*Review Article*

# Protein-Based Salivary Profiles as Novel Biomarkers for Oral Diseases

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The Global Burden of Oral Diseases affects 3.5 billion people worldwide, representing the number of people affected by the burden of untreated dental caries, severe periodontal disease, and edentulism. Thus, much more efforts in terms of diagnostics and treatments must be provided in the fight of these outcomes. In this sense, recently, the study of saliva as biological matrix has been identified as a new landmark initiative in the search of novel and useful biomarkers to prevent and diagnose these conditions. Specifically, saliva is a rich reservoir of different proteins and peptides and accessible due to recent advances in molecular biology and specially in targeted and unbiased proteomics technologies. Nonetheless, emerging barriers are an obstacle to the study of the salivary proteome in an effective way. This review aims at giving an overall perspective of salivary biomarkers identified in several oral diseases by means of molecular biology approaches.

## 1. Introduction

Saliva is a complex biological matrix generated by the salivary glands. Each salivary gland emits considerably different secretions with a highly variable composition depending on sympathetic and parasympathetic stimulation, circadian rhythm, eating habits, health-illness spectrum, drug intake, and other conditions [1]. The basic secretory units of salivary glands are clusters of cells called acini. The main three pairs of salivary glands in humans (parotid, submaxillary, and sublingual) together with the minor salivary glands generate 0.75–1.5 liters of this exocrine secretion per day.

This physiological secretion remains high during the day, reducing significantly during the night [2].

Besides water, saliva contains a large number of electrolytes (i.e., $Ca^{2+}$, $Cl^-$, $H_2PO_4^-$, $HCO_3^-$, $I^-$, $K^+$, $Mg^{2+}$, $Na^+$, and $SCN^-$), proteins (i.e., mucins, enzymes, and immunoglobulins), lipids, and other molecules [3]. Saliva plays a pivotal role in the early stages of digestion, allowing a correct physiological homeostasis in human through nutrition [4]. Salivary antioxidant capacity is mainly related to some enzymes (i.e., salivary peroxidase, superoxide dismutase, catalase, glutathione peroxidase, and myeloperoxidase), uric acid, and, to a less extent, ascorbic acid and albumin [5]. In

**Anti-bacterial**
Amylases, cystatins, histatins, mucins, peroxidases, lactoferrin, lysozyme, agglutinin

**Anti-viral**
Mucins, lactoferrin, cystatins, immunoglobulins, peroxidases

**Anti-fungal**
Lactoferrin, peroxidases, immunoglobulins, mucins, histatins

**Tissue coating**
Amylases, cystatins, mucins, proline-rich proteins, statherins

Salivary proteome

**Buffering**
Carbonic anhydrases, histatins

**Digestion**
Amylases, mucins, lipases

**Mineralization**
Cystatins, histatins, proline-rich proteins, statherins

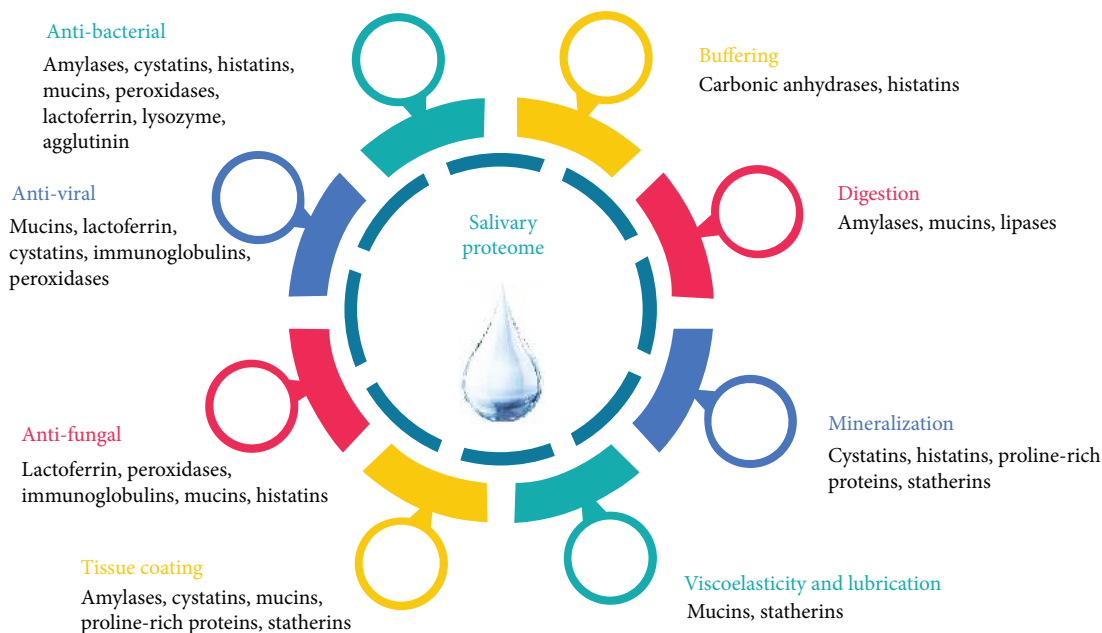**Viscoelasticity and lubrication**
Mucins, statherins

FIGURE 1: Biological function of the salivary proteome (adapted from Van Nieuw Amerongen et al. [159]).

this sense, saliva is the first line of defence against oxidative stress (OE), reactive oxygen species (ROS), and free radicals [6]. Imbalance between the systemic manifestation of ROS has been implicated in the pathogenesis of over 100 pathological conditions and also in the prevailing free-radical theory of aging [7].

Recently, the term liquid biopsy (LP) was coined in analytic chemistry as the sampling and analysis of nonsolid biological tissues, primarily blood and also saliva and other biofluids. LP methodologies allow the biomonitoring of several biomarkers such as proteins, nucleic acids, circulating tumor cells, or disease drivers related to infections which proved usefulness in the diagnosis, prognosis, and staging of a large number of pathologies [8]. In principle, when saliva is compared with other biofluids (e.g., blood serum, amniotic fluid, cerebrospinal fluid, and bronchoalveolar lavage fluid), this matrix seems attractive over the others due to its noninvasive nature, its lower economic cost, and its greater clinical safety. Although certain pathologies and adverse drug reactions may limit the bioavailability of this fluid [9], saliva remains as a window of opportunity for modern medicine [10]. In this sense, this matrix has been used by medicine for the biomonitoring of physiological functions for more than a century. A good example would be salivary cortisol determinations which have been widely used in medicine and behavioural research in the last 150 years for their easy conservation and handling. Therefore, salivary cortisol is stable at room temperature for 1–2 days and at 4°C for one week [11].

Currently, several sensitive analytical techniques allow the detection and quantification of a large number of biomarkers in saliva such as mass spectrometry (MS), reverse transcription-polymerase chain reaction (RT-PCR), microarrays, nanoscale sensors, magnetic resonance spectroscopy (MRS), Western blot, immunoassay techniques, or

enzymatic assays. A continuous and exponential growth in the saliva-related research lines has occurred throughout the last decades and new relevant concepts as point-of-care (POC) diagnostics have emerged [12]. In the past, cost-effectiveness analysis applied to these techniques showed them as not appropriate for clinical purposes; however, nowadays, these barriers are being effectively addressed, and this approaches are being progressively translated to clinical practice [13]. Currently, five alphabets (also known as "OMICS") of biomarkers present in saliva are known: proteome, transcriptome, microRNA (miRNA), metabolome, and microbiome [14].

In the field of salivanomics, the greatest advances in recent decades have focused on the analysis on nucleic acids; despite this, some interest has also been placed on protein-based techniques. Human saliva is a rich reservoir of proteins and peptides; in fact, it gathers more than 3652 proteins and 12,562 peptides and shares almost 51% of the proteins and 79% of the peptides contained in the plasma [15, 16] (Figure 1). Recent advances in proteomics techniques have brought the discovery of a large number of biomarkers and therapeutic targets in a large number of oral diseases and systemic pathologies with repercussions in the oral cavity [17]. A new landmark in salivanomics has been the discovery of the presence of exosomes and its outstanding stability in saliva. Exosomes are extracellular vesicles involved in intercellular traffic [18]. These vesicles comprise genetic material (i.e., miRNAs) and proteins. Exosomes play a pivotal role in immune system modulation, inflammation, and oncogenesis [19]. On the other hand, the discovery of the function of certain salivary peptides has helped in the development of new antibiotics [20].

In the present review, the most relevant scientific information published to date related to the salivary proteome within the spectrum of oral diseases is collected and critically

discussed. This paper is mainly focused on proteins of human origin present in saliva and not on the oral disease driver-related proteins or the ones related to the pathogen-host-environment interplay.

### 1.1. Methods for Collecting Saliva.

Protein kinetics and its concentrations in saliva are influenced by several factors. In this line, quantity and composition of extracted saliva are affected by the time of day, degree of hydration, body position, psychological stimuli, drug intake, health-related behaviours, systemic/oral health, and other factors [21]. In addition, deficits in sample collection, sample handling, and sample transport to the laboratory can trigger preprocessing problems. Thus, proteomic literature has extensively expressed the necessity of highly standardized protocols and tailored to fit the experimental design [22].

At this point, it is important to highlight that saliva can be collected under resting or stimulated conditions. Salivary gland stimulation can be achieved by means of different stimuli such as chewing (gums or swabs), taste stimuli (citric acid), or pharmacologic and electric stimulants [22]. Salivary flow is controlled by the autonomic nervous system. Parasympathetic stimulation produces a higher flow rate, while sympathetic stimulation produces a small flow but richer in proteins and peptides. This stimulation provides clear differences in the snapshot of the salivary proteome and also in the relative amount of specific proteins detected [23].

On the other side, saliva can be collected as whole saliva (WS) or individual gland saliva. Different approaches have been described in order to obtain single gland fluids. Regarding to parotid gland saliva, different methods can be used such as the Lashley's cup [24] or the modified Carlson-Crittenden device [25]. Submandibular and sublingual gland saliva can be collected by means of Truelove's V-shaped collector [26] or Fox's micropipette [27]. Minor gland secretions can be collected by pipettes, absorbent papers, or capillary tubes [28]. A relevant drawback in relation to the majority of these methodologies is the requirement of duct cauterization, which in practice is technically demanding and uncomfortable for patients [22].

In the case of WS, regardless of the approach used, patients should refrain from eating, drinking, and oral hygiene procedures for at least one hour before collection, and just before this process, use deionized water as a mouth rinse. Specifically, to collect unstimulated whole saliva (USWS), the patient must be kept comfortably seated avoiding orofacial movements during 5 minutes [29]. Navazesh described four approaches to collect WS: draining, spitting, suction, and the swab method. Due to the preference of collecting USWS, the gold standard method is draining [22]. Different devices have been developed in order to collect passive drool such as Salivette® (Sarstedt, Nümbrecht, Germany), Quantisal® (Immunalysis, Pomona, CA, USA), Orapette® (Trinity Biotech, Dublin, Ireland), and SCS® (Greiner-Bio-One, Kremsmünster, Austria) [30]. Several reports have shown that the protein coverage does not suffer relevant changes in relation to different collection devices. The only well-known WS drawback versus single gland saliva is that it has a higher proportion of certain nonsalivary materials such as desquamated epithelial cells, food debris, bacteria, or leukocyte in WS when compared to single gland saliva [1].

The published scientific literature on the effect of preanalytical variables on saliva profiling is scarce. Controversies are specially accentuated when the focus is put on centrifugation speed, addition of a protease inhibitor cocktail (PIC), and storage temperature range [31]. Schipper et al. demonstrated that in the case of MS-based techniques, centrifugation speed does not have an effect on the number of proteins but a small effect on the intensity of the peaks [31]. Mohamed et al. reported that centrifugation can compromise the identification and quantification of larger proteins [32]. PICs (e.g., aprotinin, leupeptin, antipain, pepstatin A, phenyl-methylsulfonyl fluoride, EDTA, and thimerosal) can avoid proteolysis through the inhibition of serine-, cysteine-, aspartic-, and metallo-proteases. Nevertheless, PICs cannot fully inhibit proteolysis, and this phenomenon can occur during centrifugation especially on low-molecular-weight proteins [33]. It is worth mentioning that the addition of some reagents such as sodium azide can cause interference in immunoassays with horseradish peroxidase [33]. Despite these limitations, the majority of the described protocols use PICs to stabilize this matrix [29]. Collected samples must be collected in an ice container and proceeded in the laboratory within one hour; this methodology avoids bacterial action and minimizes posttranslational modifications (PTMs) [21]. More than 700 different species of microorganism cohabit in saliva [34]. A significant part of these microorganisms produce a variety of proteolytic and other enzymes that can trigger PTMs [29]. Moreover, temperature is known to play a pivotal role in proteostasis; for example, some proteases can function as chaperones (i.e., "helper" proteins) at low temperatures, but they act as proteases at elevated temperatures [35]. After processing, storage at −80°C have shown to provide the same spectra as fresh samples, while at −20°C temperature results can be distorted [31].

