Chapter 5
The Role of Saliva in Dental Practice

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Objectives
Saliva is a valuable source of clinical information, since it contains biomarkers that may be useful for identifying patients at an increased risk of suffering oral disease. Current technology offers a valuable opportunity for developing such biological molecules as biomarkers (Giannobile et al. 2011; Schafer et al. 2014; Malamud 2011; Jiménez 2010; Chiappin et al. 2007; Baum et al. 2011; Kaufman and Lamster 2002; Llena-Puy 2006; Yoshizawa et al. 2013; Lorenzo-Pouso et al. 2018). The present chapter offers a review of saliva as a potential and effective tool for the diagnosis and monitoring of diseases of the oral cavity.

5.1 Dental Caries

Dental caries is a very common transmissible, chronic infectious disease characterized by local destruction of the hard-dental tissues as a result of the action of acids produced by the microorganisms that conform the biofilm adhered to the teeth.

From the etiological perspective there are a number of intervening factors, such as saliva, the characteristics of teeth, and a diet containing food that can be fermented by the oral microorganisms, thereby giving rise to caries. The protective function of saliva can be attributed to four effects:

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1. the dilution and elimination of sugars and other components
2. buffering capacity
3. demineralization/remineralization effects
4. antimicrobial action.

One of the most important functions of saliva is the clearing of microorganisms and food components from the oral cavity. A large salivary volume accelerates the elimination of sugars – a fact that explains the increased caries risk seen in patients with a low salivary flow (Jiménez 2010; Lorenzo-Pouso et al. 2018; Nordlund et al. 2009; Vitorino et al. 2006) (Fig. 5.1).

Caries is diagnosed from the clinical findings, since to date no saliva test has been found to be adequate for diagnosing the disease. Although combinations of factors have been suggested to predict the risk of caries, the fact is that none have demonstrated sufficient validity – probably because of the intervention of many risk factors at different levels in the development of the disease.

The caries susceptibility tests that can be made in a microbiology laboratory include:

1. Determination of the presence of caryogenic flora. It is known that bacterial growth is favored if incubation takes place under conditions of anaerobiosis, which plays a relevant role in the development of caries.
2. Isolation of acid-producing microorganisms in saliva, such as *Lactobacillus spp.*, which contribute to lower the oral pH value and thus favor tooth demineralization and increase dental susceptibility to the action of caryogenic bacteria. The Zinder test or Alban test can be used to quantitatively assess the presence of such acid-producing microorganisms in saliva. Depending on the intensity of the color shift generated in these tests, we can quantify the *Lactobacillus spp.* colonies.
3. Determination of the buffering capacity of saliva and of the salivary secretion rate. The buffering capacity of saliva is explained by the presence of different buffering systems in this body fluid, such as the phosphate system and the carbonic acid / carbonate system.
4. Quantification of fungal colonies. A number of studies have examined the interactions between the bacterial flora and *Candida albicans* in the formation of dental plaque, and it has been postulated that the concentration of this fungal species might act as an indicator of caries risk.

