Chapter

Salivary Diagnostics

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Abstract

Saliva is one of the most ideal diagnostic tools. It is inexpensive, noninvasive, and easy to use. Other advantages like ease of collection and minimal patient discomfort make it more acceptable to the patient as well as the clinician. The most challenging aspect in salivary diagnostics is to identify the biomarker that is linked to a disease. Researches are also ongoing to develop a device that can have reliable and valid clinical applications. This chapter briefly discusses the background and current scope of salivary diagnostics, technologies for the discovery of biomarkers along with a summary of salivary sample collection, and processing methods.

Keywords: saliva, metabolomics, proteomics, biomarkers, genomics, epigenomics, sampling

1. Introduction

Saliva is a slightly acidic (pH 6–7) [1, 2] secretory, digestive enzyme that is chiefly produced by the parotid, submandibular, and sublingual glands. These are all paired glands which are serous, mucous, or mixed in their nature of secretions [1–3]. These glands along with other minor salivary glands (labial, buccal, lingual, and palatal) produce approximately 1–1.5 L of saliva per day [1–3].

Functions of saliva [4–6]

- Lubrication of oral tissues and the bolus
- Mechanical cleansing of food and bacteria
- Neutralization of acidic oral constituents
- Antimicrobial activity
- Facilitation of speech, mastication, and swallowing
- Esophageal clearance and gastric buffer
- Digestive functions mediated by enzymes like amylase and lipase
- Healing properties
- Posteruptive maturation of teeth

According to Bricker, diagnosis is the process of evaluating patient's health, as well as the resulting opinions formed by the clinician [7]. Diagnostics can be defined as the art and practice of diagnosis using a series of tests in adjuvant with clinical signs and symptoms. The use of saliva and salivary biomarkers in the diagnosis of various diseases is termed as salivary diagnostics.

Limitations in diagnostics [1, 8]:

- Lack of definitive biomarkers that are specific to diseases
- Lack of an easy and inexpensive sampling method with minimal discomfort
- Lack of easy to use, portable platform to facilitate early disease detection

2. Salivary diagnostics

Saliva has been in use as a diagnostic fluid since the 1960s for conditions like cystic fibrosis [1, 9, 10]. But it was not until the early 1990s that the distinct advantages of saliva over serum were identified [10–12]. For the past two decades, salivary diagnostics have been in use to monitor oral diseases associated with the periodontium as well as dental caries [9, 13]. In the recent past, a vast number of salivary analytes and biomarkers have been discovered that represent various diseases including oral cancer, cardiovascular diseases, various bacterial, and viral infections as well as certain autoimmune disorders. Saliva contains most of the components of serum like hormones, antibodies, growth factors, enzymes, microbes, microbial products, etc. These substances enter saliva through the blood by passive diffusion [1, 8]. Although they are seen only in trace amounts, recent advances in technologies have allowed us to overcome this barrier.

Thus, salivary diagnostics have proven to have a promising future in the diagnostic arena and may offer a quite reliable platform for clinicians to make early clinical diagnosis in the upcoming years.

2.1 Advantages of salivary diagnostics

- Noninvasive, easy to use [8, 9, 14, 15]
- More economical sampling, storage, and shipping compared to serum
- Safer than serum sampling
- Real-time diagnostic value
- Specially trained staff is not necessary
- Any number of samples can be obtained easily
- Collection and screening can be done even outside a clinical setup
- Require less manipulation during diagnostic procedures compared to serum
- Commercial availability of screening assays

2.2 Disadvantages of salivary diagnostics

- Most of the biomarker levels in saliva are lower than that found in the serum. But with advancing technology, this need not be seen as a limitation anymore [8, 15].

- The need for a reliable, sensitive, and specific device that can have valid clinical applications [8].

3. Collection, storage, and processing of salivary samples

Salivary sample can be classified as:

1. Based on the source of saliva

   a. Whole saliva

   b. Specific glandular saliva

2. Based on stimulation for salivation

   a. Stimulated saliva

   b. Unstimulated saliva/passive saliva

It is very important to standardize the methods of salivary collection as several factors influence the quality and the quantity of saliva secreted. The saliva secreted by each gland has characteristics of its own. Variations are observed in the type of protein content as well as in ionic contents. Cystatin C is a biomarker that is found as a submandibular salivary gland-selective protein as it is found in all submandibular fluids but not detected in sublingual gland-specific saliva. MUC5B mucin and calgranulin B, on the other hand, are sub lingual-selective proteins.