Finally, many salivary proteins of low abundance, suffer a strong interference with other more abundant proteins (i.e., lysozyme and $\alpha$-amylase) resulting in a low ionization efficiency in MS-based analysis. There are mainly three methods for the removal of high-abundance salivary proteins: enzyme substrate absorption method used for alpha-amylase affinity removal, immunodepletion method, and the combinatorial peptide ligand library [14].

### 1.2. Analysis.

Quantitative molecular biology techniques remain as the gold standard in the study of the salivary proteome [36]. These techniques are classified into absolute quantification techniques in which the exact concentration of proteins in a matrix is detected and the relative techniques in which the difference in protein concentration between samples is measured. Relative quantification techniques fit a very broad field of experimental designs; in this sense, semiquantitative ELISA, MS, and two-dimensional gel electrophoresis (2-DE) have been widely used. Nonetheless, absolute quantification approaches such as quantitative ELISA assays or multiplexed immunobead-based assay have also been used [37]. Recently, in the search for salivary biomarkers, nontargeted techniques have been successfully introduced. In

this sense, the current state-of-art techniques are 2-DE techniques coupled to matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) or liquid chromatography tandem-mass spectrometry (LC-MS/MS) [38]. Moreover, other non-gel-based approaches such as isobaric tags for relative and absolute quantitation (iTRAQ) or label-free quantification have been used for the quantitative analysis of the salivary proteome [39]. Minority, surface-enhanced laser desorption/ionization-time of flight (SELDI-TOF) MS was also used [40].

# 2. The Salivary Proteome in the Health-Illness Spectrum

*2.1. Salivary Proteomic Profile in Health.* A recent collaborative study among three reference centres in the saliva research revealed the presence of 1939 different proteins obtained from 19,474 unique peptides in whole saliva [41]. Despite this, there may be variations in this number depending on the equipment and techniques used [42]. Zhao et al. recently studied the number of matching proteins in five body fluids (i.e., plasma, urine, cerebrospinal fluid, amniotic fluid, and saliva) finding a total of 564 common proteins [43]. It has been hypothesized that the common proteins present in both plasma and saliva may be due to the intimate contact of saliva with crevicular fluid present at the periodontal pocket of sulcus level (such as albumin, transferrin, and immunoglobulins G and M) [34]. Nevertheless, several transport mechanisms capable to allow this communication have been identified such as passive diffusion, pinocytosis, and fusion pores at acinar cells [44]. Most of the salivary proteins have a low molecular weight. Specifically, 70% of the salivary proteome is made up of proline-rich proteins (PRPs) synthesized from the genome contained in chromosome 12 [45]; the rest of the proteins are synthesized from genome belonging to chromosomes 4 and 20 [46]. The salivary proteome is highly dynamic. Its proteins are affected by a large number of PTMs such glycosylation, phosphorylation, acetylation, ubiquitination, methylation, deamidation, sulfation, or proteolysis. The homeostatic mechanisms that regulate these modifications are not well known, but they constitute a particular "biological signature" not included in the genome [47]. ROS can also affect salivary proteins; in this sense, they can damage proteoglycans and can cause the oxidation of some relevant proteases. Some of these PTMs may increase the molecular weight of these proteins [48]. In addition, the salivary "interactome" of these proteins has been recently investigated. In this sense, most proteins interact with others creating protein complexes (e.g., amylase with MUC 5B, MUC 7, histatin 1, and histatin 5) [49].

Due to the limitations that the use of single OMIC technique entails, recently, they tend to be combined in order to obtain a better vision of the disease and its progression [50–53]. In this regard, current theories point to a bidirectional relationship between salivary microbiome and proteome. The salivary proteome thereby confers long-term stability to the composition and activity of the oral microbiota [50].

*2.2. Dental and Periodontal Diseases.* Table 1 summarizes the use of protein-based techniques for salivary biomarker identification in dental and periodontal diseases.

*2.2.1. Periodontal and Peri-Implant Diseases.* The most common forms of periodontal disease are gingivitis and periodontitis. Gingivitis is defined as a plaque-induced inflammation of the marginal gingiva, whereas periodontitis (PD) implies a chronic inflammation that causes the destruction of the connective tissue of the tooth and surrounding alveolar bone [54]. PD is one of the most frequent inflammatory events in humans; in fact, one of every two Americans aged 30 or older are affected by PD (i.e., 64.7 million people) [55].

Schenck et al. demonstrated that high levels of salivary IgA were related with higher susceptibility to gingivitis when the host response to several bacteria was investigated [56]. Another nontargeted salivary proteomics research designed with Löe's concept of experimental gingivitis analysed using 2-DE found that, in patients suffering from gingivitis, there was a greater presence of serum-related proteins such as immunoglobulins and keratins in relation to the control group [57]. Nonetheless, the majority of the investigations analyse the inflammatory condition proteome in the gingival crevicular fluid and not in the saliva [39, 58]. A problem reflected in the literature regarding MS (specifically LC-ion trap MS, LC-Orbitrap MS, or LC-FTMS) is its lower sensitivity to detect certain proinflammatory and anti-inflammatory cytokines versus ELISA techniques [59]. These cytokines are very relevant in the genesis of the periodontium pathology [60, 61]. If we take a closer look at the studies that use ELISA techniques to detect different levels of proteins in saliva from patients with gingivitis compared to controls, we will find a large number of overexpressed proteins in affected subjects: TNF-$\alpha$, IL-1, Annexin-1, HBD-1, HBD-2, HBD-3, 25-hydroxy-vitamin D3, PGE2, Cystatin C, etc. [62–66]. Due to the reversible character of this outcome, the two most used patients' subgroups in this type of research have been children and pregnant women.

Chemical studies applied in the study of PD have been constant in the medical literature for the last 70 years [67]. However, due to the lack of stable criteria and classification to diagnose this family of pathologies [68], all these investigations went through great biases until the last 30 years. PD-related salivary proteins have been classified in four subgroups [69].

The most specific salivary group biomarkers are the immunoglobulin (Ig) family proteins. Igs are glycoproteins of the $\gamma$-globulin type that acts at the saliva level in the identification and neutralization of bacterial agents. Immunofluorescence studies have shown that these Igs are synthesized by plasma B cells located at the level of salivary glands [12]. In this regard, countless studies have studied the differential levels of IgA, IgG, and IgM expression in control patients versus patients with different forms of PD [70, 71]. The main analytical techniques used to determine these Igs in saliva are radial immunodiffusion (RID), nephelometry, and ELISA [72]. Several studies have shown that the levels of these Igs in both chronic and aggressive periodontitis are higher than in healthy patients [69]. At the same time, it has also been

TABLE 1: Use of protein-based techniques for biomarkers identification in dental and periodontal diseases mucosa diseases.

| Base disease | Number of patients | Age of patients | Matrix | Analytical technique | Determined parameter | Range of concentrations | Endpoints | Reference |
|---|---|---|---|---|---|---|---|---|
| Gingivitis | 12 | AR: 22–37 | Unstimulated parotid saliva | ELISA | IgA | NR | ↑IgA in patients as a response to experimental gingivitis | [56] |
| Gingivitis | 20 (10 controls, 10 cases) | M: 35.6 | USWS | 2-DE and MALDI-TOF-MS | Total proteins | RQ | ↑Blood-related proteins, immunoglobulin peptides, and keratins in gingivitis patients vs. controls | [57] |
| Pregnancy-associated gingivitis | 54 (30 cases, 20 controls) | M: 29.3 | SWS | ELISA | hBD-1, hBD-2, and HNP-1 | * | ↑hBD-1, ↑hBD-2, and ↑HNP-1 in pregnancy-related gingivitis patients vs. controls | [62] |
| Pregnancy-associated gingivitis | 178 (78 cases, 69 controls) | NR | USWS | ELISA | IL-1$\beta$, and ANXA1 | Pregnant: IL-1$\beta$ (566.0 ± 445.1 pg/ml); ANXA1 (1184 ± 1495 pg/ml). Control: IL-1$\beta$ (258.3 ± 225.0 pg/ml); ANXA1 (495.2 ± 990.9 pg/ml) | ↑IL-1$\beta$ and ANXA1 in pregnancy-related gingivitis patients vs. controls | [64] |
| Pregnancy-associated gingivitis | 176 (59 pregnant, 47 postpartum, and 70 controls) | AR: 18–48 | USWS | ELISA | PGE2 and TNF-$\alpha$ | Pregnant: PGE2 (505.6 ± 198.5 pg/ml); TNF-$\alpha$ (61.21 ± 11.75 pg/ml). Postpartum: PGE2 (621.7 ±191 pg/ml); TNF-$\alpha$ (72.96 ±58.77 pg/ml). Control group: PGE2 (569.2 ± 297.7 pg/ml); TNF-$\alpha$ (76.67 ± 33.54 pg/ml) | ↑TNF-$\alpha$ in patients with pregnancy-induced gingivitis vs. postpartum and controls | [65] |
| Gingivitis | 35 (10 controls, 25 cases) | M: 13.6 | USWS | ELISA | Cystatin C, IL-1$\beta$, and TNF-$\alpha$ | Control: cystatin C (3.721 ± 2.1740 mg/ml); IL-1$\beta$ (2.270 ± 1.595 pg/ml); TNF-$\alpha$ (623.386 ±395.101 pg/ml). Case: cystatin C (2.987 ± 1.139 mg/ml); IL-1$\beta$ (5.587 ± 5.441 pg/ml); TNF-$\alpha$ (321.163 ± 335.043 pg/ml) | No significant alterations | [66] |
| ChP | 46 (21 controls, 25 cases) | M: 53 | USWS | ELISA | IgA, IgG, IgE, and IgM | Controls: IgA (596.9 ± 103.6 ng/l), IgG (369.6 ± 44.6 ng/l), IgE (0.24 ± 0.89 IU/ml), IgM (779 ± 49.5 ng/ml). Case: IgA (670 ± 110.0 ng/ml), IgG (370 ± 60.9 ng/ml), IgE (0.20 ± 1.0 IU/ml), IgM (791.4 ± 43.7 ng/ml) | ↑IgA in chronic periodontitis patients vs. controls | [70] |

TABLE 1: Continued.

| Base disease | Number of patients | Age of patients | Matrix | Analytical technique | Determined parameter | Range of concentrations | Endpoints | Reference |
|---|---|---|---|---|---|---|---|---|
| ChP | 70 (20 controls, 50 cases) | AR: 18–45 | USWS | ELISA | IgA, IL-1β, and MMP-8 | Control: IgA (81.23 ± 24.61 μg/ml); IL-1β (89.93 ± 25.48 pg/ml); MMP-8 (57.95 ± 31.64 ng/ml). Case: IgA (196.48 ± 54.61 μg/ml); IL-1β (530.76 ± 343.85 pg/ml); MMP-8 (672.18 ± 411.0 ng/ml) | ↑IgA, ↑IL-1β, and ↑MMP-8 in chronic periodontitis patients vs. controls | [71] |
| Aggressive periodontitis | 10 (5 controls and 5 cases) | M: 24 | USWS | 2-DE and LC-MS/MS validation via ELISA | Total proteins | RQ | ↓Lactoferrin, ↑IgA2, and ↑albumin in aggressive periodontitis patients vs. controls | [74] |
| ChP | 20 (10 controls and 10 cases) | AR: 26–50 | USWS | 2-DE and MALDI-TOF-MS | Total proteins | RQ | ↑α-Amylase variants in chronic periodontitis patients vs. controls | [75] |
| ChP | 57 (38 obese patients (13 periodontitis and 33 nonperiodontitis) and 19 healthy patients) | AR: 35–65 | SWS | SELDI-TOF-MS | Total proteins | RQ | ↑Albumin, ↑a and b hemoglobin, ↑ α-defensins 1, 2, and 3 in periodontitis obese patients vs. controls | [76] |
| ChP | 25 (15 periodontitis patients with type two diabetes and 10 controls) | AR: 40–60 | USWS | 2-DE and LC-MS/MS | Total proteins | RQ | ↑Immunoglobulin J chain, ↓polymeric immunoglobulin receptor, ↑plastin-2, ↓actin-related protein, ↓interleukin-1 receptor antagonist, and ↑leukocyte elastase chronic periodontitis patients vs. controls | [77] |
| ChP and gingivitis | 67 (17 chronic periodontitis, 17 gingivitis, and 33 healthy) | AR: 20–64 | USWS | LC-MS/MS validation via MRM | Total proteins | RQ | ↑Matrix metalloproteinase-9, ↑Ras-related protein-1, ↑actin-related protein 2/3 complex subunit 5, ↓clusterin, and ↓deleted in malignant brain tumors 1 in chronic periodontitis and gingivitis patients vs. controls | [78] |
| ChP | 54 (20 controls, 43 cases) | AR: 20–73 | USWS | ELISA | IL-1β, IL-1ra, platelet-derived growth factor-BB, VEGF, MMP-8, MMP-9, CRP, and lactoferrin | Control: MMP-8 (36.8 (16.9–295.5 ng/ml); lactoferrin (10,877 (5808–20,937 ng/ml)). Case: MMP-8 (203.7 (86.8–609.2 ng/ml); lactoferrin (10,877 15,801 (12707–18,687 ng/ml)) | ↑IL-1β and ↑MMP-8 in chronic periodontitis and gingivitis patients vs. controls | [80] |