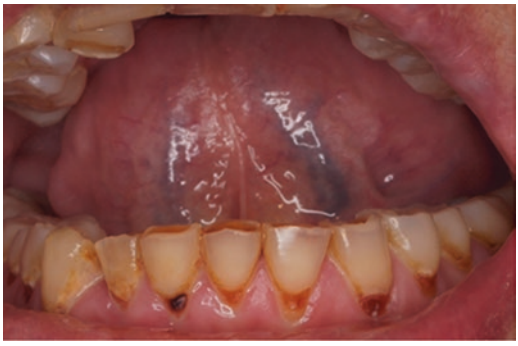

Fig. 5.1 Dental caries as a result of hyposalivation

Many studies have attempted to relate the prevalence of caries to the salivary phenotype, but the results have been contradictory. A recent field of interest in the investigation of caries risk has been the measurement of oligosaccharides, the concentrations of which have been found to be correlated to caries in young adults. In turn, significant associations have been observed between patient age and the salivary (submandibular/sublingual glands) concentrations of lactoferrin, albumin, lysozyme, mucin, cystatin, potassium, calcium, sodium and chloride. Some studies have adopted a proteomic approach to examine whether salivary proteins can act as biomarkers in the evaluation of caries risk. Their data suggest that statherin and cystatin are the best predictors of occlusal caries in saliva, though supragingival plaque and the total oral bacterial count must also be taken into account (Vitorino et al. 2006; Wang et al. 2018; Bhalla et al. 2010).

5.2 Periodontal Diseases

Periodontal disease is a chronic inflammatory disorder of bacterial origin that affects the hard and soft tissues supporting the teeth. Over 300 pathogens possibly implicated in periodontal destruction have been described in the literature. The term "periodontal disease" includes plaque-related gingivitis and periodontitis. Gingivitis is characterized by inflammation of the gums only, while in the case of periodontitis the inflammatory process extends to the alveolar bone and periodontal ligament – with progressive destruction of these elements (Slots and Slots 2011; Prakasam and Srinivasan 2014; de Lima et al. 2016; Ebersole et al. 2013; Kaufman and Lamster 2000; Miller et al. 2006; Grigoriadou et al. 2010; Canakci et al. 2009; AL-abbagh et al. 2012; Schenck et al. 1993; Ulker et al. 2008; Olayanju et al. 2012; Gonçalves Lda et al. 2010; Lee et al. 2018; Wilczynska-Borawska et al. 2006; Kaushik et al. 2011; Meschiari et al. 2013).

Periodontal disease is highly prevalent 20–50% of global population and exhibits a worldwide distribution. In most cases, periodontitis is preceded by gingivitis, and its progression towards bone and attachment loss is modulated by microbiological and immunological factors. In fact, periodontitis is regarded as a chronic inflammatory disease caused by microorganisms that form part of the subgingival biofilm, and which require a susceptible host in order to initiate the chronic inflammatory reaction that gives rise to periodontal destruction (Ebersole et al. 2013; Kaufman and Lamster 2000; Miller et al. 2006; Grigoriadou et al. 2010; Canakci et al. 2009; AL-abbagh et al. 2012; Schenck et al. 1993; Ulker et al. 2008) (Figs. 5.2 and 5.3).

In the healthy individual, the oral flora is in ecological equilibrium with the host, and this allows periodontal health to be maintained. However, this stable relationship can become altered as a result of different factors such as antimicrobial therapy or changes in host susceptibility secondary to alterations in the defense mechanisms.

Saliva contains over 1×10^8 bacteria/ml and is the usual environment of microorganisms from the supra- and subgingival biofilm, the dorsal surface of the tongue, and other surfaces of the oral mucosa. In this regard, the tongue is one of the main sources of most of the salivary bacteria. Periodontal disease can be described as one

