

Review Article

SALIVA AS A DETECTIVE BIOFLUID

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Abstract

Saliva is a complex fluid consisting of secretions from the major and minor salivary glands. Saliva has been described as the mirror of the body and indeed as a mirror of our blood as these biofluids and their molecular components share many similarities. As a diagnostic fluid, saliva offers distinctive advantages over serum including the simple and non-invasive method of collection and its easy, low-cost storage. So, this review examines the diagnostic application of saliva for hereditary disorders, autoimmune diseases, malignancies, infectious diseases and endocrine disorders, as well as in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use. This paper explores what saliva can reveal about general health, drawing examples from recent research on salivary biomarkers of systemic illness and highlighting the current use and potential clinical and research applications, of diagnostics based on oral fluids.

KEYWORDS: Albumin, Autoantibodies, Hormones, Immunoglobulins, PCR, Saliva

INTRODUCTION

Saliva is the mixed product of three major salivary glands (parotid, submandibular, and sublingual) and minor salivary glands located throughout the oral cavity.^[1] Minor salivary glands are mainly Von Ebner glands (entirely serous organs situated in the connective tissue below the circumvallatae papillae) and Blandin-Nuhm mucous glands.^[2] Saliva is sterile when it leaves the salivary glands but ceases to be so as soon as it mixes with the crevicular fluid, remains of food, microorganisms, desquamated oral mucous cells, etc. Daily secretion rates range between 500 and 700 ml and the average volume in the mouth is 1.1 ml. 99% of saliva is water and the other 1% is composed of organic and inorganic molecules.^[3] Saliva is a good indicator of the plasma levels of various substances and molecules and drugs and can therefore be used as a non-invasive method for diagnosis and treatment planning. The purpose of this article is to review the literature on the diagnostic applications of saliva.



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COMPOSITION & FUNCTIONS

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FUNCTIONS	SALIVARY COMPONENTS INVOLVED
Lubrication	Mucins, proline-rich glycoproteins, water
Antimicrobial	Amylase, complement, defensins, lysozyme, lactoferrin,
	lactoperoxidase, mucins, cystatins, histatins, proline-rich
	glycoproteins, secretory IgA, secretory leukocyte protease inhibitor,
	statherin, thrombospondin
Growth factors	EGF, TGF-, TGF-, FGF, IGF-I & IGF-II, NGF
Mucosal integrity	Mucins, electrolytes, water
Lavage/cleansing	Water
Buffering	Bicarbonate, phosphate ions, proteins
Remineralization	Calcium, phosphate, statherin, anionic proline-rich proteins
Food preparation	Water, mucins
Digestion	Amylases, lipase, ribonuclease, proteases, water, mucins
Taste & speech	Water, gustin, mucins

Adopted from FDI Working Group 10, Core (1992), and Fox (1989).^[4]

Several pathways, both intra and extracellular, like passive and active diffusion and ultra filtration enable the saliva to be reached by some substances that are not among its normal components. This raises the prospect of using saliva in the diagnosis of certain pathologies.^[1]

SALIVA vs. SERUM SAMPLING

- 1. Increased flexibility.
- 2. Cost effective.
- 3. Convenient.
- 4. Less invasive.
- 5. Less technique sensitive.
- 6. Collection of biological material in a stress-free, painless manner.
- 7. Multiple sampling.
- 8. Samples can be stored and transported easily.
- 9. Easy for children.
- 10. Less risk of infection to the technical staff.^[5]

DIAGNOSTIC APPLICATIONS OF SALIVA:

Autoimmune disorders:

Sjogren's syndrome-

A low resting flow rate and stimulated flow rate is indicative.^[4] Levels of salivary IL-2, IL-6,^[6] sodium and chloride, IgA, IgG, lactoferrin albumin,^[7] 2 microglobulin,^[8] lipids, cystatin C and cystatin S^[4] are significantly elevated and phosphate is decreased.^[9] Autoantibodies to the La and Ro ribonucleoprotein antigens are evident especially of IgA class.^[4, 10] A remarkable alteration of salivary carbonic anhydrase VI is seen in Sjogrens syndrome cases.^[11]



Systemic lupus erythematosus-

Systemic lupus erythematosus is an autoimmune disease characterized by multisystemic involvement. SLE patients have elevated levels of calcium, magnesium, salivary IgA, IgM and lowered resting salivary flow rates.^[1]