The composition of saliva varies according to whether it is basal or stimulated. High flow of saliva has been reported following parasympathetic stimulation with low levels of organic and inorganic compounds.

Low volume of protein-rich and K^+-rich saliva is seen following sympathetic stimulation. Salivary composition is also affected by the presence of food in the mouth. After a meal, total protein and α-amylase in saliva have been seen to increase [16, 17].

3.1 Whole saliva

Whole saliva consists of mainly water and a mix of salivary secretions from all the glands in varying concentrations along with epithelial cells, microbes, and their products [1, 18, 19].

A representation of the entire oral environment, whole saliva is the easiest, feasible, and most convenient mode of salivary sample collection. There is no additional training of the personnel and can even be collected by the study participant.

Whole saliva can be either unstimulated or stimulated. Unstimulated whole saliva is collected by passive drooling or spitting, although care has to be taken to avoid blood and sputum contamination during the spitting method. Studies show that spitting method of salivary sampling contains 14 times more bacteria than when collected by passive drooling. This can influence the storage and further analysis of the sample.

3.2 Stimulated saliva

It is commonly used in patients who have difficulty producing saliva [1, 18, 19]. This method specifically induces salivary production by manual massaging of the gland or with masticatory action such as chewing on paraffin wax, gum, or sterile cotton rolls. Such induction often affects the quantity and pH of the saliva. Use of citric acid specifically lowers the pH of the sample which may interfere with the antibody binding, thus altering the results in hormonal immunoassay. It also interferes with measurement of certain analytes such as testosterone [16].

Chewing on sterile cotton rolls has shown to induce variations in testosterone, DHEA, estradiol, 17-OH hydroxyprogesterone (seen as exceptionally high levels), and S-IgA (maybe seen as exceptionally low). Binding of cortisol to cotton wool fibers is also recorded in literature [16].

There are non-cotton based alternatives such as polystyrene foam swabs, rayon balls, and polyester Salivette. However, studies show that these bind to and remove 87% of progesterone from saliva.

3.2.1 Processing of various salivary samples

Type of sample collected [18]	**Materials required [18]**	**Procedure [18]**
1. Whole saliva	1. 50 ml sterile tube and paper/styrofoam cups 2. Crushed ice and container 3. Distilled water	1. Inform patient of the intended time of saliva collection. Advice to refrain from eating, drinking, or using oral hygiene procedures 1 hour prior to the said time. 2. Time preferred is between 8:00 am and 10:00 am to avoid disturbances due to the circadian rhythm. 3. The subject is asked to rinse well with distilled water for 1 min. 4. 5 min after rinse, the subject is asked to spit into the 50 ml collecting tube. The tube is to be kept in crushed ice between sample collections. 5. Approximately 5 ml of saliva needs to be collected. 6. Processing should occur within 1 hour of collection.
2. Ductal secretion	1. Sterile-modified Carlson-Crittenden/ Lashley cup with appropriate PVC tubing 2. Low-affinity conical plastic collecting tubes in ice 3. 5 ml of sterile 20% w/v aqueous citric acid solution (store at room temperature) 4. Cotton tip applicators	1. A modified Carlson-Crittenden tube is positioned near the orifice of the duct 2. Unstimulated saliva is collected for about 15–20 min. 3. Stimulated saliva is collected by intermittent application of 20% aqueous citric acid solution over the dorsum of the tongue.
3. Parotid secretions [18, 20, 21]	1. Sterile-modified Carlson-Crittenden/ Lashley cup with appropriate PVC tubing 2. Low-affinity conical plastic collecting tubes in ice 3. 5 ml of sterile 20% w/v aqueous citric acid solution (store at room temperature) 4. Cotton tip applicators	1. A modified Carlson-Crittenden tube is positioned near the orifice of the duct. 2. Unstimulated saliva is collected for about 15–20 min. 3. Stimulated saliva is collected by intermittent application of 20% aqueous citric acid solution over the dorsum of the tongue.