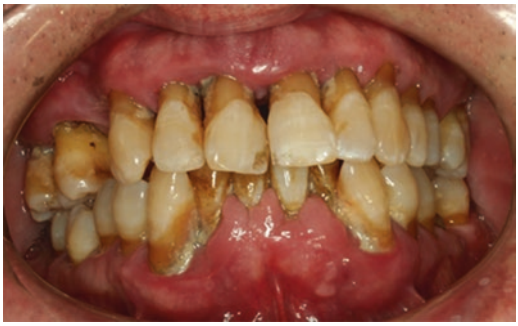

Fig. 5.2 Patient with generalized periodontitis

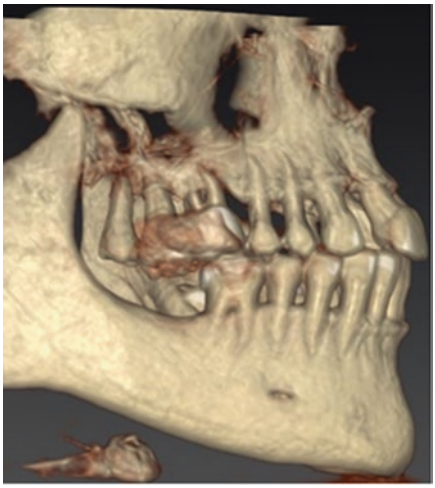

Fig. 5.3 Cone beam radiological image in a periodontal patient

of the predominant polymicrobial infections in humans. A range of organisms are implicated in the origin of periodontitis, such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Fusobacterium nucleatum spp., Prevotella intermedia, Prevotella nigrescens, Tannerella forsythia, Treponema spp. Capnocytophaga spp., Campylobacter rectus, Eikenella corrodens* and many other bacteria (Slots and Slots 2011).

The mucins (MG1 and MG2) in turn exert cytoprotective, lubricating and protective actions. Mucin MG2 influences bacterial aggregation and adherence, and in this respect a decrease in salivary MG2 levels can increase colonization by *Aggregatibacter actinomycetemcomitans*.

Different studies have pointed to periodontal infection as a risk factor for certain systemic disorders such as cardiovascular diseases, certain respiratory conditions, obesity and metabolic syndrome. In addition, it has been postulated to alter adequate diabetes control and give rise to premature delivery and/or infants with low birth weight (de Lima et al. 2016; Ebersole et al. 2013; Kaufman and Lamster 2000; Miller et al. 2006).

Increased oxidative damage has been observed in the more advanced stages of periodontal disease, since reactive oxygen species (ROS) are found to be increased. Salivary markers of oxidative stress and antioxidant status constitute a promising tool for the investigation of oral diseases, in view of the importance of ROS in the pathogenesis of periodontal disease. An increase in ROS gives rise to periodontal tissue destruction, and this is one of the leading causes of periodontal disease. There is evidence of significant changes in oxidative stress, with increased levels of different oxidative stress markers (8-OHdG, malondialdehyde [MDA], glutathione peroxidase [GPx], superoxide dismutase [SOD] and total antioxidant capacity [TAOC]) in individuals with worsened periodontal health (Kaufman and Lamster 2000; AL-abbagh et al. 2012).

Oxidative stress increases with the number of different bacteria present in the environment. Reactive oxygen species are related to polymorphonuclear cell (PMN) destruction of periodontal pathogens, and in this regard the increase in ROS levels as a consequence of PMN action would lead to tissue degeneration and worsened periodontal disease.

In sum, the saliva of patients with periodontitis has been found to contain gingival tissue cells, crevicular fluid and a series of inflammatory mediators and molecules implicated in tissue destruction. Consequently, saliva can be expected to contain biomarkers specific of all three key aspects of periodontitis (inflammation, collagen degradation and bone turnover) and which are correlated to the clinical features of the disease. The application of salivary proteomic biomarkers to the diagnosis of periodontal disease is still in the experimental phase, and is based on the changes in profile of molecules implicated in inflammation, collagen degradation and bone loss. Some of these salivary biomarkers are enzymes such as alkaline phosphatase, esterase, metalloproteinases (MMP), β-glucuronidase, aminopeptidase, cystatin, prolactin and α-amylase, while others are immunoglobulins such as IgA and IgG, cytokines such as interleukin-1β (IL-1β), interleukin-8 (IL-8) and steroid hormones (Prakasam and Srinivasan 2014).

Recently, macrophage inflammatory protein-1α (MIP-1α) has been identified as the biomarker of greatest diagnostic capacity, followed by IL-1β and IL-6 (Grigoriadou et al. 2010; Canakci et al. 2009; AL-abbagh et al. 2012; Schenck et al. 1993; Ulker et al. 2008; Olayanju et al. 2012).