Table 1: Continued.

| Base disease | Number of patients | Age of patients | Matrix | Analytical technique | Determined parameter | Range of concentrations | Endpoints | Reference |
|---|---|---|---|---|---|---|---|---|
| ChP | 46 (20 controls, 26 cases) | M: 49 | USWS | ELISA | HGF | Control: HGF (0.06 to 5.38 ng/ml). Case: HGF (0.68 ng/ml (range: 0–7.33)) | ↑HGF in chronic periodontitis patients vs. controls | [83] |
| ChP | 52 (24 controls, 28 cases) | AR: 20–50 | USWS | ELISA | IL-1$\beta$ | Control: IL-1$\beta$ (161.51 pg/ml). Case: IL-1$\beta$ (1312.75 pg/ml) | ↑IL-1$\beta$ in chronic periodontitis patients vs. controls | [84] |
| ChP | 42 (15 controls, 27 cases) | AR: 35–55 | SWS | ELISA | MMP-8, MMP-9, TIMP-1, TIMP-2, and MPO | NR | ↑MMP-8, ↑TIMP-1, and ↑MPO in chronic periodontitis patients vs. controls | [88] |
| Orthodontically induced inflammatory root resorption | 72 (24 controls, 48 cases) | AR: 10–30 | USWS | 2-DE and LC-MS/MS validation via Western blot | Total proteins | RQ | ↑P21-ARC and ↑CDC42 in orthodontically induced inflammatory root resorption vs. controls | [91] |
| Caries | 32 (16 controls, 16 cases) | AR:18–29 | USWS | 2-DE and LC-MS/MS | Total proteins | RQ | ↑Amylase, ↑IgA, and ↑lactoferrin, ↓cystatins, ↓acidic PRPs, and ↓lipocalin-1 in dental caries patients vs. controls | [99] |
| Caries | 30 (10 controls, 20 cases) | AR: 10–12 | USWS | iTRAQ-LC MS/MS validation via MRM | Total proteins | RQ | ↑Mucin 7, ↑mucin 5B, ↑histatin 1, ↑cystatin S, ↑cystatin SN, and ↑basic salivary ↑proline-rich protein 2 in dental caries patients vs. controls | [100] |
| Caries | 100 (50 controls, 50 cases) | AR: 4–6 | USWS | 2-DE | Total proteins | RQ | ↑Proline-rich protein bands in dental caries patients vs. controls | [101] |
| Caries | 26 (13 controls, 13 cases) | AR: 3–4 | SWS | MALDI-TOF MS combined with magnetic beads | Total proteins | RQ | ↑2 specific peptides with m/z values 3162.0 Da and 3290.4 Da in caries patients vs. controls | [104] |
| Caries | 30 (10 controls, 20 cases) | AR: 4.7 | SWS | MALDI-TOF MS combined with magnetic beads | Total proteins | RQ | ↑Histatin-1 in caries patients vs. controls | [105] |

Abbreviations: 2-DE: two-dimensional gel electrophoresis; ANXA: annexin; AR: age range; ARC: activity-regulated cytoskeleton-associated protein; CDC: cell-division cycle protein; ChP: chronic periodontitis; CRP: c-reactive protein; ELISA: enzyme-linked immunosorbent assay; HDB: hemoglobin subunit delta; HGF: hepatocyte growth factor; HNP: human neutrophil peptide; Ig: immunoglobulin; IL: interleukin; ITRAQ: isobaric tags for relative and absolute quantitation; LC-MS/MS: liquid chromatography tandem-mass spectrometry; M: mean; NR: not reported; MALDI-TOF: matrix-assisted laser desorption/ionization; MMP: matrix metalloproteinase; MPO: myeloperoxidase; MRM: multiple reaction monitoring; PG: prostaglandin; PRP: proline-rich protein; RQ: relative quantification; SWS: stimulated whole saliva; TIMP: tissue inhibitor of metalloproteinases; TNF: tumor necrosis factor; UWSW: unstimulated whole saliva; VEGF: vascular endothelial growth factor. *for additional data see original source.

shown that the level of these proteins decreases significantly with periodontal treatment. In addition, oral dysbiosis may trigger the production of specific proteases against Igs [73].

The second group comprises nonspecific markers. In this regard, there is an innumerable amount of nonspecific proteins that have been found altered in patients with periodontal disease versus healthy patients. Among them, we find, for example, albumins, amylases, mucins, lactoferrins, lysozymes, histatins, or proteins related to oxidative stress (OS). Nontargeted proteomic techniques are the most used to identify these nonspecific biomarkers [74–77]. Bostanci et al. demonstrated through label-free quantitative proteomics that patients with PD had lower levels of lactoferrin, lacritin, sCD14, Mucin 5B, and Mucin 7 vs. control [78]. This finding points to a reduction in the salivary antimicrobial and defence properties among PD-affected patients.

The third group comprises proteins related to systemic and local inflammation at the soft gingival tissues level. In this sense, C-reactive protein (CRP) and cytokines stand out. At the same time, within the group of cytokines, there are several remarkable subfamilies such as those of IL-1 (11 proteins), TNF-$\alpha$ (19 proteins), chemokines, growth factors, or bone metabolism-related cytokines (i.e., RANK/RANKL/OPG) [79].

CRP is an acute phase protein, whose levels rise in response of inflammation. This analyte can be detectable in saliva by means of ELISA [80] and integrated microfluidic platforms [81]. According to a recent systematic review, high salivary levels of CRP have been correlated with local inflammation (PD) and systemic inflammation [82]. At present, the most widely studied PD-related cytokines have been interleukin-1 beta and hepatocyte growth factor. Several case-control studies confirmed that both proteins are overexpressed in PD-affected patients vs. control [83, 84].

The RANKL/RANK/OPG pathway is responsible for controlling osteoclastogenesis [85]. Apparently, at the salivary level, high and low levels of RANKL of OPG, respectively, have being found during PD [86].

The last groups of proteins are metalloproteinases (MMPs). MMPs are a subfamily of zinc-dependent proteases responsible of extracellular matrix (ECM) remodelling. Aside from their initial role as ECM modifiers, MMPs also interact with several cell-surface molecules (i.e., chemokines, cytokines, growth factors, intercellular junction proteins, other proteases, and cell receptors). Imbalance in the ECM equilibrium has been linked to alterations at tissue remodelling, inflammatory response, cell growth, and migration [87]. Many scientific reports have given insight into MMPs and their relationship with periodontal inflammation and destruction due to the pivotal role of these proteases in collagen degradation.

The MMPs 8 and 9 are the main detectable ones in saliva. One of the actual gold standard biomarkers of PD is salivary MMP8, as several ELISA and POC platforms have ascertained [87]. Meschiari et al. demonstrated that salivary MMP9 (also known as gelatinase B) is overexpressed in PD-affected patients by zymography approaches [88].

Recent reports have used proteomic techniques in the search of salivary biomarkers in peri-implant diseases (i.e., peri-implantitis and peri-implant mucositis). These reports have shown a series of markedly overexpressed proteins in these pathological conditions, especially cytokines (i.e., IL-1b and RANK/RANKL/OPG) and MMPs (MMP8) [89]. These biomarkers are very close to those described in the PD; this finding supports the epidemiological relationship between PD and peri-implant diseases [90]. A particular proteomic signature has been also detected in the processes of root resorption induced by orthodontic movements by means of 2-DE coupled to MALDI-TOF-MS [91, 92].

*2.2.2. Caries.* Caries is a biofilm-mediated carbohydrate-driven pathological condition. This outcome produces the mineral breakdown of the dental tissues [93]. Dental caries at permanent dentition is the most common human diseases, affecting 2.4 billion people (40% of the global population) [94]. Classically, the diagnosis of this condition has been made through conventional clinical diagnosis and radiological techniques [95]; however, recent studies at the salivary level have also served to find new useful biomarkers in the diagnosis and response to treatment of this outcome [14]. Different salivary parameters outside the proteome have been studied and correlated with the predisposition to dental caries such as dysbiosis of microbiota, evaluation of pH, buffering capacity, viscosity, and flow rate levels [96]. The biomarkers currently detected at the salivary proteome level were recently classified by Gao et al. into three subgroups: Igs, innate (nonimmune) host defence proteins and peptides, and proteins and peptides implicated on calcium phosphate chemistry.

In relation to Igs, the evidence is limited in relation to IgA and salivary IgG [97]. Nonetheless, Fidalgo et al. recently developed a meta-analysis of case-control studies to explore salivary IgA levels in dental caries concluding that high levels of IgA were higher in patients with caries (0.27 OR [0.17–0.38]) [98].

Regarding nonspecific proteins, different case-control studies with nontargeted proteomic techniques have found differential expression of different proteins [99, 100]. Numerous investigations have pointed out that a low number of PRPs is associated with an increased risk of dental caries [101, 102]. On the other hand, different studies have shown that the presence of mucins in patients with caries was significantly higher than in patients without this pathology [103]. Regarding other proteins (i.e., agglutinins, amylase, lactoferrin, and lysozyme), the results have been disparate and contradictory. Finally, in relation to salivary antibacterial peptides, there are contradictory results regarding their diagnostic value (i.e., alpha-defensins, cathelicidins, histatins, and staterins) [104, 105].

*2.3. Diseases of the Oral Mucosa.* Table 2 summarizes the use of protein-based techniques for salivary biomarker identification in oral mucosa diseases.

*2.3.1. Recurrent Aphthous Stomatitis.* Recurrent aphthous stomatitis (RAS) is accompanied by recurrent oral ulcerations, commonly called aphthae [106]. Approximately 20% of the general population suffers from RAS [107]. Several

TABLE 2: Use of protein-based techniques for biomarkers identification in oral mucosa diseases.

| Base disease | Number of patients | Age of patients | Matrix | Analytical technique | Determined parameter | Range of concentrations | Endpoints | Reference |
|---|---|---|---|---|---|---|---|---|
| RAS | 30 (10 controls, 20 cases) | M: 35.9 | USWS | ELISA | Cortisol | Control: cortisol (0.64 ± 0.36 mg/dl); case: cortisol (0.57 ± 0.25 mg/dl) | ↑Cortisol in recurrent aphthous stomatitis patients vs. controls | [108] |
| RAS | 68 (34 controls, 34 cases) | M: 23.29 | USWS | ELISA | Cortisol and amylase | Control: cortisol (3.65 ± 2.5 ng/dl); amylase (128.74 ± 86.3 U/ml); case: cortisol (3.35 ± 1.8 ng/dl); amylase (155.09 ± 116.1 U/ml) | No significant differences | [109] |
| RAS | 75 (25 controls, 50 cases) | M: 27.5 | USWS | ELISA | MPO | Control: MPO (21.36 ± 14.73 U g$^{-1}$); case: MPO (19.22 ± 18.97 U g$^{-1}$). | No significant differences | [111] |
| RAS | 62 (30 controls, 32 cases) | AR: 14–46 | Unstimulated parotid saliva | ELISA | Superoxide dismutase, glutathione peroxidase, and catalase | Control: superoxide dismutase (0.56 ± 0.11 U/mg protein); catalase (0.78 ± 0.03 U/mg); glutathione peroxidase (2.88 ± 0.18 U/mg). Case: superoxide dismutase 0.90 ± 0.04 (U/mg); catalase (0.90 ± 0.04 U/mg); glutathione peroxidase (1.70 ± 0.1 U/mg). | ↑Superoxide dismutase, ↑glutathione peroxidase, and ↓catalase in recurrent aphthous stomatitis patients vs. controls | [112] |
| RAS | 52 (26 controls, 26 cases) | AR: 22–64 | USWS | ELISA | IL-6 and TNF-$\alpha$ | Control: IL-6 (9.38 ± 9.23 pg/ml); TNF-$\alpha$ (7.88 ± 8.45 pg/ml); case: IL-6 (12.5 ± 17.51 pg/ml); TNF-$\alpha$ (28 ± 26.19 pg/ml). | ↑IL-6 in recurrent aphthous stomatitis vs. controls | [114] |
| Behçet's disease and RAS | 119 (60 controls and 59 cases (33 Behçet's disease and 16 recurrent aphthous stomatitis)) | AR: 16–45 | USWS | ELISA | Salivary epidermal growth factor | Control: salivary epidermal growth factor (2758.7 ± 81,657.9 pg/ml). Behçet's disease: salivary epidermal growth factor (1939.7 ± 81,561.5 pg/ml). Recurrent aphthous stomatitis: salivary epidermal growth factor (1650.5 ± 8704.7 pg/ml) | ↓Salivary epidermal growth factor in recurrent aphthous stomatitis, and Behçet's disease vs. controls | [115] |