Type of sample collected [18]	**Materials required [18]**	**Procedure [18]**
4. Sub mandibular and sublingual secretions [18, 20]	1. Submandibular and sublingual collector (as described by Wolfe et al.) fitted with sterile 100 ml pipette and low-affinity conical plastic collecting tube 2. Distilled water 3. Sterile cotton pads 4. Dental mirror and forceps 5. 5 ml of sterile 20% w/v aqueous citric acid solution (store at room temperature) 6. Cotton tip applicators	Submandibular and sublingual salivary samples are collected separately using the collecting tubes described by Wolfe et al. 1. Subject is asked to rinse with distilled water for 1 min following which cotton pads are place on the floor of the mouth and the buccal mucosa. This is done to prevent contamination from sublingual and parotid secretions. 2. Stimulated saliva is collected using citric acid, by placing the micropipette of the device at the opening of the Wharton's duct. 3. Sublingual saliva is collected in a similar method, except that Wharton's and Stenson's duct orifices are closed with cotton pads.

4. Processing and storage of samples

4.1 Materials required

- Laboratory vortex mixer [18, 21]
- Refrigerated centrifuge able to accommodate 50 ml tubes.
- Cryotubes able to accommodate −80°C temperatures.
- −80°C freezer for long-term storage purposes.
- Aprotinin stored at 4°C (from standard commercial stock solution).
- 400 mM Na_3OV_4 standard stock: 147.12 mg/2 ml water.

Adjust the pH to 10.0. Let the solution boil for about 10 min till it remains colorless. Store at room temperature.

- 10 mg/ml phenylmethylsulfonyl fluoride (PMSF) standard: Dissolve 100 mg PMSF in 10 ml isopropanol by gentle inversion. Store at room temperature.
- SUPERase Inhibitor (Ambion) stored at −20°C.

4.2 Crucial points to remember during sample collection

1. No mucous contamination should be allowed in the salivary sample [18, 19, 21].
2. Sample should be processed as soon as possible after collection and preferably within 1 hour.
3. During stimulated saliva collection, if citric acid is used as stimulant on the dorsal surface of the tongue, lateral surface of the tongue should be swabbed every 30 s.

4. Reduce the number of freeze/thaw cycles to as low as possible to avoid degradation of nucleic acids and proteins.

5. Avoid exposure of sample to air as this may result in oxidation and inactivation of RNAse inhibitors.

4.3 General considerations for salivary storage

1. Saliva fractions are preferred to be stored immediately without any processing [18, 19].
   - Specimens can be stored at room temperature (when analysis is carried out immediately or in 30–90 min from collection).
   - At +4°C (when analysis is carried out in 3–6 h from collection).
   - At −20°C and better at −80°C (when analysis is carried out days to months after collection) [16].

2. Snap freezing of saliva in liquid nitrogen: mix each salivary fraction with 80% glycerol in water in 1:1 ratio, and then dip the sample in liquid nitrogen.
   This inhibits the bacterial protease activity degrading some salivary protein compounds, such as s-IgA.

3. Inhibition of the enzyme activity present in saliva: Mix each salivary fraction with enzyme inhibitors 10:1 (leupeptin, aprotinin, and 4-[2-aminoethyl] benzene sulfonyl fluoride).
   A mixture of protease inhibitors and stabilizing substances like aprotinin, leupeptin, antipain, pepstatin A, phenyl methyl sulfonyl fluoride, EDTA, and thimerosal has been documented.

4. Addition of sodium azide (NaN3) to saliva specimens in attempt to retard bacterial growth.
   But sodium azide may interfere with horseradish peroxidase, a common component of enzyme immunoassays.

5. Addition of trifluoroacetate at 10% water solution, to denature salivary enzymes that could degrade several salivary compounds, such as proteins and steroid hormones.

6. For protein analysis, the following protease inhibitors are added:

   i. 0.33 μl aprotinin. Invert gently to mix.

   ii. 1 μl Na_3OV_4 (from standard stock of 400 mM). Invert gently to mix.

   iii. 3.3 μl PMSF (standard stock of 10 mg/ml). Invert gently to mix.

   For RNA analysis following RNAse inhibitors are added:

   - 65 μl of SUPERase Inhibitor (Ambion) stored at −20°C

Samples should be preferably kept on ice, fractioned and frozen as soon as possible to maintain sample integrity. Refrigeration prevents degradation of some of

the biomolecules in saliva. Bacterial proteases are seen in saliva which may degrade several salivary proteins and in turn affect protein analyses.

S-IgA can be degraded at room temperature by bacterial protease, while at −30°C a decrease of 10% is seen after 8 months of storage. A 9.2% decrease is seen per month at room temperature which can be avoided by storage at 5°C, for up to 3 months.

4.4 Possible contaminants during saliva sampling

1. Blood: May compromise quantitative estimates of salivary molecules [19, 22].
2. Food products: Steroid antibodies may cross-react with certain food products.
3. Sodium azide: May interfere with procedures like ELISA and EIA.