5.3 Peri-implantitis

Dental implants are currently one of the safest options for replacing lost teeth. Osseointegration is the direct structural and functional connection between the bone and the rough surface of the dental implant, needed to resist occlusal loading. The term peri-implant disease is currently a general reference to the inflammatory reactions occurring in tissues surrounding dental implants (Gomes et al. 2018; Liskmann et al. 2007) (Fig. 5.4).

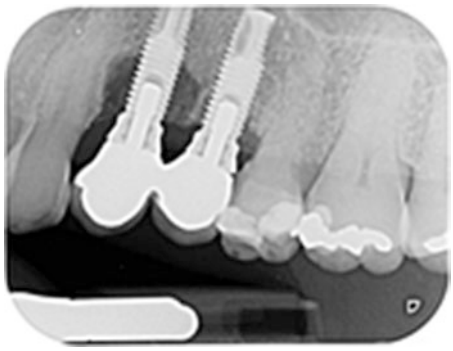

Fig. 5.4 Peri-implantitis

The causes of early implant failure include surgical trauma, bacterial contamination of the implant bed, and smoking. In turn, late implant failure can be caused by bacterial infections, while the contributing role of occlusal overload or biomechanical imbalances is more controversial. Mucositis is defined as the presence of inflammation of the peri-implant mucosa, without the loss of bone support, while peri-implantitis is characterized by inflammation with the loss of supporting bone.

The salivary interleukin-1β levels are seen to be lower in patients with healthy implants than in those with inflamed dental implants. In turn, a significant positive correlation has been observed between salivary IL-6 concentration and peri-implant inflammatory disorders.

Salivary total antioxidant capacity (TAOC) and the salivary levels of urate and ascorbate have been shown to be higher in individuals with healthy implants than in those with peri-implantitis.

5.4 Viral Diseases (Balamane et al. 2010)

The diagnosis of viral diseases of the oral cavity is based on the clinical findings and laboratory test results. In this respect, saliva can be used to detect and evaluate host immunization to measles, mumps and rubella, for example (see Chap. 12).

5.5 Fungal Diseases

Infections due to *Candida spp.* are among the most frequent mycoses. The great majority of Candida species (90–95%) isolated in patients with candidiasis correspond to *Candida albicans, Candida glabrata, Candida parapsilosis, Candida tropicalis* and *Candida krusei* (Salvatori et al. 2016; Thein et al. 2006; Canabarro et al. 2013).

The development of infection due to Candida depends on a number of factors:

(a) Damage to the skin or mucous membrane barriers.
(b) The dose or magnitude of the fungal inoculum.
(c) The defensive immune condition of the host.
(d) Fungal virulence (crucial role of dimorphism). Colonization, adhesion, invasion and damage are the successive stages involved in the pathogenesis of Candida infection.

There are a number of clinical presentations of oral candidiasis: pseudomembranous candidiasis, erythematous candidiasis, hyperplasic candidiasis, associated lesions (prosthetic stomatitis, angle cheilitis, rhomboid glossitis, exfoliativa cheilitis), and mucocutaneous candidiasis (chronic). When two or more of these clinical presentations coexist, we speak of multifocal oral candidiasis. The diagnosis of any of the forms of oral candidiasis is essentially based on the clinical findings. The observed clinical lesions in turn need to be confirmed by the microscopic identification of *Candida* in the oral samples and/or its isolation in culture. It should be noted that in the case of Candida, mere identification of the organism in the oral cavity is not indicative of infection, since it is a common component of the normal oral microflora. The definitive diagnosis of candidiasis requires the confirmation of tissue invasion by *Candida* (Fig. 5.5).

Saliva can also be used for the detection of fungal infections. Specifically, the salivary fungal count can reflect colonization of the oral mucosa.