Table 2: Continued.

| Base disease | Number of patients | Age of patients | Matrix | Analytical technique | Determined parameter | Range of concentrations | Endpoints | Reference |
|---|---|---|---|---|---|---|---|---|
| Pemphigus vulgaris | 127 (77 controls, 50 cases) | M: 46.84 | USWS | ELISA | Desmoglein 1 and 3 | Control: all controls were below cut-off value; case: desmoglein 1 (58.25 ± 47.52 index value); desmoglein 3 (144.47 ± 53.42 index value) | ↑Desmoglein 1 and ↑3 in pemphigus vulgaris patients vs. controls | [118] |
| BP | 100 (50 controls, 50 cases) | AR: 38–91 | USWS | ELISA | BP180 NC16a and BP230-C3 | NR | BP180 NC16a useful as diagnostic marker for pemphigoid | [119] |
| Mucous membrane pemphigoid | 114 (50 controls, 50 cases) | AR: 26–87 | USWS and stimulated parotid saliva | ELISA | IgG and IgA | NR | IgA useful as diagnostic marker for pemphigoid | [120] |
| BMS | 60 (30 controls, 30 cases) | M: 63.8 | USWS | ELISA | Cortisol and α-amylase | Control: cortisol (3.69 ± 3.07 ng/ml); amylase (146.22 ± 130.4 IU/l); case: cortisol (4.50 ± 3.68 ng/ml); amylase (351.68 ± 142.5 IU/l) | ↑Cortisol and ↑α-amylase in BMS patients vs controls | [122] |
| BMS [123] | 29 (14 controls, 15 cases) | M: 65.7 | USWS and SWS | ELISA | Cortisol, 17b-estradiol, progesterone, dehydroepiandrosterone, and α-amylase | | * | ↑Cortisol in USWS, and of ↑17b-estradiol in SWS in BMS vs. controls |
| BMS | 270 (90 controls, 180 cases) | AR: 15–88 | USWS | ELISA | Albumin, lysozyme, amylase, IgM, IgG, and IgA | Control: albumin (9.36 ± 3.44 mg/dl); lysozyme (24.03 ± 3.38 mg/ml); amylase (1638.0 ± 372.0 IU/l); IgM (1.02 ± 0.06 mg/dl); IgG (0.79 ± 0.15 mg/dl); IgA (24.34 ± 1.26 mg/dl). Case: albumin (18.04 ± 2.56 mg/dl); lysozyme (28.10 ± 3.48 mg/dl); amylase (3030.0 ± 470.0 IU/l); IgM (2.19 ± 0.68 mg/dl); IgG (4.47 ± 0.76 mg/dl); IgA (33.15 ± 3.53 mg/dl) | ↑Albumin, ↑IgA, ↑IgG, ↑IgM, and ↑lysozyme in BMS patients vs. controls | [124] |

TABLE 2: Continued.

| Base disease | Number of patients | Age of patients | Matrix | Analytical technique | Determined parameter | Range of concentrations | Endpoints | Reference |
|---|---|---|---|---|---|---|---|---|
| BMS | 45 (30 controls, 15 cases) | M: 55.2 | USWS | ELISA | IgA | Control: IgA (164.71 ± 158.80 μg/ml). Case: IgA (176.14 ± 97.23 μg/ml). | No significant differences | [125] |
| BMS | 97 (50 controls, 47 cases (BMS, oral lichen planus, and RAS)) | NR | USWS | ELISA | IgE | Control: IgE (20.6 ± 66.6 mg/dl). Case: IgE (8.07 ± 30.4 mg/dl) | No significant differences | [126] |
| BMS | 38 (19 controls, 19 cases) | NR | USWS | LC-MS/MS validation via ELISA | Total proteins | RQ | ↑Alpha-enolase, ↑IL-18, and ↑KLK13 in BMS vs. controls | [127] |

Abbreviations: AR: age range; BMS: burning mouth syndrome; BP: bullous pemphigoid; ELISA: enzyme-linked immunosorbent assay; Ig: immunoglobulin; IL: interleukin; KLK: kallikrein-related peptidase; LC-MS/MS: liquid chromatography tandem-mass spectrometry; M: mean NR: not reported; MPO: myeloperoxidase; NC: noncollagenous; RAS: recurrent aphthous stomatitis; RQ: relative quantification; SWS: stimulated whole saliva; TNF: tumor necrosis factor; UWSW: unstimulated whole saliva. *for additional data see original source.

reports have investigated the salivary proteome of patients suffering from this pathology. In particular, the most studied molecules have been cortisol, the OE-related peptides, Igs, and certain cytokines.

Different ELISA-based reports have found higher cortisol levels in patients with RAS than healthy controls [108, 109]. It has been hypothesized that these altered levels may be linked to the stress and anxiety present in these patients, establishing a neurobiological basis for this pathology. Total antioxidant capacity (TAC) is not related to the aetiology of this pathology; however, patients with RAS do tend to have altered levels of molecules related to OS [110–112]. Numerous studies have shown that levels of IgA and IgG increase considerably in RAS disease outbreaks [113]. Different inflammatory mediators, especially cytokines, can stimulate the production of MHC class I and II antigens in epithelial cells [106]. These cells trigger a cytotoxic response in T lymphocytes causing ulceration. In relation to this etiopathogenic model, numerous cytokines are found in greater amounts in patients with RAS (i.e., TNF-$\alpha$, PGE2, VEGF, and IL-6) [114–116].

### 2.3.2. Pemphigus and Pemphigoid.
Vesiculobullous disorders are autoimmune-based pathologies characterized by the presence of antibodies against epithelial tissue-specific adhesion molecules. Its prevalence is 0.2 to 3 people out of every 100,000 [117].

Hallaji et al. demonstrated that by ELISA techniques, in the case of pemphigus, salivary desmoglein 1 and desmoglein 3 had sensitivities of 70% and 94%, respectively, in the diagnosis of this dermatological condition [118]. In the case of the pemphigoid, Esmaili et al. proved that the salivary concentration of BP180-NC16a is useful in the diagnosis of this disease [119]. It has also been shown that IgA and IgG salivary are markedly increased during pemphigoid and can be good alternatives in its diagnosis [120].

### 2.3.3. Glossodynia or Burning Mouth Syndrome.
The International Headache Society (IHS) defines burning mouth syndrome (BMS) as an intraoral burning or dysesthetic sensation, which is repeated daily for more than 2 hours/day for more than 3 months, without clinically evident causing lesions. BMS prevalence is barely 4% in the general population but reaches 18%–33% of postmenopausal women [121].

Due to the psychosomatic profile of this aetiology of this disease, stress-related proteins (such as cortisol and $\alpha$-amylase) have been related to its presentation [122, 123]. There are few studies investigating the role of salivary Igs in this pathology, and the existing ones have contradictory results. Regarding cytokine-based investigations, the results are also contradictory for a large number of proteins (i.e., IL-1$\beta$, IL-6, IL-8, and TNF-$\alpha$) [124–126].

Recently, nontargeted proteomic techniques have discovered other novel biomarkers for this pathology. A recent case-control study based on the LC-MS/MS and iTRAQ found 50 altered proteins (39 overexpressed and 11 subexpressed); three of them were validated through ELISA: alpha-enolase, IL-18, and KLK13 [127].

### 2.4. Oral Cancer and Potentially Malignant Oral Lesions.
Table 3 summarizes the use of protein-based techniques for salivary biomarker identification in oral cancer and potentially malignant disorders.

### 2.4.1. Oral Lichen Planus.
Oral lichen planus (OLP) is a relatively common mucocutaneous disorder. OLP is originated through a chronic inflammation triggered by the epithelial cells apoptosis mediated by autocytotoxic T lymphocytes. According to the World Health Organization (WHO), OLP is considered an oral potentially malignant oral disorder (OPMD). There are several prospective long-term studies that show a malignant transformation rate of 1% over a 5-year average period [128]. Despite the progress of molecular biology in recent decades, there is no useful biomarker in assessing the risk of malignant transition of this entity; however, recent research based on salivary proteome analysis may be a step forward. The protein-based biomarkers most widely investigated in relation to the diagnosis of OLP have been cortisol, OS-related molecules, Igs, and cytokines.

In relation to cortisol, numerous investigations have investigated the relationship between psychological status and levels of this hormone in patients with OLP. Some case-control studies suggest that the elevated levels of this glucocorticoid are common among affected individuals [129, 130]. However, some reports do not find significant differences [131] or even find lower cortisol levels in OLP-affected patients [132]. Theoretically, cortisol generates a reduction in the number of lymphocytes and other immune cells and also dysfunctions in the hypothalamus-pituitary-adrenal (HPA) axis which trigger reduction in its production [133]. Lopez-Jornet et al. demonstrated that the levels of adiponectin were higher in OLP patients. In relation to Igs analysed via ELISA, IgA and IgG are considerably increased in patients with OLP compared to controls [129].

OLP aetiology is based on an imbalance between Th1/Th2 lymphocytes. The proinflammatory mediators that justify this imbalance are significantly increased in OLP-affected patients: IL-4, IL-10, IL-18, TNF-$\alpha$, NF-$\kappa$B-related cytokines, CD44, and CD14 [113]. Interestingly, treatment with immunosuppressants such as corticosteroids or nonantibiotic macrolides and alternative therapies such as plant extracts and polyphenols have shown a relevant reduction in these inflammation-based biomarkers [133–135]. It should be noted that no research has yet provided a valid salivary biomarker to predict OLP malignant transformation.

Recently, nontargeted proteomic studies based on MS-based studies have provided new perspectives regarding the aetiology and diagnosis of the OLP [136, 137].

### 2.4.2. Oral Leukoplakia.
Oral leukoplakia (OL) is defined as "a white plaque of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer" [138]. The pooled estimated prevalence rate of OL varies between 1.7 and 2.7% in general population. OL is considered by the WHO as OPMD. Malignant transformation of oral leukoplakia in annual average is 1%. Despite the molecular biology progress to date, there is no certain marker to predict OL malignant transformation.

TABLE 3: Use of protein-based techniques for biomarkers identification in oral cancer and potentially malignant disorders.

| Base disease | Number of patients | Age of patients | Matrix | Analytical technique | Determined parameter | Range of concentrations | Endpoints | Reference |
|---|---|---|---|---|---|---|---|---|
| OLP | 65 (32 controls, 33 cases) | M: 57 | USWS | ELISA | Cortisol, IgA, and adiponectin | Control: IgA (48.9 ± 32.8 mg/l); cortisol (0.4 ± 0.2 μg/dl); adiponectin (20.1 ± 24.9 mg/ml). Case: IgA (80.3 ± 51.3 mg/l); cortisol (0.5 ± 0.3 μg/dl); adiponectin (38.2 ± 63.5 mg/ml) | ↑Cortisol and ↑IgA in OLP patients vs. controls | [129] |
| OLP | 61 (31 controls, 30 cases) | M: 54 | USWS | ELISA | Cortisol | Control: cortisol (5.21 ± 2.54 mg/ml). Case: cortisol (4.67 ± 0.33 mg/ml) | No significant differences | [130] |
| OLP | 62 (31 controls, 31 cases) | M: 30 | USWS | ELISA | Cortisol and dehydroepiandrosterone | Control: cortisol (14.10 [8.60–18–30] nmol/l); dehydroepiandrosterone (0.66 (0.51–1.22) nmol/l); case: cortisol (13.50 (10.50–21–30), nmol/l); dehydroepiandrosterone (0.75 (0.46–0.99) nmol/l) | No significant differences | [131] |
| OLP | 20 (10 controls, 10 cases) | M: 58.1 | Unstimulated parotid saliva | ELISA | Cortisol | * | No significant differences | [132] |
| OLP | 20 (10 controls, 10 cases) | M: 57 | USWS | LC-MS/MS validation via MRM | Total proteins | RQ | ↑S100A8, ↑S100A9, and ↑haptoglobin in OLP vs. controls | [136] |
| OLP | 12 (6 controls, 6 cases) | AR: 23–60 | USWS | 2-DE and MALDI-TOF-MS | Total proteins | RQ | ↑Urinary prokallikrein and ↓PLUNC in OLP vs. controls | [137] |
| OL and OSCC | 88 (31 controls, 29 OL, 28 OSCC) | M: 60.89 | USWS | ELISA | IL-1β, IL-6, and TNF-α | Control: IL-1β (354 ± 61.51 pg/ml); IL-6 (16 ± 3.91 pg/ml); TNF-α (38 ± 3.23 pg/ml). OL: IL-1β (143 ± 54.74 pg/ml); IL-6 (18 ± 5.19 pg/ml); TNF-α (30 ± 3.01 pg/ml). OSCC: IL-1β (906 ± 62.21 pg/ml); IL-6 (129 ± 66.59 pg/ml); TNF-α (34 ± 21.58 pg/ml) | ↑IL-1β and ↑IL-6 in OL and OSCC patients vs. controls | [139] |
| OL and OSCC | 75 (25 controls, 25 OL/oral submucosa fibrosis (OPMDs), 25 oral cancer) | M: 53.2 | USWS | ELISA | IL-8 | Control: IL-8 (210.096 ± 142.302 pg/ml). OL/oral submucosa fibrosis: IL-8 (299.513 ± 158.165 pg/ml). OSCC: IL-8 (1718.610 ± 668.294 pg/ml) | ↑IL-8 in OSCC patients vs. controls and OL | [140] |

