicator of stomatitis in cancer therapy patients. Cancer 1981; 48 : 1450-4.

46. Tenovuo J. The biochemistry of nitrates, nitrites, nitrosamiines and other potential carcinogens in human saliva. J Oral Pathol 1986; 15 : 303-7.

47. Panjaminthy K, Manoharan S, Ramchandran CR. Lipid peroxidation and antioxidant status in patients with periodontitis. Cell Mol Biol Lett 2005; 10 : 255-64.

48. Li Y, Zhou X, St John MA, Wong DT. RNA profiling of cell free saliva using micro assay technology. J Dent Res 2004; 83: 199-2003.

49. Rai B.salivary levels Vitamin E and C in different histological grading of oral cancer. Pesq Bras Odontoped Clin Integr, João Pessoa, 8(1):123-125, jan./abr. 2008

50. Rai B, Kharb S, Jain R, Anand SC. Biomarkers of periodontitis in oral fluids.J.oral Sci. 50 (2008) , 53-56

51. Rai B. Acute Phase Protein: Pregnancy Periodontitis. Turk J Biochem. 2008; 33 (3) ; 126–127.

52. Rai B. Salivary melatonin leveling preganancy in periodental diseases. Pak J Med Sci 2007 ; 23 (5):823.

53. Rai B, Anand SC.Serum and Salivary Vitamin C in Periodontal Disease. AMDS.2008 ;2(2): 26-27]

54. Rai B.Salivary Levels of Tumor Necrosis Factor-Alpha in Periodontitis. AMDS 2008; 2(2): 40-41

55. Rai B.Total Salivary Glutathione Levels: Periodontitis in Smoker and Non-Smoker. AMDS. 2008;2(2): 47-49

56. Rai B, Anand SC.Saliva as a Diagnostic Tool in Medical Science : a Review Study. AMDS 2008;2 (1): 9-12

57. Rai B, Jain R, Anand SC, Kharb S.Salivary Lactate Dehydrogenase Isoenzymes in Oral Lichenplanus. AMDS. 2008;2(1):. 1-3

58. Rai B, Anand SC. Gingivitis and periodontitis : 8-hydroxydeoxyguanosine. AMDS. 2008; 2(1): 4-6

59. Rai B. Salivary Lipid Peroxidation Product Malonaldehyde in Pre-Cancer and Cancer. AMDS.2008; 2(1): 7-8

60. Rai B, Kharb S, Anand S C. Effect of scaling and root planning on salivary 8-hydroxydeoxyguanosine levels : periodontitis

J Pak Dent Assoc. 2007;16(4):189-91

61. Rai B, Kharb S, Anand S C. Thiocynate, a salivary marker of periodontitis among smokers and non-smokers: a pilot study

J Pak Dent Assoc . 2007;16(1):35-7

62. Rai B, Jain R, Anand S C, Kharb S. 8-hydroxydeoxyguanosine levels: Periodontitis in smoker and non-smoker: a pilot study

J Pak Dent Assoc . 2006;15(2):89-90

63. Rai B, Kharb S, Anand SC. Salivary Enzymes and Thiocynate: Salivary Markers of Periodontitis among Smokers and Non-smokers; a Pilot Study. AMDS.2007 ;1(1): 1-4.

64. Rai B, Kharb S, Jain R , Anand SC: Effect of Scaling and Root Planning on Salivary 8-Hydroxydeoxyguanosine
Levels: Periodontitis: The Internet Journal of Dental Science. 2007; Volume 5, Number 2.

65. Rai B. Salivary Lipid Peroxidation Product Malonaldehyde In Periodontal Diseases. The Internet Journal of Laboratory Medicine. 2007. Volume 2 Number 2

66. Rai B, Anand SC, Kharb S (2006). 8hydroxydeoxyguanosine levels : Periodontitis in Smoker and Non-smoker : A Pilot Study. JPDA. 15 (2): 89-90.

67. Rai B, Anand SC, Kharb S, (2006). Salivary Lipid Perioxidation Products Malonaldehyde in Various Dental Diseases, World Journal of Medical Science, 1 (2) (In Press), accepted.
68. Slavkin HC. Toward molecular based diagnostics for the oral cavity. J Am Dent Assoc 1998; 129: 1138-43.
69. Milla SM. Saliva testing – a nontraditional diagnostic tool. Clin Lab Sci 1994; 7: 39-44.