Systemic sclerosis-

Systemic sclerosis or systemic <u>scleroderma</u> is an <u>autoimmune</u> or <u>connective tissue disease</u>. According to a study, salivary keratin 6L, psoriasin, TPI, and Arp2/3 complex, might have a pathological role in systemic sclerosis.^[11]

Hereditary diseases: Cystic fibrosis (CF)-

Saliva of CF patients contains increased calcium levels, decreased protease activity and poor biological activity of epidermal growth factor.^[4] Elevated levels of neutral lipids, phospholipids, glycolipids,^[12] sodium, chloride, potassium,^[13] calcium, phosphorus, urea, uric acid, and total protein seen.^[12] Decrease in flow rate was reported for minor salivary glands.^[4]

Coeliac disease-

Saliva shows presence of increased IgA antigliadin,^[14] and transglutaminase autoantibody.^[15] No difference in saliva flow rate is observed, but total protein, albumin, IgA, IgG, salivary peroxidase and myeloperoxidase are elevated.^[16]

21-Hydroxylase deficiency-

An inherited disorder of steroidogenesis. Early morning salivary levels of 17-hydroxyprogesterone (17-OHP) were reported to be an excellent screening test for the diagnosis of non-classic 21-hydroxylase deficiency, since the salivary levels accurately reflected the serum levels of 17-OHP.^[12]

Cardiovascular diseases -

Low salivary amylase levels in preoperative patients with ruptured aortic aneurysm is indicative of increased mortality^[17] and is a more sensitive indicator of catecholamine activity than heart rate in stressed cardiac patients. AMI patients show higher levels of creatinine phosphokinase, myeloperoxidase, TNF-, and proinflammatory matrix metalloproteinases.^[18] Unstimulated whole saliva C-reactive protein, unstimulated whole saliva soluble CD40 ligand and unstimulated creatinine kinase myoglobin fraction yielded a sensitivity of 86%, specificity of 81% and negative predictive value of 86%.^[19]

Endocrinology-

Saliva levels reflect the free level of hormones. Hormones like cortisol, dehydroepiandrosterone, estradiol, estriol, progesterone and testosterone can be assessed.^[20] Salivary cortisol levels help in identifying Cushing's syndrome, Addison's disease, monitoring the hormone response to exercise and effect of acceleration stress. Cortisone levels do not bear any diagnostic significance.^[4] Salivary aldosterone levels are increased in primary aldosteronism.^[21] Salivary testosterone levels indicate status of testicular function and may also be useful in behavioral studies of aggression, depression, abuse and violent and antisocial behavior. Salivary insulin levels helps in monitoring serum insulin levels. To study



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pineal function in newborn infants, measurement of melatonin in saliva offers a good alternative.^[12] Decreased salivary estriol acts as a marker of fetal growth retardation. An increased salivary estriol-to-progesterone ratio may be a predictor of pre-term delivery.^[4]

Infectious diseases:

Viral diseases-

Researchers have demonstrated that saliva tests for antibodies to HIV represent a noninvasive alternative to quantification of antibodies in blood.^[22] As compared with serum, sensitivity and specificity of antibody to HIV in saliva are between 99% and 100%.^[23] Saliva can be used to measure beta-2 microglobulin and/or soluble tumor necrosis factor -receptor levels, to assess HIV disease activity. Polymerase chain reaction measures herpes viruses 6, 7, 8, Epstein- Barr, cytomegalovirus and human rabies virus.^[20] Acute hepatitis A (HAV) and hepatitis B (HBV) has IgM antibodies in saliva.^[24] The ratio of IgM to IgG anti-HAV antibody correlated with the time interval from onset of infection.^[4] Comparison of serum and saliva levels of antibody to HAV; sensitivity = 98.7% and specificity = 99.6%,^[25] Hepatitis B and C revealed excellent agreement. Saliva determines immunization and detects infection with measles, mumps, and rubella.^[4] Oliveira et al, showed that salivary IgM detection may be a convenient non-invasive alternative to serum for investigation of recent rubella cases with a specificity of 96%.^[26] For newborn infants, salivary IgA response is a better marker of rotavirus (RV) infection which persists for 3 months.^[4] Salivary levels of anti-dengue IgM and IgG demonstrates sensitivity of 92% and specificity of 100%, and salivary levels of IgG differentiates between primary and secondary infection.^[27] Saliva was also found to be a reliable alternative to serum for identification of the antibody to parvovirus B with a sensitivity of 100% and specificity of 95%.^[4] HPV types 16 and 18 can be detected by PCR.^[1] Another application of saliva testing is in epidemiology of viral diseases.^[28]