5. Biomarkers

According to the National Institutes of Health, a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmaceutical responses to a therapeutic intervention [13, 19].

It is the term given to a change in a biological molecule due to intervention by reactive oxygen, nitrogen, or halide species [23].

5.1 Benchmarks of a biomarker

- A major product of oxidative modification that may be implicated directly in the development of disease [13, 23].
- A stable product, not susceptible to artifactual induction or loss during storage.
- Representative of the balance between oxidative damage generation and clearance (i.e., the steady state but also possibly applicable to the measurement of cumulative oxidative damage).
- Determined by an assay that is specific, sensitive, reproducible, and robust.
- Free of confounding factors from dietary intake.
- Accessible in a target tissue or a valid surrogate tissue such as a leukocyte.
- Measurable within the limits of detection of a reliable analytical procedure.

5.2 Comparison between circulatory and salivary markers

Most of the commonly investigated markers in plasma are seen in a narrow range and is well documented over the years [19]. Due to the novelty of salivary diagnostics, this is a shortcoming, as the markers are still in the process of discovery and documentation. Also, the markers vary through a wide range, quantitatively as well as qualitatively. These variations, physiologically and/or pathologically, make the standardization of salivary analyses a challenge.

Many of the components from plasma reach the saliva mainly through passive diffusion. To compare and reach a successful clinical conclusion, it is important that

the concentration of the analyte in the plasma and that in the saliva be highly correlated. Studies have shown that the concentrations of proteins and certain ions are comparable to their level in plasma in a stimulated salivary sample than the sample collected passively.

A number of molecules are produced directly by the salivary glands, which include secretory IgA and lysozyme, the presence of which may be sparse or nil in the respective serum sample. On the other hand, concentration of transferrin, iron, bilirubin, cholesterol, lipoproteins, IgG, and IgM is seen to be 4–15 times more than that seen in saliva.

Certain compounds like blood urea nitrogen, C4, and total bilirubin have high correlation between salivary and serum concentration. This suggests a passive diffusion of these compounds from plasma.

Steroid hormones such as cortisol, cortisone, and testosterone are seen in saliva in their free and unbound state, making salivary analysis a much more preferred method over serum analysis. Although they are also found in their free state in the urine, the sampling of urine is often done and measured over 24 h which is more tedious than salivary sampling.

Nonsteroidal hormones such as melatonin and thyroid hormones can also be analyzed through a salivary sample. However, polypeptide hormones are not as easily detected in saliva due to their large size which hinders in passive diffusion. Plasma leakage through lesions or other modes such as active or facilitated transport may also contribute to detections of polypeptide hormones in saliva.

6. Technologies for discovery of salivary biomarkers

6.1 Proteomic technology

Proteome is the protein component of a genome [1, 8, 24, 25]. Proteomes are found in bodily fluids and are potential sources of disease biomarkers. Proteomics refers to the analysis of the portion of the genome that is expressed. Proteome analysis helps in the identification of any signs of morbidity during early stages of a disease as well as to monitor the progression of the disease. Few analyses used in proteomics are:

- Polyacrylamide gel electrophoresis (PAGE)
- Mass spectrometry (MS)
- Electro spray ionization (ESI)
- Matrix-assisted laser desorption ionization (MALDI)
- Mass analyzers like time-of-flight (TOF), quadrupole TOF, Fourier-transform ion cyclotron resonance (FT-ICR), etc.

Disadvantages: functional changes of the protein may occur due to posttranslational modifications which may affect the reflection of the physiologic or pathologic conditions. To evaluate such posttranslational modifications of proteins as biomarkers, comprehensively analytical methodologies like dendrimer-associated MS/MS, MALDI-MS, and targeted HPLC-ESI-MS/MS are used.

6.2 Transcriptomic technology

Salivary transcriptomes were discovered in 2004 which are basically RNA molecules (mostly mRNA) that are exceptionally stable in saliva [1, 8, 26, 27].

Salivary transcriptome is an emerging concept, and various studies are being conducted all over the world in extraction, purification, amplification, and microarray screening.

Although salivary transcriptomes are still under research, a comparative study done at UCLA regarding accuracy of salivary RNA and serum RNA proved that salivary transcriptomes have a slight upper hand compared to that of serum in certain cases like oral cancer.

As a biomarker, transcriptomes are highly robust and reliable.