TABLE 3: Continued.

| Base disease | Number of patients | Age of patients | Matrix | Analytical technique | Determined parameter | Range of concentrations | Endpoints | Reference |
|---|---|---|---|---|---|---|---|---|
| OL and OSCC | 110 (48 OL, 62 OSCC) | NR | USWS | ELISA | C4d | OL: Cd4 IL-8 (0.04 ± 0.03 $\mu$g/ml$^{-1}$). OSCC: Cd4 (0.07 ± 0.07 $\mu$g/ml$^{-1}$) | ↑C4d in OSCC patients *vs.* OL patients | [141] |
| OL and OSCC | 69 (20 OSCC, 15 OSCC cured, 15 OL, and 20 controls) | AR: 32–89 | USWS | ELISA | Endothelin-1 | NR | No significant differences | [142] |
| OL | 25 (10 controls, 15 cases) | M: 73.8 | USWS | 2-DE and MALDI-TOF-MS | Total proteins | RQ | ↓Apolipoporotein A-1 and ↑cystatin SN precursor in OL patients *vs.* controls | [143] |
| Head and neck cancer | 24 (8 controls, 16 cases) | M: 51.88 | USWS | 2-DE and LC-MS/MS | Total proteins | RQ | ↑Beta fibrin, ↑S100 calcium-binding protein, ↑transferrin, ↑ immunoglobulin heavy chain constant region gamma, ↑coflilin-1 and, ↓transthyretin in Head and neck cancer patients *vs.* controls | [150] |
| OSCC | 38 (19 controls, 19 cases) | M: 66 | USWS | ELISA | Maspin, phosphor Src, CycD1, Ki67, MMP-9, and LDH | * | ↓Maspin, ↓phospho Src, ↑CycD1, ↑Ki67, ↑MMP-9, and ↑LDH in OSCC patients *vs.* controls | [151] |
| OSCC | 53 (26 controls, 27 cases) | M: 53 | USWS | ELISA | ErbB2 and CEA | Control: ErbB2 (4.9 ± 2.1 ng/ml); CEA (22.6 ± 22.1 ng/ml); Case: ErbB2 (5.2 ± 1.8 ng/ml); CEA (42.6 ± 21.1 ng/ml) | ↑CEA in OSCC patients *vs.* controls | [153] |
| OSCC | 54 (25 controls, 29 cases) | M: 61.9 | USWS | LC-MS/MS validation via ELISA | IL-1α, IL-1β, IL-6, IL-8, TNF-α, VEGF, catalase, profilin-1, S100A9, CD59, galectin-3-bindig protein, CD44, thioredoxin, and keratin-19 | * | ↑S100A9 and ↑IL-6 in OSCC patients *vs.* controls | [154] |
| OSCC | 128 (64 controls, 64 cases) | M: 54 | USWS | 2-DE and LC-MS/MS validation via ELISA | Total proteins | RQ | ↑M2BP, ↑MRP14, ↑CD59, ↑catalase, and ↑profilin in OSCC patients *vs.* controls | [156] |
| OSCC | 77 (30 controls, 47 cases) | M: 53.3 | USWS | MALDI-TOF MS combined with magnetic beads validation via ELISA | Total proteins | RQ | ↑24-mer ZNF510 peptide in OSCC *vs.* controls | [156] |
| OSCC | | M: 50.7 | USWS | | Total proteins | RQ | | [157] |

TABLE 3: Continued.

| Base disease | Number of patients | Age of patients | Matrix | Analytical technique | Determined parameter | Range of concentrations | Endpoints | Reference |
|---|---|---|---|---|---|---|---|---|
| | 460 (96 controls, 103 low-risk OPMDs, 130 high-risk OPMDs, and 131 OSCC) | | | LC–MS/MS validation via MRM | | | ↑MMP1, ↑KNG1, ↑ANXA2, and ↑HSPA5 in OSCC patients vs. controls | |
| OSCC | 37 (17 controls, 20 cases) | M: 57 | USWS | LC–MS/MS validation via ELISA | Total proteins | RQ | No significant differences | [158] |

Abbreviations: 2-DE: two-dimensional gel electrophoresis; ANXA: annexin; AR: age range; C4d: complement component 4; CD: cell-surface protein; CEA: carcinoembryonic antigen; CycD1: cyclin D1; ELISA: enzyme-linked immunosorbent assay; erbB-2: human epidermal growth factor receptor 2; HSP: heat shock protein; Ig: immunoglobulin; IL: interleukin; KNG: kininogen; LC-MS/MS: liquid chromatography tandem-mass spectrometry; LDH: lactate dehydrogenase; M2BP: human Mac-2-binding protein; M: mean; MALDI-TOF: matrix-assisted laser desorption/ionization; Maspin: mammary serine protease inhibitor; MMP: matrix metalloproteinases; MRP: migration inhibitory factor-related protein; NR: not reported; OL: oral leucoplakia; OLP: oral lichen planus; OPMD: oral potentially malignant disorder; OSCC: oral squamous cell carcinoma; phosphor-SRC: phosphorylated SRC; PLUNC: palate, lung, and nasal epithelium clone protein; RQ: relative quantification; SWS: stimulated whole saliva; TNF: tumor necrosis alpha; UWSW: unstimulated whole saliva; VEGF: vascular endothelial growth factor; ZNF: zinc finger protein. *for additional data see original source.

Proteomic studies focused on saliva to anticipate this malignancy are scarce, and the study of cytokines is based on ELISA techniques (i.e., IL-6, IL-8, and TNF-$\alpha$) [139, 140]. Other reported proteins that were also useful to discern between OL and oral squamous cell carcinoma have been C4d, MDA, endothelin-1, and lactate dehydrogenase [141, 142]. Camisasca et al. recently reported that in a 2-DE gel-based proteomic study, 22 spots are much more abundant in patients with OL than in controls. One spot corresponded to CK10. Later, the authors validated this marker by means of immunohistochemistry [143].

*2.4.3. Oral Squamous Cell Carcinoma.* Oral squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide. Oral carcinogenesis is modulated by environmental and genetic factors [144]. The most extensively modifiable risk factors for this entity are tobacco and alcohol consumption [145, 146]. In the last 20 years, the study of HPV as a carcinogenic factor has also taken on strength [147]. The most extensively described OSCC-related modifiable risk factors are tobacco and alcohol consumption. In the last 20 years, the study of HPV as a carcinogenic factor has also raised. Despite all efforts on the side of public health and transnational research, a significant improvement in the 5-year survival rate of this neoplasm has not been achieved [144].

In relation to oral diseases, OSCC is by far the one in which proteomics research has employed its greatest efforts. A recent meta-analysis suggested that the use of simple or combined salivary biomarkers for the OSCC may be useful for diagnostic purposes [148]. One of the first family of proteins that aroused interest as OSCC biomarkers was interleukins family; in this sense, there are a large number of studies that ascertained their concentrations in saliva. Specifically, the most studied interleukins have been IL-6, IL-8, IL-1, and TNF-$\alpha$. High levels of these proteins in saliva have been associated with OSCC. The biological plausibility of these high levels is found in the proangiogenic and proinflammatory functions of these analytes [149]. Elevated levels of IgG have also been detected in OSCC-affected patients versus controls, which ascertains the pivotal role of angiogenesis in oral carcinogenesis [150]. On the other hand, by means of ELISA techniques, Shpitzer et al. found that the salivary levels of Ki-67 and Cyclin D1 were also altered in these patients [151]. These findings are compatible with numerous immunohistochemistry reports in OSCC [152]. On the other hand, different investigations mainly based on Western blot, or MRS-based targeted proteomics techniques have found cell-surface glycoproteins overexpressed in patients with OSCC such as CD44, CD59, or CEA. Other biomarkers related to the zinc finger protein family (ZNF) such as ZNF510, Cyfra 21-1, and CK19 have also been reported [153–155]. In this sense, Jou et al. reported a sensitivity and specificity greater than 95% for salivary ZNF510 in the discrimination of tumors in early stages (T1 + T2) vs. advanced stages (T3 + T4) [156].

Nontargeted proteomic techniques have provided other unique proteins or panels useful as oncological markers. Hu et al. reported in a ROC curve analysis that a panel consisting of 5 proteins (M2BP, MRP14, CD59, catalase, and profilin) had a sensitivity of 90% and a specificity of 83% in the diagnosis of the OSCC via LC-MS/MS [155]. A Taiwanese group composed another panel with 4 proteins (MMP1, KNG1, ANXA2, and HSPA5) that able to diagnose OSCC and also to predict OPMDs malignant transformation [157]. Csosz et al. failed to validate some of the biomarkers described by other authors; this Hungarian group justified this fact by the ethnic and geographical variability of the target populations [158].

## 3. Conclusion and Future Perspectives

The advance in the field of salivanomics is a teragnostic revolution in oral pathology. The salivary proteome has a Janus role in oral pathology; oral proteins can provide cytoprotective functions in many of the oral diseases, and, at the same time, they can contribute to inflammation, infection, and even tumorigenesis in this cavity. In this sense, salivary proteome plays a pivotal role in oral homeostasis; imbalances at immunological and nonimmunological salivary defence systems can cause a myriad of possible mechanisms leading to oral pathologies.

Moreover, the salivary proteome is an immense source of useful biomarkers in the diagnosis and prognosis of this burden of diseases. However, the precise mechanisms underlying the role of oral proteins in the initiation and progression of these conditions are still largely unknown. Further research and a standardization of the analytical processes involved in its study are necessary to give a step forward. The study of the salivary proteome will mean an inexorable change in current dentistry.

## Disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Alejandro I. Lorenzo-Pouso and María García-Vence wrote the main body of the paper. Pía López-Jornet, Susana B. Bravo, Javier Carballo, Mario Pérez-Sayáns, Abel García-García, and Manuela Alonso-Sampedro helped in the literature search and correction. All authors have approved the final version of the article.

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# References

[1] E. Kaufman and I. B. Lamster, "The diagnostic applications of saliva—a review," *Critical Reviews in Oral Biology and Medicine*, vol. 13, no. 2, pp. 197–212, 2002.

[2] K. V. Holmberg and M. P. Hoffman, "Anatomy, biogenesis and regeneration of salivary glands," *Monographs in Oral Science*, vol. 24, pp. 1–13, 2014.

[3] S. P. Humphrey and R. T. Williamson, "A review of saliva: normal composition, flow, and function," *The Journal of Prosthetic Dentistry*, vol. 85, no. 2, pp. 162–169, 2001.

[4] I. Lombaert, M. M. Movahednia, C. Adine, and J. N. Ferreira, "Concise review: salivary gland regeneration: therapeutic approaches from stem cells to tissue organoids," *Stem Cells*, vol. 35, no. 1, pp. 97–105, 2017.

[5] P. Zukowski, M. Maciejczyk, and D. Waszkiel, "Sources of free radicals and oxidative stress in the oral cavity," *Archives of Oral Biology*, vol. 92, pp. 8–17, 2018.

[6] M. Battino, M. S. Ferreiro, I. Gallardo, H. N. Newman, and P. Bullon, "The antioxidant capacity of saliva," *Journal of Clinical Periodontology*, vol. 29, no. 3, pp. 189–194, 2002.

[7] I. L. C. Chapple, "Reactive oxygen species and antioxidants in inflammatory diseases," *Journal of Clinical Periodontology*, vol. 24, no. 5, pp. 287–296, 1997.

[8] G. Siravegna, S. Marsoni, S. Siena, and A. Bardelli, "Integrating liquid biopsies into the management of cancer," *Nature Reviews Clinical Oncology*, vol. 14, no. 9, pp. 531–548, 2017.

[9] E. Csosz, G. Kallo, B. Markus, E. Deak, A. Csutak, and J. Tozser, "Quantitative body fluid proteomics in medicine—a focus on minimal invasiveness," *Journal of Proteomics*, vol. 153, pp. 30–43, 2017.

[10] M. Greabu, M. Battino, M. Mohora et al., "Saliva–a diagnostic window to the body, both in health and in disease," *Journal of Medicine and Life*, vol. 2, no. 2, pp. 124–132, 2009.

[11] U. Turpeinen and E. Hamalainen, "Determination of cortisol in serum, saliva and urine," *Best Practice & Research. Clinical Endocrinology & Metabolism*, vol. 27, no. 6, pp. 795–801, 2013.