Bacterial diseases-

Saliva can be used for diagnosis of Helicobacter pylori infection, which is associated with peptic ulcer. Immunologic assay detects H. pylori antibodies predicting risk for gastric adenocarcinoma^[20] and pneumococcal C polysaccharide antibodies. Shigella infection revealed higher titers of anti-lipopolysaccharide and anti-Shiga toxin antibody. A correlation coefficient of 0.58 was observed between IgG antibody levels in serum and saliva in Pigeon breeder's disease.^[4] Lymes disease can be detected by anti-tick antibody in saliva.^[10] Specific antibody to Taenia solium larvae in serum demonstrated greater sensitivity than antibody in saliva for identification of neurocysticercosis.^[4] Beuno et al did a study on neurocysticercosis where the search for antibodies revealed the presence of IgG > IgA > IgE in CSF, serum & saliva samples, with IgG being present in all phases of the disease, and IgA/IgE in the inactive form.^[29]

Dental caries & periodontal diseases-

Oral diagnostic aids can be used to identify and quantify bacteria associated with dental caries (Streptococcus mutans and Lactobacillus acidophilus), Porphymonas gingivalis, associated with periodontitis,^[20] and candida species associated with candidiasis.^[30] Statherin and truncated cystatin S act as potential risk indicator for the development of caries.^[11] Periodontal disease pathogens lead to higher salivary concentrations of IgA, IgG and IgM.^[31] Saliva of patients with adult periodontitis and localized juvenile periodontitis show higher



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enzymatic activities of alkaline phosphatase, ester -glucoronidase, aminopeptidases, butyrate esterase, cystenine aminopeptidase, salivary collagenase, gelatinase, tissue inhibitor of metalloproteinase, lactoferrin, myeloperoxidase, cysteine proteinases, aspartate and alanine aminotransferases (AST and ALT), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT), creatine kinase (CK) alkaline phosphatase (ALP), acidic phosphatase (ACP),^[1, 32] and decreased concentration of MG2.^[31] Salivary proteins rise by 1.8 & 1.3 times in periodontitis and gingivitis subgroups. Notable rise in salivary albumin,^[33] lactoferrin, peroxidase enzyme concentration is seen in gingivitis and periodontitis.^[31] LDH and AST can help monitor the progression of the periodontal disease. Increased activity of CK, LDH, AST, ALT and GGT indicates the pathological changes located in soft tissues only. Increased activity of ACP, especially ALP, indicates that the pathological destructive process has affected the alveolar bone.^[32] Cystatin and lysozyme levels in saliva from those with periodontal disease were lower than that in the healthy group.^[11] Osteopontin concentrations increase proportionally with the progression of disease. High levels of Creactive protein are seen in chronic and aggressive periodontal diseases. During the initiation of an inflammatory response in the periodontal connective tissue, numerous cytokines, such as prostaglandin E2, interleukin-1beta, interleukin-6 and tumor necrosis factor-alpha are upregulated in saliva along with enzymes such as MMP-8, 9 and 13.^[31]

Nephrology-

Salivary pH shows an increase due to high urea concentration in dialysis patients.^[19] Salivary creatinine concentrations show a high sensitivity and specificity for determining the presence of renal disease.^[20]