6.3 Point-of-care technologies for salivary diagnostics

It includes the application of microfluid and micro/nanoelectromechanical system (MEMS/NEMS) [1, 8, 28]. This is an integrated system that allows the analysis of multiple markers in a drop of the salivary sample, simultaneously with the help of ultrasensitive biosensors. Recently, the UCLA Collaborative Oral Fluid Diagnostic Research Center along with UCLA School of Engineering has developed a MEMS-based electrochemical detection platform that has been named oral fluid nanosensor test (OFNASET) [2, 3]. It is highly specific and sensitive and allows simultaneous detection of multiple salivary analytes and RNA markers [2, 3].

6.4 Metabolomics

Metabolomics has been defined as a global holistic overview of the metabolic status [29]. It facilitates the measurement of comprehensively small metabolites and endogenous markers in bodily fluids and aids in biomarker discovery. Salivary metabolomics has been used successfully in physiology, diagnostics, functional genomics, pharmacology, toxicology, and nutrition.

γ-Aminobutyric acid, phenylalanine, valine, n-eicosanoic acid, and lactic acid have been used in the metabolic profiling of oral squamous cell carcinoma, oral lichen planus, and oral leukoplakia.

GC/MS profiling has proven excellent reproducibility with salivary compounds, such as alcohols, aldehydes, ketones, carboxylic acids, esters, amines, amides, lactones, and hydrocarbons. Reproducibility is an extremely important characteristic in diagnostics that shows way for use of this method to analyze larger sample sizes.

6.5 Salivary microbiome

The microbial population that exists within the oral cavity is collectively referred to as the microbiome [30]. The salivary microbiome provides a unique doorway to analyze the symbiotic and/or pathologic existence between the individual and colonizing microbes. This emerging research field is providing new insights into the functioning of the human immune system and defense mechanisms.

6.6 Salivary genomics

The isolation of high-quality and high-molecular-weight DNA from saliva and its analysis in various clinical purposes can be termed as salivary genomics [30, 31]. Technology has led to the development of various DNA collection kits, stabilizing reagents and purification procedures. These in turn have improved the quantity as well as the quality of DNA comparable to that obtained from blood. The ease of sampling and reliability of the assays have made salivary genomics widely accepted and is opening doors to discovery as well as application of salivary epigenomes in diagnostics.

6.7 Salivary epigenomics

The environmental regulation of the genome and its structural and chemical adaptation without altering the DNA base pair sequence are highlighted by the epigenome [30]. The epigenetic changes such as the length of the telomere, microRNAs, etc. are measurable in saliva. DNA in the blood is derived from a variety of cells including the immune cells, which vary significantly over time. However, contradictory to the DNA obtained in blood, the cellular components as well as the DNA in saliva are representatives of PMNLs and shed epithelial cells of the oral cavity, which are much more homogenous in nature.

Two main epigenetic mechanisms that regulate gene patterns through various tissues are methylation and demethylation of genes. The blood DNA methylation patterns have been noted as highly similar to those that are seen in salivary DNA methylation patterns. The methylation changes have been correlated to a number of systemic diseases including diabetes mellitus, muscular dystrophy, and even early life adversities. It is important in diagnostics, as it shows a map of changes in the human body over a period of time.

7. Salivary biomarkers in various diseases

7.1 Oral squamous cell carcinoma

OSCC has seen an increase in occurrence since the past two decades [13, 17, 32–36]. There has been 5.3 times increase for men and 2 times the increase for women [10]. Several studies have been done to discover and analyze the potential salivary biomarkers for oral cancer [10].

Various salivary biomarkers for OSCC:

- CEA, carcinogenic embryonic carcinogen
- CA19-9, carcino-antigen
- TPS, tissue polypeptide specific antigen
- IGF, growth factor
- MMP-2, MMP-11, metalloproteinase
- HA3, cell proliferation regulator
- CA125, serum tumor marker
- Cyfra 21-1, intermediate filament protein
- IL8, IL 1B DUSP1, chemokine-mediator of inflammatory response
- OAZ1, oncogene
- S100P, polyamine synthesis regulator
- SAT, calcium binding protein, cell cycle, and differentiation regulator polyamine metabolism

- IAP, apoptosis inhibitor
- SCC, squamous cell carcinoma-associated antigen
- RNS, reactive nitrogen species
- 8-OHdG, DNA damage marker
- IgG, immunoglobulin
- Sec IgA, mucosal immunoglobulin
- LOH, loss of heterozygosity loss of specific chromosomal regions
- DNA, hypermethylation gene inactivation

An increase is seen in the following biomarkers in patients with OSCC: carbonyls, lactate dehydrogenase, metalloproteinase-9 (MMP9), Ki67, cyclin D1 (CycD1) [10].