A decrease in salivary secretion can cause dysbiosis, favoring the excessive growth of *C. albicans*. In this regard, there is an inverse correlation between the salivary flow rate and the oral Candida burden. Furthermore, it has been shown that patients with Sjögren's syndrome and oral candidiasis have high salivary levels of calprotectin – an antimicrobial peptide – possibly due to transudation of the inflamed mucosa. Therefore, salivary dysfunction may cancel the host defenses in the oral cavity secondary to changes in the levels of salivary proteins or due to loss of the protective salivary layer on the mucosal surfaces – thereby reducing epithelial barrier function (Salvatori et al. 2016; Thein et al. 2006).

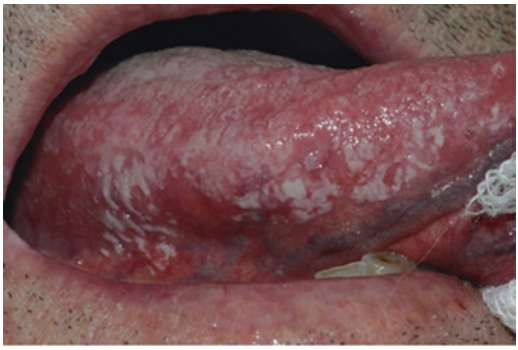

Fig. 5.5 Oral candidiasis

The development of diagnostic techniques independent of culture procedures appears to be a promising option for the early diagnosis of candidiasis. There are many limitations in this context, however, related mainly to the low yield of such techniques when used on an individual basis and to the high incidence of false-positive results, which in turn may contribute to an excessive use of antifungal agents.

Alterations of the salivary proteome related to proteins with antifungal activity, such as immunoglobulins, histatin, mucins, peroxidases and proline-rich proteins, may be of diagnostic utility, especially in recurrent cases.

Although largely regarded as a disorder of immunocompromised individuals, oral *C. albicans* infections are common among people wearing dentures and in cases of hyposalivation. Consequently, many immunocompetent individuals are susceptible to oral fungal infections. In this regard, it would be particularly interesting to know how the oral microbiome is altered in immunocompetent subjects with salivary dysfunction.

5.6 Orthodontics

Orthodontics has become one of the most popular dental treatments, though the application of appliances such as brackets can cause changes in saliva. The integrity and balance of the oral mucosa depend on the quality of saliva, the pH value and the concentration of proteins, which are factors that allow saliva to protect the hard and soft tissues of the oral cavity. The pH value and concentration of proteins may become altered in orthodontic patients, since the appliances used modify the oral environment, making saliva more acidic – though the total protein concentration is not affected (Koch et al. 2010; Eliades et al. 2003).

One of the main concerns among investigators in the last decade has been the performance of alloys in the environments in which they are intended to operate. The corrosion of orthodontic appliances raises two main concerns: (a) whether corrosion products are really produced and are absorbed in the body, causing local or systemic effects; and (b) the impact of corrosion upon the physical properties and clinical performance of the orthodontic appliances. Corrosion is characterized by deterioration of the surface of metals or alloys. In the concrete case of the metal appliances used in orthodontics, corrosion is a consequence of oxidation – reduction mechanisms.

A number of characteristics of the oral medium influence metal corrosion, such as temperature, humidity, pH changes, food, etc. Electrolytic corrosion is explained by the difference in potential between different metals, resulting in genuine degradation of the metal structure. Most studies on the release of metals from orthodontic appliances have focused on the biocompatibility of these materials, with assessment of the concentrations of Co, Cr, Ni and Fe in saliva.

Eliades et al. (Eliades et al. 2003) used inductively coupled plasma atomic emission spectroscopy (ICP-AES) to analyze the saliva of patients wearing orthodontic appliances with the aim of assessing the presence of metal traces (Ni, Cr, Fe). The authors concluded that there are no significant differences in the salivary levels of these metals between the patients and controls. In some cases the salivary metal concentrations were below the limit of detection of the measurement system used.

5.7 Conclusions

Diagnosis based on saliva can be greatly facilitated by the new technologies – becoming a powerful future tool for the diagnosis of oral and systemic diseases.

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