[12] M. Groschl, "Saliva: a reliable sample matrix in bioanalytics," *Bioanalysis*, vol. 9, no. 8, pp. 655–668, 2017.

[13] K. Aro, F. Wei, D. T. Wong, and M. Tu, "Saliva liquid biopsy for point-of-care applications," *Frontiers in Public Health*, vol. 5, 2017.

[14] K. E. Kaczor-Urbanowicz, C. Martin Carreras-Presas, K. Aro, M. Tu, F. Garcia-Godoy, and D. T. W. Wong, "Saliva diagnostics - current views and directions," *Experimental Biology and Medicine*, vol. 242, no. 5, pp. 459–472, 2016.

[15] F. Amado, M. J. C. Lobo, P. Domingues, J. A. Duarte, and R. Vitorino, "Salivary peptidomics," *Expert Review of Proteomics*, vol. 7, no. 5, pp. 709–721, 2010.

[16] F. M. L. Amado, R. M. P. Vitorino, P. M. D. N. Domingues, M. J. C. Lobo, and J. A. R. Duarte, "Analysis of the human saliva proteome," *Expert Review of Proteomics*, vol. 2, no. 4, pp. 521–539, 2005.

[17] N. Spielmann and D. T. Wong, "Saliva: diagnostics and therapeutic perspectives," *Oral Diseases*, vol. 17, no. 4, pp. 345–354, 2011.

[18] Y. Ogawa, Y. Miura, A. Harazono et al., "Proteomic analysis of two types of exosomes in human whole saliva," *Biological & Pharmaceutical Bulletin*, vol. 34, no. 1, pp. 13–23, 2011.

[19] M. Yáñez-Mó, P. R.-M. Siljander, Z. Andreu et al., "Biological properties of extracellular vesicles and their physiological functions," *Journal of Extracellular Vesicles*, vol. 4, no. 1, article 27066, 2015.

[20] F. Trindade, F. Amado, J. Pinto da Costa et al., "Salivary peptidomic as a tool to disclose new potential antimicrobial peptides," *Journal of Proteomics*, vol. 115, pp. 49–57, 2015.

[21] W. L. Siqueira and C. Dawes, "The salivary proteome: challenges and perspectives," *Proteomics. Clinical Applications*, vol. 5, no. 11-12, pp. 575–579, 2011.

[22] M. Navazesh, "Methods for collecting saliva," *Annals of the New York Academy of Sciences*, vol. 694, pp. 72–77, 1993.

[23] G. B. Proctor and G. H. Carpenter, "Salivary secretion: mechanism and neural regulation," *Monographs in Oral Science*, vol. 24, pp. 14–29, 2014.

[24] K. Inenaga, T. Inangaki, R. Hosokawa, and K. Ono, "Parotid salivary secretion induced by stimulation of periodontal regions with toothbrush in humans," *The Journal of Medical Investigation*, vol. 56, p. 277, 2009.

[25] A. Sproles and L. D. Schaeffer, "An advanced design of the Carlson-Crittenden cup for collection of parotid fluid," *Biomaterials, Medical Devices, and Artificial Organs*, vol. 2, no. 2, pp. 95–101, 1974.

[26] E. L. Truelove, D. Bixler, and A. D. Merritt, "Simplified method for collection of pure submandibular saliva in large volumes," *Journal of Dental Research*, vol. 46, no. 6, pp. 1400–1403, 1967.

[27] P. C. Fox, P. F. van der Ven, B. C. Sonies, J. M. Weiffenbach, and B. J. Baum, "Xerostomia: evaluation of a symptom with increasing significance," *Journal of the American Dental Association*, vol. 110, no. 4, pp. 519–525, 1985.

[28] A. H. Kutscher, I. D. Mandel, E. V. Zegarelli et al., "A technique for collecting the secretion of minor salivary glands: I. Use of capillary tubes," *Journal of Oral Therapeutics and Pharmacology*, vol. 3, no. 5, pp. 391-392, 1967.

[29] B. S. Henson and D. T. Wong, "Collection, storage, and processing of saliva samples for downstream molecular applications," *Methods in Molecular Biology*, vol. 666, pp. 21–30, 2010.

[30] Z. Khurshid, S. Zohaib, S. Najeeb, M. Zafar, P. Slowey, and K. Almas, "Human saliva collection devices for proteomics: an update," *International Journal of Molecular Sciences*, vol. 17, no. 6, 2016.

[31] R. Schipper, A. Loof, J. de Groot, L. Harthoorn, E. Dransfield, and W. van Heerde, "SELDI-TOF-MS of saliva: methodology and pre-treatment effects," *Journal of Chromatography B*, vol. 847, no. 1, pp. 45–53, 2007.

[32] R. Mohamed, J. L. Campbell, J. Cooper-White, G. Dimeski, and C. Punyadeera, "The impact of saliva collection and processing methods on CRP, IgE, and myoglobin immunoassays," *Clinical and Translational Medicine*, vol. 1, no. 1, p. 19, 2012.

[33] S. Chiappin, G. Antonelli, R. Gatti, and E. F. De Palo, "Saliva specimen: a new laboratory tool for diagnostic and basic investigation," *Clinica Chimica Acta*, vol. 383, no. 1-2, pp. 30–40, 2007.

[34] A. Simon-Soro, I. Tomas, R. Cabrera-Rubio, M. D. Catalan, B. Nyvad, and A. Mira, "Microbial geography of the oral cavity," *Journal of Dental Research*, vol. 92, no. 7, pp. 616–621, 2013.

[35] C. Leidhold and W. Voos, "Chaperones and proteases—-guardians of protein integrity in eukaryotic organelles," *Annals of the New York Academy of Sciences*, vol. 1113, no. 1, pp. 72–86, 2007.

[36] Y. C. Hsiao, L. J. Chu, Y. T. Chen et al., "Variability assessment of 90 salivary proteins in intraday and interday samples from healthy donors by multiple reaction monitoring-mass spectrometry," *Proteomics. Clinical Applications*, vol. 12, no. 2, 2018.

[37] R. Aebersold and M. Mann, "Mass spectrometry-based proteomics," *Nature*, vol. 422, no. 6928, pp. 198–207, 2003.

[38] S. Hu, Y. Xie, P. Ramachandran et al., "Large-scale identification of proteins in human salivary proteome by liquid chromatography/mass spectrometry and two-dimensional gel electrophoresis-mass spectrometry," *Proteomics*, vol. 5, no. 6, pp. 1714–1728, 2005.

[39] N. Bostanci, P. Ramberg, A. Wahlander et al., "Label-free quantitative proteomics reveals differentially regulated proteins in experimental gingivitis," *Journal of Proteome Research*, vol. 12, no. 2, pp. 657–678, 2013.

[40] S. Shintani, H. Hamakawa, Y. Ueyama, M. Hatori, and T. Toyoshima, "Identification of a truncated cystatin SA-I as a saliva biomarker for oral squamous cell carcinoma using the SELDI ProteinChip platform," *International Journal of Oral and Maxillofacial Surgery*, vol. 39, no. 1, pp. 68–74, 2010.

[41] W. Yan, R. Apweiler, B. M. Balgley et al., "Systematic comparison of the human saliva and plasma proteomes," *Proteomics. Clinical Applications*, vol. 3, no. 1, pp. 116–134, 2009.

[42] S. Bandhakavi, M. D. Stone, G. Onsongo, S. K. Van Riper, and T. J. Griffin, "A dynamic range compression and three-dimensional peptide fractionation analysis platform expands proteome coverage and the diagnostic potential of whole saliva," *Journal of Proteome Research*, vol. 8, no. 12, pp. 5590–5600, 2009.

[43] M. Zhao, Y. Yang, Z. Guo et al., "A comparative proteomics analysis of five body fluids: plasma, urine, cerebrospinal fluid, amniotic fluid and saliva," *Proteomics-Clinical applications*, no. article 1800008, 2018.

[44] X. Cong, Y. Zhang, Q. H. He et al., "Endothelial tight junctions are opened in cholinergic-evoked salivation in vivo," *Journal of Dental Research*, vol. 96, no. 5, pp. 562–570, 2017.

[45] E. A. Azen, P. A. Goodman, and P. A. Lalley, "Human salivary proline-rich protein genes on chromosome 12," *American Journal of Human Genetics*, vol. 37, no. 2, pp. 418–424, 1985.

[46] E. A. Azen, "Genetic polymorphism of basic proteins from parotid saliva," *Science*, vol. 176, no. 4035, pp. 673-674, 1972.

[47] M. Castagnola, T. Cabras, A. Vitali, M. T. Sanna, and I. Messana, "Biotechnological implications of the salivary proteome," *Trends in Biotechnology*, vol. 29, no. 8, pp. 409–418, 2011.

[48] S. Tsuchida, M. Satoh, H. Umemura et al., "Proteomic analysis of gingival crevicular fluid for discovery of novel periodontal disease markers," *Proteomics*, vol. 12, no. 13, pp. 2190–2202, 2012.

[49] K. T. B. Crosara, D. Zuanazzi, E. B. Moffa, Y. Xiao, M. A. d. A. M. Machado, and W. L. Siqueira, "Revealing the amylase interactome in whole saliva using proteomic approaches," *BioMed Research International*, vol. 2018, Article ID 6346954, 15 pages, 2018.

[50] N. Grassl, N. A. Kulak, G. Pichler et al., "Ultra-deep and quantitative saliva proteome reveals dynamics of the oral microbiome," *Genome Medicine*, vol. 8, no. 1, p. 44, 2016.

[51] S. Hu, Y. Li, J. Wang et al., "Human saliva proteome and transcriptome," *Journal of Dental Research*, vol. 85, no. 12, pp. 1129–1133, 2006.

[52] K. Bao, N. Bostanci, N. Selevsek, T. Thurnheer, and G. N. Belibasakis, "Quantitative proteomics reveal distinct protein regulations caused by Aggregatibacter actinomycetemcomitans within subgingival biofilms," *PLoS One*, vol. 10, no. 3, article e0119222, 2015.

[53] J. Groenink, E. Walgreen-Weterings, K. Nazmi et al., "Salivary lactoferrin and low-Mr mucin MG2 in Actinobacillus actinomycetemcomitans-associated periodontitis," *Journal of Clinical Periodontology*, vol. 26, no. 5, pp. 269–275, 1999.

[54] B. L. Pihlstrom, B. S. Michalowicz, and N. W. Johnson, "Periodontal diseases," *Lancet*, vol. 366, no. 9499, pp. 1809–1820, 2005.

[55] P. I. Eke, B. A. Dye, L. Wei, G. O. Thornton-Evans, R. J. Genco, and CDC Periodontal Disease Surveillance workgroup: James Beck (University of North Carolina, Chapel Hill, USA), Gordon Douglass (Past President, American Academy of Periodontology), Roy Page (University of Washin, "Prevalence of periodontitis in adults in the United States: 2009 and 2010," *Journal of Dental Research*, vol. 91, no. 10, pp. 914–920, 2012.

[56] K. Schenck, D. Poppelsdorf, C. Denis, and T. Tollefsen, "Levels of salivary IgA antibodies reactive with bacteria from dental plaque are associated with susceptibility to experimental gingivitis," *Journal of Clinical Periodontology*, vol. 20, no. 6, pp. 411–417, 1993.

[57] R. Goncalves Lda, M. R. Soares, F. C. Nogueira et al., "Analysis of the salivary proteome in gingivitis patients," *Journal of Periodontal Research*, vol. 46, no. 5, pp. 599–606, 2011.

[58] M. M. Grant, A. J. Creese, G. Barr et al., "Proteomic analysis of a noninvasive human model of acute inflammation and its resolution: the twenty-one day gingivitis model," *Journal of Proteome Research*, vol. 9, no. 9, pp. 4732–4744, 2010.

[59] J. A. Stenken and A. J. Poschenrieder, "Bioanalytical chemistry of cytokines–a review," *Analytica Chimica Acta*, vol. 853, pp. 95–115, 2015.

[60] N. Bostanci, G. Emingil, B. Afacan et al., "Tumor necrosis factor-$\alpha$-converting enzyme (TACE) levels in periodontal diseases," *Journal of Dental Research*, vol. 87, no. 3, pp. 273–277, 2008.

[61] F. D'Aiuto, L. Nibali, M. Parkar, K. Patel, J. Suvan, and N. Donos, "Oxidative stress, systemic inflammation, and severe periodontitis," *Journal of Dental Research*, vol. 89, no. 11, pp. 1241–1246, 2010.

[62] M. Gursoy, U. K. Gursoy, A. Liukkonen, T. Kauko, S. Penkkala, and E. Kononen, "Salivary antimicrobial defensins in pregnancy," *Journal of Clinical Periodontology*, vol. 43, no. 10, pp. 807–815, 2016.

[63] G. Dogusal, B. Afacan, E. Bozkurt, and I. Sonmez, "Gingival crevicular fluid and salivary resistin and tumor necrosis factor-alpha levels in obese children with gingivitis," *Journal of Periodontology*, vol. 89, no. 8, pp. 973–982, 2018.