7.1.1 Periodontal diseases

Periodontitis is a group of inflammatory diseases that is characterized by loss of connective tissue [13, 32, 37] attachment and bone around the teeth in conjunction with the formation of periodontal pockets due to the apical migration of the junctional epithelium [32].

Inflammation of the gingiva and periodontal tissues is the first sign of a periodontal disease, usually in response to plaque accumulation [10, 12].

The biomarkers in periodontal diseases can be broadly divided into [32, 37, 38]:

- Inflammatory markers
- Markers of connective tissue destruction
- Markers of bone remodeling

a. Inflammatory markers

- β-glucuronidase—Elevated
- CRP—Elevated
- IL-1β—Elevated
- IL-16—Elevated
- MIP-1α—Elevated in aggressive periodontitis
- TNFα—Elevated

b. Markers of connective tissue destruction

- α2 Macroglobulin—Decreased

- MMP 8—Elevated
- MMP 9—Elevated
- AST—Elevated
- ALT—Elevated
- TIMPs—Decreased

c. Markers for bone remodeling

- Alkaline phosphatase—Elevated
- β C-terminal type 1 collagen telopeptide—Intermediate
- C telopeptide pyridinoline crosslinks of type 1 collagen—Intermediate
- Osteoprotegrin—Elevated
- Osteocalcin—Reduced
- Osteonectin—Reduced
- RANKL—Intermediate
- HGF—Elevated

Some other biomarkers of periodontal disease include [13, 32, 33, 37]:
Fibronectin, elastase, albumin, C-reactive protein, hyaluronic acid, CD44, aspartate aminotransferase, C3 complement

7.2 Biomarkers for breast cancer in saliva

- C-erbB-2 [13, 39]
- VEGF
- EGF
- CEA

7.3 Biomarkers of Sjogren's syndrome

- a-Amylase [13, 40, 41]
- Carbonic anhydrase VI
- Proline-rich proteins (PRPs)
- Prolactin-inducible protein precursor (PIP)

- Lactoferrin b-2- microglobulin
- lg k light chain
- Polymeric Ig receptor

7.4 Biomarkers of cardiac diseases

- Cardiac enzymes like CK-MB, MYO and Tnl [32]
- Inflammatory markers like CRP TNFα MMP-9 and myeloperoxidase
- Adhesion markers like soluble CD40 ligand and slCAM-1

7.5 Biomarkers for HIV

- HIV antibody
- Anti-HIV immunoglobulin [16]

7.6 Biomarkers (transcriptomic) of pancreatic cancer

- KRAS [42, 43]
- MBD3L2
- ACRV1
- CDKL3

7.7 Biomarkers (transcriptomic) of ovarian cancer

- H3F3A [44]
- SRGN
- B2M
- BASP1
- AGPATI
- ILIB
- IER3

7.8 Biomarkers of infectious disease

- Dental caries: presence of *Lactobacillus* sp. and *Streptococcus mutans*
- Candidiasis: presence of *Candida* sp.

- Hepatitis: presence of HBV DNA levels
- Peptic ulcers, gastritis, and cancrum: presence of *H. pylori* [16]

7.9 Pharmaceutical and illicit drugs

- Lithium
- Carbamazepine
- Barbiturates
- Benzodiazepines
- Phenytoin
- Cyclosporine
- Alcohol
- Tobacco
- Marijuana
- Cocaine
- Amphetamines [16]

7.10 Hormones

- Steroidal hormones: cortisol, testosterone, estrogen
- Nonteroidal: melatonin, thyroid hormones [16]

8. Conclusions

Saliva is rightly termed "the mirror of the human body." Saliva is already being used in various scenarios such as investigations for monitoring pharmaceutical as well as illicit drug usage, various malignant neoplasia, hormonal analysis, and even as forensic aids.

The use of saliva as an adjunct to clinical and laboratory investigations in diagnostics is no more a distant dream. The development of more reliable and affordable tests is paving way to a promising future in salivary diagnostics. With increasing awareness towards disease prevention and early diagnosis and intervention, salivary diagnostics could, in the future, be included as a part of routine clinical investigations.

Conflict of interest

None.

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