Oncology-

Saliva of patients with oral SCC demonstrates p53 antibody. Elevated levels of salivary defensin-1 were found to be indicative of oral SCC.^[1] CD44 salivary protein acts as a potential molecular marker.^[30] Li et al. showed that malignant tumors located in the head and neck can be diagnosed through saliva with 91% precision.^[34] Antiapoptotic protein bcl-2 expression is higher in invasive cancers. EGF receptor overexpressed in oral cancers correlating with aggressive tumor behavior.^[5] Levels of MicroRNAs-125a and MicroRNAs-200a are significantly lower in OSCC patients.^[19] A study identified alpha-1- -glycoprotein and complement factor B proteins in OSCC patients. Cystatin S, parotid secretory factor and poly-4-hydrolase beta-subunit proteins were detected in most normal patient's saliva, but not in that from OSCC patients.^[11] HPV integration yields oncoproteins E6 and E7 which can be identified with PCR method. Endothelin 1 (ET-1); a vasoactive peptide is overproduced in oral cancer. Cyfra 21-1, tissue polypeptide antigen, and CA125,^[5] and carcinoembryonic antigen^[1] increased whereas transthyretin (-2.92 fold) decreased. Dowling et al. identified beta fibrin (+2.77-fold), \$100 calcium binding protein (+5.35-fold), transferrin (+3.37-fold), immunoglobulin heavy chain constant region gamma (+3.28 fold) and cofilin-1 (+6.42 fold) in oral cancer patients.^[11] Elevated salivary sialic acid has been reported in OSCC. PR Sanjay et al observed increased salivary levels of total protein and sugar.^[35] Elevated statherin, protein, interleukines-1, -6, -8, and TNF- levels^[36] and altered carbohydrate expression seen.^[35] Up-regulation of CD 59,^[37] M2BP, MRP14 seen along with downregulation of clusterin (protein involved in apoptosis) and polymeric immunoglobulin receptor. Profilin 1 is upregulated in early progressive stage of tumor formation. Altered levels of catalase

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involved in carcinogenesis and tumor progression.^[38] El- Naggar et al., (2001) showed highest incidence of microsatellite LOH of chromosome 9p, 3p &17p.^[39] Righini et al., (2007) stated that six methylated genes are frequently found in OSCC patients saliva; TIMP (40%), ECAD (36%), MGMT (29%), p16 (29%), DAPK (27%) & RASSF1A (20%).^[40] Zhong et al., (2005) detected telomerase positivity in 75% of patients with OSCC.^[41] Higher levels of salivary nitrate and nitrite, and increased activity of nitrate reductase is seen in OSCC patients.^[19] Saliva may be useful as an adjunct diagnostic test for systemic cancers as well. Higher levels of salivary kallikrein are associated with malignant tumors. Salivary CA 125, a glycoprotein complex, is often used as a marker for ovarian cancer. Increased salivary EGF concentrations seen in women with primary or recurrent breast cancer. The protein product of the oncogene c-erbB-2, also known as HER-2/neu, is elevated in the saliva of women diagnosed with breast cancer. Relationship of proteins like transketolase, Dim1p, v-Ha-ras oncogene, type I collagen pro alpha, tumor necrosis factor (ligand) superfamily member 4, and pirin with tumor metastasis has been established. Transketolase modulator recognition factor 2, Dim1p homolog, splicing factor (arginine/serine-rich) and v-Ha-ras 1 oncogene hypo-expressed in poorly metastatic tumors and significantly up-regulated in highly metastatic tumor. Type I collagen pro alpha and tumor necrosis factor showed a high expression in non-metastatic neoplasms. Pirin was detected only in non-metastatic lesions, while retinal home box protein was only detected in metastatic tumors. Using mass spectrometry it has been found that 74 protein masses and the levels of four peptides in saliva (1472.78 Da, 2936.49 Da, 6556.81 Da and 7081.17 Da) were higher in gastric cancer patients as compared to healthy subjects.^[11]

Drug monitoring-

Saliva has been proposed for monitoring of systemic levels of drugs. Factors such as molecular size, lipid solubility, and the degree of ionization of the drug molecule, as well as the effect of salivary pH and the degree of protein binding of the drug, are important determinants of drug availability in saliva. Unbound fraction of drug is available for diffusion into saliva.^[4] Currently, saliva can be used to detect and/or monitor cotinine, cannabinoids, cocaine, phencyclidine, opioids, barbiturates, diazepines, amphetamines, and ethanol. Salivary and serum levels correlated better with cyclosporine, theophylline, irinotecan, and lithium.^[20] A defined correlation between salivary and serum cisplatin levels has not been established. Salivary ethanol concentration may be used as an index of the blood ethanol concentration, provided that the salivary sample is obtained at least 20 min following ingestion. Other recreational drugs that can be identified in saliva are amphetamines, barbiturates, benzodiazepines, cocaine, phencyclidine, and opioids. Saliva can also be used to detect recent marijuana use by means of radiommunoassay. D9-Tetrahydrocannabinol, a major psychoactive component of marijuana, can be detected in saliva for at least 4 hours after marijuana is smoked.^[4] Salivary cotinine levels were found to be indicative of active and passive smoking.^[10]