[64] M. N. Hassan, G. N. Belibasakis, P. Gumus, V. O. Ozturk, G. Emingil, and N. Bostanci, "Annexin-1 as a salivary biomarker for gingivitis during pregnancy," *Journal of Periodontology*, vol. 89, no. 7, pp. 875–882, 2018.

[65] P. Gumus, V. O. Ozturk, E. Bozkurt, and G. Emingil, "Evaluation of the gingival inflammation in pregnancy and postpartum via 25-hydroxy-vitamin D3, prostaglandin E2 and TNF-$\alpha$ levels in saliva," *Archives of Oral Biology*, vol. 63, pp. 1–6, 2016.

[66] A. E. Ulker, O. Tulunoglu, N. Ozmeric, M. Can, and S. Demirtas, "The evaluation of cystatin C, IL-1$\beta$, and TNF-$\alpha$ levels in total saliva and gingival crevicular fluid from 11- to 16-year-old children," *Journal of Periodontology*, vol. 79, no. 5, pp. 854–860, 2008.

[67] M. Berg, D. Y. Burrill, and L. S. Fosdick, "Chemical studies in periodontal disease III: putrefaction of salivary proteins," *Journal of Dental Research*, vol. 25, no. 4, pp. 231–246, 1946.

[68] J. G. Caton, G. Armitage, T. Berglundh et al., "A new classification scheme for periodontal and peri-implant diseases and conditions - introduction and key changes from the 1999 classification," *Journal of Clinical Periodontology*, vol. 45, Supplement 20, pp. S1–S8, 2018.

[69] W. V. Giannobile, T. Beikler, J. S. Kinney, C. A. Ramseier, T. Morelli, and D. T. Wong, "Saliva as a diagnostic tool for periodontal disease: current state and future directions," *Periodontology 2000*, vol. 50, no. 1, pp. 52–64, 2009.

[70] O. A. Olayanju, S. K. Rahamon, I. O. Joseph, and O. G. Arinola, "Salivary immunoglobulin classes in Nigerians with periodontitis," *The Journal of Contemporary Dental Practice*, vol. 13, no. 2, pp. 163–166, 2012.

[71] V. Rangbulla, A. Nirola, M. Gupta, P. Batra, and M. Gupta, "Salivary IgA, interleukin-1$\beta$ and MMP-8 as salivary biomarkers in chronic periodontitis patients," *The Chinese Journal of Dental Research*, vol. 20, no. 1, pp. 43–51, 2017.

[72] C. Schneider, M. Illi, M. Lotscher, M. Wehrli, and S. von Gunten, "Isolation of antibodies from human plasma, saliva, breast milk, and gastrointestinal fluid," *Methods in Molecular Biology*, vol. 1643, pp. 23–31, 2017.

[73] M. Nakayama and N. Ohara, "Molecular mechanisms of Porphyromonas gingivalis-host cell interaction on periodontal diseases," *Japanese Dental Science Review*, vol. 53, no. 4, pp. 134–140, 2017.

[74] Y. Wu, R. Shu, L. J. Luo, L. H. Ge, and Y. F. Xie, "Initial comparison of proteomic profiles of whole unstimulated saliva obtained from generalized aggressive periodontitis patients and healthy control subjects," *Journal of Periodontal Research*, vol. 44, no. 5, pp. 636–644, 2009.

[75] L. Da Rós Gonçalves, M. R. Soares, F. C. S. Nogueira et al., "Comparative proteomic analysis of whole saliva from chronic periodontitis patients," *Journal of Proteomics*, vol. 73, no. 7, pp. 1334–1341, 2010.

[76] H. Range, T. Leger, C. Huchon et al., "Salivary proteome modifications associated with periodontitis in obese patients," *Journal of Clinical Periodontology*, vol. 39, no. 9, pp. 799–806, 2012.

[77] H. H. Chan, Z. H. A. Rahim, K. Jessie, O. H. Hashim, and T. B. Taiyeb-Ali, "Salivary proteins associated with periodontitis in patients with type 2 diabetes mellitus," *International Journal of Molecular Sciences*, vol. 13, no. 4, pp. 4642–4654, 2012.

[78] N. Bostanci, N. Selevsek, W. Wolski et al., "Targeted proteomics guided by label-free quantitative proteome analysis in saliva reveal transition signatures from health to periodontal disease," *Molecular & Cellular Proteomics*, vol. 17, no. 7, pp. 1392–1409, 2018.

[79] K. M. Jaedicke, P. M. Preshaw, and J. J. Taylor, "Salivary cytokines as biomarkers of periodontal diseases," *Periodontology 2000*, vol. 70, no. 1, pp. 164–183, 2016.

[80] C. H. Lee, Y. W. Chen, Y. K. Tu, Y. C. Wu, and P. C. Chang, "The potential of salivary biomarkers for predicting the sensitivity and monitoring the response to nonsurgical periodontal therapy: a preliminary assessment," *Journal of Periodontal Research*, vol. 53, no. 4, pp. 545–554, 2018.

[81] N. Christodoulides, S. Mohanty, C. S. Miller et al., "Application of microchip assay system for the measurement of C-reactive protein in human saliva," *Lab on a Chip*, vol. 5, no. 3, pp. 261–269, 2005.

[82] V. Gohel, J. A. Jones, and C. J. Wehler, "Salivary biomarkers and cardiovascular disease: a systematic review," *Clinical Chemistry and Laboratory Medicine*, vol. 56, no. 9, pp. 1432–1442, 2018.

[83] M. Wilczynska-Borawska, J. Borawski, O. Kovalchuk, L. Chyczewski, and W. Stokowska, "Hepatocyte growth factor in saliva is a potential marker of symptomatic periodontal disease," *Journal of Oral Science*, vol. 48, no. 2, pp. 47–50, 2006.

[84] R. Kaushik, R. K. Yeltiwar, and K. Pushpanshu, "Salivary interleukin-1$\beta$ levels in patients with chronic periodontitis before and after periodontal phase I therapy and healthy controls: a case-control study," *Journal of Periodontology*, vol. 82, no. 9, pp. 1353–1359, 2011.

[85] M. Perez-Sayans, J. M. Somoza-Martin, F. Barros-Angueira, J. M. G. Rey, and A. Garcia-Garcia, "RANK/RANKL/OPG role in distraction osteogenesis," *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, vol. 109, no. 5, pp. 679–686, 2010.

[86] N. Buduneli and D. F. Kinane, "Host-derived diagnostic markers related to soft tissue destruction and bone degradation in periodontitis," *Journal of Clinical Periodontology*, vol. 38, Supplement 11, pp. 85–105, 2011.

[87] T. Sorsa, U. K. Gursoy, S. Nwhator et al., "Analysis of matrix metalloproteinases, especially MMP-8, in gingival crevicular fluid, mouthrinse and saliva for monitoring periodontal diseases," *Periodontology 2000*, vol. 70, no. 1, pp. 142–163, 2016.

[88] C. A. Meschiari, A. M. Marcaccini, B. C. Santos Moura, L. R. Zuardi, J. E. Tanus-Santos, and R. F. Gerlach, "Salivary MMPs, TIMPs, and MPO levels in periodontal disease patients and controls," *Clinica Chimica Acta*, vol. 421, pp. 140–146, 2013.

[89] P. Emecen-Huja, I. Hasan, and C. S. Miller, "Biologic markers of failing implants," *Dental Clinics of North America*, vol. 59, no. 1, pp. 179–194, 2015.

[90] L. J. A. Heitz-Mayfield, "Peri-implant diseases: diagnosis and risk indicators," *Journal of Clinical Periodontology*, vol. 35, Supplement 8, pp. 292–304, 2008.

[91] K. E. Kaczor-Urbanowicz, O. Deutsch, B. Zaks, G. Krief, S. Chaushu, and A. Palmon, "Identification of salivary protein biomarkers for orthodontically induced inflammatory root resorption," *Proteomics Clinical Applications*, vol. 11, no. 9-10, 2017.

[92] M. F. Ellias, S. H. Zainal Ariffin, S. A. Karsani, M. Abdul Rahman, S. Senafi, and R. Megat Abdul Wahab, "Proteomic analysis of saliva identifies potential biomarkers for orthodontic tooth movement," *The Scientific World Journal*, vol. 2012, Article ID 647240, 6 pages, 2012.

[93] N. B. Pitts, D. T. Zero, P. D. Marsh et al., "Dental caries," *Nature Reviews Disease Primers*, vol. 3, article 17030, 2017.

[94] N. J. Kassebaum, E. Bernabe, M. Dahiya, B. Bhandari, C. J. L. Murray, and W. Marcenes, "Global burden of untreated caries: a systematic review and metaregression," *Journal of Dental Research*, vol. 94, no. 5, pp. 650–658, 2015.

[95] R. H. Selwitz, A. I. Ismail, and N. B. Pitts, "Dental caries," *Lancet*, vol. 369, no. 9555, pp. 51–59, 2007.

[96] C. Martins, A. K. Buczynski, L. C. Maia, W. L. Siqueira, and G. F. B. A. Castro, "Salivary proteins as a biomarker for dental caries—a systematic review," *Journal of Dentistry*, vol. 41, no. 1, pp. 2–8, 2013.

[97] X. Gao, S. Jiang, D. Koh, and C. Y. S. Hsu, "Salivary biomarkers for dental caries," *Periodontology 2000*, vol. 70, no. 1, pp. 128–141, 2016.

[98] T. K. da Silva Fidalgo, L. B. Freitas-Fernandes, M. Ammari, C. T. Mattos, I. P. R. de Souza, and L. C. Maia, "The relationship between unspecific s-IgA and dental caries: a systematic review and meta-analysis," *Journal of Dentistry*, vol. 42, no. 11, pp. 1372–1381, 2014.

[99] R. Vitorino, S. de Morais Guedes, R. Ferreira et al., "Two-dimensional electrophoresis study of in vitro pellicle formation and dental caries susceptibility," *European Journal of Oral Sciences*, vol. 114, no. 2, pp. 147–153, 2006.

[100] K. Wang, Y. Wang, X. Wang et al., "Comparative salivary proteomics analysis of children with and without dental caries using the iTRAQ/MRM approach," *Journal of Translational Medicine*, vol. 16, no. 1, p. 11, 2018.

[101] S. Bhalla, S. Tandon, and K. Satyamoorthy, "Salivary proteins and early childhood caries: a gel electrophoretic analysis," *Contemporary Clinical Dentistry*, vol. 1, no. 1, pp. 17–22, 2010.

[102] A. S. Hemadi, R. Huang, Y. Zhou, and J. Zou, "Salivary proteins and microbiota as biomarkers for early childhood caries risk assessment," *International Journal of Oral Science*, vol. 9, no. 11, article e1, 2017.

[103] B. L. Slomiany, J. Piotrowski, A. Czajkowski, and A. Slomiany, "Control of mucin molecular forms expression by salivary protease: differences with caries," *The International Journal of Biochemistry*, vol. 25, no. 5, pp. 681–687, 1993.

[104] C. Tian, X. Sun, X. Liu, X. Huang, F. Chen, and S. Zheng, "Salivary peptidome profiling analysis for occurrence of new carious lesions in patients with severe early childhood caries," *PLoS One*, vol. 12, no. 8, article e0182712, 2017.

[105] X. Sun, X. Huang, X. Tan et al., "Salivary peptidome profiling for diagnosis of severe early childhood caries," *Journal of Translational Medicine*, vol. 14, no. 1, p. 240, 2016.

[106] S. R. Porter, C. Scully, and A. Pedersen, "Recurrent aphthous stomatitis," *Critical Reviews in Oral Biology and Medicine*, vol. 9, no. 3, pp. 306–321, 1998.

[107] I. I. Ship, "Epidemiologic aspects of recurrent aphthous ulcerations," *Oral Surgery, Oral Medicine, and Oral Pathology*, vol. 33, no. 3, pp. 400–406, 1972.

[108] A. Eguia-del Valle, R. Martinez-Conde-Llamosas, J. Lopez-Vicente, A. Uribarri-Etxebarria, and J. M. Aguirre-Urizar, "Salivary cortisol determination in patients from the Basque Country with recurrent aphthous stomatitis. A pilot study," *Medicina Oral Patología Oral y Cirugía Bucal*, vol. 18, pp. e207–e211, 2013.

[109] U. K. Kunikullaya, M. A. Kumar, V. Ananthakrishnan, and G. Jaisri, "Stress as a cause of recurrent aphthous stomatitis and its correlation with salivary stress markers," *The Chinese Journal of Physiology*, vol. 60, no. 4, pp. 226–230, 2017.

[110] S. Tugrul, A. Kocyigit, R. Dogan et al., "Total antioxidant status and oxidative stress in recurrent aphthous stomatitis," *International Journal of Dermatology*, vol. 55, no. 3, pp. e130–e135, 2016.

[111] F. Caglayan, Miloglu, O. Altun, Erel, and A. B. Yilmaz, "Oxidative stress and myeloperoxidase levels in saliva of patients with recurrent aphthous stomatitis," *Oral Diseases*, vol. 14, no. 8, pp. 700–704, 2008.

[112] Y. Karincaoglu, K. Batcioglu, T. Erdem, M. Esrefoglu, and M. Genc, "The levels of plasma and salivary antioxidants in the patient with recurrent aphthous stomatitis," *Journal of Oral Pathology & Medicine*, vol. 34, no. 1, pp. 7–12, 2005.

[113] Y. Hassona and C. Scully, "Salivary changes in oral mucosal diseases," *Periodontology 2000*, vol. 70, no. 1, pp. 111–127, 2016.

[114] V. V. Boras, J. Lukac, V. Brailo, P. Picek, D. Kordic, and I. A. Zilic, "Salivary interleukin-6 and tumor necrosis factor-alpha in patients with recurrent aphthous ulceration," *Journal of Oral Pathology & Medicine*, vol. 35, no. 4, pp. 241–243, 2006.

[115] E. Adisen, A. Aral, C. Aybay, and M. A. Gurer, "Salivary epidermal growth factor levels in Behçet's disease and recurrent aphthous stomatitis," *Dermatology*, vol. 217, no. 3, pp. 235–240, 2008.

[116] K. Chaudhuri, K. K. Nair, and L. Ashok, "Salivary levels of TNF-$\alpha$ in patients with recurrent aphthous stomatitis: a cross-sectional study," *Journal of Dental Research, Dental Clinics, Dental Prospects*, vol. 12, no. 1, pp. 45–48, 2018.

[117] H. C. Nousari and G. J. Anhalt, "Pemphigus and bullous pemphigoid," *The Lancet*, vol. 354, no. 9179, pp. 667–672, 1999.

[118] Z. Hallaji, H. Mortazavi, V. Lajevardi et al., "Serum and salivary desmoglein 1 and 3 enzyme-linked immunosorbent assay in pemphigus vulgaris: correlation with phenotype and severity," *Journal of the European Academy of Dermatology and Venereology*, vol. 24, no. 3, pp. 275–280, 2010.

[119] N. Esmaili, H. Mortazavi, K. Kamyab-Hesari et al., "Diagnostic accuracy of BP180 NC16a and BP230-C3 ELISA in serum and saliva of patients with bullous pemphigoid," *Clinical and Experimental Dermatology*, vol. 40, no. 3, pp. 324–330, 2015.

[120] S. Ali, C. Kelly, S. J. Challacombe et al., "Salivary IgA and IgG antibodies to bullous pemphigoid 180 noncollagenous domain 16a as diagnostic biomarkers in mucous membrane pemphigoid," *The British Journal of Dermatology*, vol. 174, no. 5, pp. 1022–1029, 2016.

[121] "Headache Classification Committee of the International Headache Society (IHS). The International Classification of Headache Disorders, 3rd edition," *Cephalalgia*, vol. 38, no. 1, pp. 1–211, 2018.

[122] T. Nosratzehi, S. Salimi, and A. Parvaee, "Comparison of salivary cortisol and $\alpha$-amylase levels and psychological profiles in patients with burning mouth syndrome," *Special Care in Dentistry*, vol. 37, no. 3, pp. 120–125, 2017.

[123] H. I. Kim, Y. Y. Kim, J. Y. Chang, J. Y. Ko, and H. S. Kho, "Salivary cortisol, 17β-estradiol, progesterone, dehydroepiandrosterone, and α-amylase in patients with burning mouth syndrome," *Oral Diseases*, vol. 18, no. 6, pp. 613–620, 2012.

[124] O. Hershkovich and R. M. Nagler, "Biochemical analysis of saliva and taste acuity evaluation in patients with burning mouth syndrome, xerostomia and/or gustatory disturbances," *Archives of Oral Biology*, vol. 49, no. 7, pp. 515–522, 2004.

[125] H. Imura, M. Shimada, Y. Yamazaki, and K. Sugimoto, "Characteristic changes of saliva and taste in burning mouth syndrome patients," *Journal of Oral Pathology & Medicine*, vol. 45, no. 3, pp. 231–236, 2016.

[126] G. Campisi and C. Di Liberto, "Role of total IgE in unspecified burning oral symptoms. Serum and salivary comparative levels in a case-control study," *Minerva Stomatologica*, vol. 52, no. 7-8, pp. 381–391, 2003.

[127] E. H. Ji, C. Diep, T. Liu et al., "Potential protein biomarkers for burning mouth syndrome discovered by quantitative proteomics," *Molecular Pain*, vol. 13, 2016.

[128] G. Lodi, M. Carrozzo, S. Furness, and K. Thongprasom, "Interventions for treating oral lichen planus: a systematic review," *The British Journal of Dermatology*, vol. 166, no. 5, pp. 938–947, 2012.

[129] P. Lopez-Jornet, C. A. Cayuela, A. Tvarijonaviciute, F. Parra-Perez, D. Escribano, and J. Ceron, "Oral lichen planus: salival biomarkers cortisol, immunoglobulin A, adiponectin," *Journal of Oral Pathology & Medicine*, vol. 45, no. 3, pp. 211–217, 2016.

[130] A. Mansourian, S. Najafi, N. Nojoumi, P. Parhami, and M. S. Moosavi, "Salivary cortisol and salivary flow rate in clinical types of oral lichen planus," *Skinmed*, vol. 16, no. 1, pp. 19–22, 2018.

[131] C. Girardi, C. Luz, K. Cherubini, M. A. Z. . Figueiredo, M. L. T. Nunes, and F. G. Salum, "Salivary cortisol and dehydroepiandrosterone (DHEA) levels, psychological factors in patients with oral lichen planus," *Archives of Oral Biology*, vol. 56, no. 9, pp. 864–868, 2011.

[132] P. O. Rodstrom, M. Jontell, M. Hakeberg, U. Berggren, and G. Lindstedt, "Erosive oral lichen planus and salivary cortisol," *Journal of Oral Pathology & Medicine*, vol. 30, no. 5, pp. 257–263, 2001.

[133] M. A. Gonzalez-Moles and C. Scully, "HPA-suppressive effects of aqueous clobetasol propionate in the treatment of patients with oral lichen planus," *Journal of the European Academy of Dermatology and Venereology*, vol. 24, no. 9, pp. 1055–1059, 2010.

[134] E. M. Varoni, G. Lodi, A. Sardella, A. Carrassi, and M. Iriti, "Plant polyphenols and oral health: old phytochemicals for new fields," *Current Medicinal Chemistry*, vol. 19, no. 11, pp. 1706–1720, 2012.

[135] P. Lopez Jornet and C. Aznar-Cayuela, "Efficacy of topical chamomile management vs. placebo in patients with oral lichen planus: a randomized double-blind study," *Journal of the European Academy of Dermatology and Venereology*, vol. 30, no. 10, pp. 1783–1786, 2016.

[136] M. M. Souza, G. P. Florezi, M. Nico, F. de Paula, F. M. Paula, and S. V. Lourenco, "Salivary proteomics in lichen planus: a relationship with pathogenesis?," *Oral Diseases*, vol. 24, no. 5, pp. 784–792, 2018.

[137] L.-L. Yang, X.-Q. Liu, W. Liu, B. Cheng, and M.-T. Li, "Comparative analysis of whole saliva proteomes for the screening of biomarkers for oral lichen planus," *Inflammation Research*, vol. 55, no. 10, pp. 405–407, 2006.

[138] S. Warnakulasuriya, N. W. Johnson, and I. van der Waal, "Nomenclature and classification of potentially malignant disorders of the oral mucosa," *Journal of Oral Pathology & Medicine*, vol. 36, no. 10, pp. 575–580, 2007.

[139] V. Brailo, V. Vucicevic-Boras, J. Lukac et al., "Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer," *Medicina Oral Patología Oral y Cirugia Bucal*, vol. 17, pp. e10–e15, 2012.

[140] S. R. Punyani and R. S. Sathawane, "Salivary level of interleukin-8 in oral precancer and oral squamous cell carcinoma," *Clinical Oral Investigations*, vol. 17, no. 2, pp. 517–524, 2013.

[141] D. Ajona, M. J. Pajares, M. D. Chiara et al., "Complement activation product C4d in oral and oropharyngeal squamous cell carcinoma," *Oral Diseases*, vol. 21, no. 7, pp. 899–904, 2015.

[142] R. R. Hoffmann, L. S. Yurgel, and M. M. Campos, "Evaluation of salivary endothelin-1 levels in oral squamous cell carcinoma and oral leukoplakia," *Regulatory Peptides*, vol. 166, no. 1-3, pp. 55–58, 2011.

[143] D. R. Camisasca, L. da Rós Gonçalves, M. R. Soares et al., "A proteomic approach to compare saliva from individuals with and without oral leukoplakia," *Journal of Proteomics*, vol. 151, pp. 43–52, 2017.

[144] S. Warnakulasuriya, "Global epidemiology of oral and oropharyngeal cancer," *Oral Oncology*, vol. 45, no. 4-5, pp. 309–316, 2009.

[145] Y. C. Ko, Y. L. Huang, C. H. Lee, M. J. Chen, L. M. Lin, and C. C. Tsai, "Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan," *Journal of Oral Pathology & Medicine*, vol. 24, no. 10, pp. 450–453, 1995.

[146] N. E. Allen, V. Beral, D. Casabonne et al., "Moderate alcohol intake and cancer incidence in women," *Journal of the National Cancer Institute*, vol. 101, no. 5, pp. 296–305, 2009.

[147] R. Herrero, X. Castellsagué, M. Pawlita et al., "Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study," *Journal of the National Cancer Institute*, vol. 95, no. 23, pp. 1772–1783, 2003.

[148] E. N. S. Guerra, A. C. Acevedo, A. F. Leite, D. Gozal, H. Chardin, and G. de Luca Canto, "Diagnostic capability of salivary biomarkers in the assessment of head and neck cancer: a systematic review and meta-analysis," *Oral Oncology*, vol. 51, no. 9, pp. 805–818, 2015.

[149] F. Balkwill and A. Mantovani, "Inflammation and cancer: back to Virchow?," *The Lancet*, vol. 357, no. 9255, pp. 539–545, 2001.

[150] P. Dowling, R. Wormald, P. Meleady, M. Henry, A. Curran, and M. Clynes, "Analysis of the saliva proteome from patients with head and neck squamous cell carcinoma reveals differences in abundance levels of proteins associated with tumour progression and metastasis," *Journal of Proteomics*, vol. 71, no. 2, pp. 168–175, 2008.

[151] T. Shpitzer, Y. Hamzany, G. Bahar et al., "Salivary analysis of oral cancer biomarkers," *British Journal of Cancer*, vol. 101, no. 7, pp. 1194–1198, 2009.

[152] C. Rivera, A. K. Oliveira, R. A. P. Costa, T. De Rossi, and A. F. Paes Leme, "Prognostic biomarkers in oral squamous cell carcinoma: a systematic review," *Oral Oncology*, vol. 72, pp. 38–47, 2017.

[153] M. Honarmand, L. Farhad-Mollashahi, A. Nakhaee, and M. Nehi, "Salivary levels of ErbB2 and CEA in oral squamous cell carcinoma patients," *Asian Pacific Journal of Cancer Prevention*, vol. 17, Supplement 3, pp. 77–80, 2016.

[154] E. Csosz, P. Labiscsak, G. Kallo et al., "Proteomics investigation of OSCC-specific salivary biomarkers in a Hungarian population highlights the importance of identification of population-tailored biomarkers," *PLoS One*, vol. 12, no. 5, article e0177282, 2017.

[155] S. Hu, M. Arellano, P. Boontheung et al., "Salivary proteomics for oral cancer biomarker discovery," *Clinical Cancer Research*, vol. 14, no. 19, pp. 6246–6252, 2008.

[156] Y. J. Jou, C. D. Lin, C. H. Lai et al., "Salivary zinc finger protein 510 peptide as a novel biomarker for detection of oral squamous cell carcinoma in early stages," *Clinica Chimica Acta*, vol. 412, no. 15-16, pp. 1357–1365, 2011.

[157] J. S. Yu, Y. T. Chen, W. F. Chiang et al., "Saliva protein biomarkers to detect oral squamous cell carcinoma in a high-risk population in Taiwan," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 113, no. 41, pp. 11549–11554, 2016.

[158] E. Csosz, B. Markus, Z. Darula et al., "Salivary proteome profiling of oral squamous cell carcinoma in a Hungarian population," *FEBS Open Bio*, vol. 8, no. 4, pp. 556–569, 2018.

[159] A. Van Nieuw Amerongen, J. G. M. Bolscher, and E. C. I. Veerman, "Salivary proteins: protective and diagnostic value in cariology?," *Caries Research*, vol. 38, no. 3, pp. 247–253, 2004.