Haematopoietic stem cell transplantation (HCT)-

Imanguli et al stated that lactoferrin and secretory leukocyte protease inhibitor in saliva demonstrated elevations 1 month post-HCT that persisted at least 6 months. Secretory IgA (sIgA) levels were decreased 1 month post transplant, with recovery at approximately 6



months. Levels of salivary 2-microglobulin were elevated at 6 months and correlated with sIgA levels.^[11]

Forensic analysis-

The capacity of detecting human DNA in saliva has also been useful in forensics. Saliva can be found in bites marks left in objects or victims of violent crimes, cigars, postage stamps, envelopes, and other objects.^[30] Salivary mRNA has been used in forensic determination of body fluids from crime scenes even weeks after the sample has been deposited. It has also been suggested for determination of sleep driving as a potential cause of traffic accidents by detecting mucin expression level.^[36] Five saliva RNA markers (SPRR3, SPRR1A, KRT4, KRT6A and KRT13), which can be stable for up to 180 days, can be used for the identification of blood and saliva stains in forensic practice. Biomarker, salivary amylase, is highly correlated with sleep drive. Importantly, both salivary amylase activity and mRNA levels are also responsive to extended waking in humans.^[31]

Oral disease with relevance for systemic diseases-

Monitoring of gland-specific secretions important for the differential diagnosis of diseases that may have an effect on specific salivary glands, like obstruction or infection. Quantitative alterations in saliva may be a result of medications- Diuretics, antihypertensives, antipsychotics, antihistamines, antidepressants, anticholinergics, antineoplastics, and recreational drugs such as opiates, amphetamines, barbiturates, hallucinogens, cannabis, and alcohol. Monitoring increased salivary albumin level can assist in the identification of chemotherapy induced stomatitis at a pre-clinical stage. Reduced salivary EGF levels may be important for the progression of radiation-induced mucositis.^[4] In Diabetes mellitus higher levels of salivary IgA, perioxidase, glucose content, potassium, total protein and amylase is seen.^[1] Levels of statherin, proline-rich peptide P-B, P-C peptide and histatins are significantly less concentrated in the saliva of diabetic subjects than in controls, while the levels of defensins 1, 2 and 4 and S100A9 are higher.^[11] It has been well established that IL-1 polymorphism has a distinct influence on the initiation and progression of periodontal disease. Studies have linked this polymorphism in the periodontal patient to glycemic events in type 1 and type 2 Diabetes, and increased risk in patients with chronic atherosclerotic disease.^[18]

Oral Mucosal diseases-

Nitric oxide levels in saliva are increased in patients with oral mucosal disease like oral lichen planus and recurrent aphthous stomatitis and free radicals.^[1]

Psychiatry-

Saliva has been used to monitor therapeutic responses in the treatment of anxiety by measuring salivary levels of 3-methoxy-4-hydroxyphenylglycol (MHPG). Aphel M.D. et al demonstrated that participants with childhood trauma showed a trend towards higher MHPG. Higher MHPG levels were associated with higher levels of peritraumatic distress and post traumatic stress disorder symptoms.^[42] Castagnola M. et al studied salivary peptides in subjects with a diagnosis of autism and stated that phosphorylation level of four specific salivary phospho-peptides (statherin, histatin, acid proline rich proteins) was significantly lower in a sub-group of autistic patients. Increase of salivary amylase is a known proteomic

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indicator of psychological stress and sympathetic activation.^[11] Mantella et al demonstrated that elderly individuals with generalized anxiety disorder have elevated salivary cortisol levels.^[43]

CONCLUSION

Saliva lacks the drama of blood, sincerity of sweat and emotional appeal of tears. Despite the absence of charisma practitioners are finding that saliva provide an easily available, non invasive diagnostic medium for a rapidly widening range of diseases and clinical situations. Thus saliva based diagnostics is in the cutting edge of diagnostic technology and can act as an alternative for clinicians to use in the near future to make clinical decisions and to predict post treatment outcomes.

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