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Salivary Analysis

Biomarkers, Clinical Applications,
and Emerging Technologies

*Edited by Juan Aitken-Saavedra, Paulo Braz-Silva
and Gonzalo Rojas-Alcayaga*



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Paulo Braz-Silva and Gonzalo Rojas-Alcayaga*

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Aims and Scope of the Series

This book series will offer a comprehensive overview of recent research trends as well as clinical applications within different specialties of dentistry. Topics will include overviews of the health of the oral cavity, from prevention and care to different treatments for the rehabilitation of problems that may affect the organs and/or tissues present. The different areas of dentistry will be explored, with the aim of disseminating knowledge and providing readers with new tools for the comprehensive treatment of their patients with greater safety and with current techniques. Ongoing issues, recent advances, and future diagnostic approaches and therapeutic strategies will also be discussed. This series of books will focus on various aspects of the properties and results obtained by the various treatments available, whether preventive or curative.

Meet the Series Editor



Dr. Sergio Alexandre Gehrke is a doctorate holder in two fields. The first is a Ph.D. in Cellular and Molecular Biology from the Pontificia Catholic University, Porto Alegre, Brazil, in 2010 and the other is an International Ph.D. in Bioengineering from the Universidad Miguel Hernandez, Elche/Alicante, Spain, obtained in 2020. In 2018, he completed a postdoctoral fellowship in Materials Engineering in the NUCLEMAT of the Pontificia Catholic University, Porto Alegre, Brazil. He is currently the Director of the Postgraduate Program in Implantology of the Bioface/UCAM/PgO (Montevideo, Uruguay), Director of the Cathedra of Biotechnology of the Catholic University of Murcia (Murcia, Spain), an Extraordinary Full Professor of the Catholic University of Murcia (Murcia, Spain) as well as the Director of the private center of research Biotecnos – Technology and Science (Montevideo, Uruguay). Applied biomaterials, cellular and molecular biology, and dental implants are among his research interests. He has published several original papers in renowned journals. In addition, he is also a Collaborating Professor in several Postgraduate programs at different universities all over the world.

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Preface

Saliva is an organic fluid whose properties go beyond its digestive functions or the protection of oral tissues. In addition to these well-known roles, it plays a fundamental part in all oral functions, such as phonation, taste, and swallowing, as well as in local immune defense. It contains a wide range of molecules and ions involved in local homeostatic regulation, while also serving as indicators of overall systemic function. Alterations in either its production, through increased or decreased flow, or its composition have a significant impact on the integrity of oral tissues, oral functions, and, consequently, on health-related quality of life.

Changes in the quantity and quality of saliva have led to the need to design therapeutic strategies to restore its properties or, at the very least, reduce the impact of such alterations on oral tissues. However, the results of these strategies are often partial and variable, representing an ongoing challenge for researchers and clinicians in the search for new therapeutic alternatives.

The impact of saliva alterations on health-related quality of life is critical for individuals experiencing reduced or increased flow, often accompanied by qualitative changes. The importance of this fluid for daily life and overall well-being is evident in how its alterations can promote local infectious processes and lead to changes in personal identity perception. From the patient's perspective, xerostomia and hyposalivation represent a disruption experienced as a life-cycle crisis, forcing individuals to develop adaptive mechanisms to maintain a satisfactory life despite the challenges posed by reduced salivary flow.

The diagnostic potential of saliva for both oral and systemic diseases has been a growing area of research, opening promising perspectives for its use as a disease indicator in both medical and dental fields. In recent years, scientific evidence has increasingly shown that saliva analysis offers a consistent, accessible, potentially cheaper, and non-invasive source of biological information capable of reflecting both systemic and local processes of great clinical relevance. Moreover, it could serve as an indicator of therapeutic response and disease progression.

This volume brings together a collection of chapters that explore, from multiple perspectives, the role of saliva and its components in assessing health and disease. Each contribution provides a complementary perspective on current advances and outstanding challenges in this exciting research field.

The book begins with the systematic review “Salivary Biomarkers in Primary Sjögren's Syndrome: A Systematic Review of Diagnostic and Prognostic Accuracy” which

examines how salivary biomarkers can support the diagnosis and monitoring of this complex autoimmune disease. It is followed by the chapter “Role of Antioxidant and Oxidants in Saliva”, which explores the delicate balance between oxidative and antioxidative species and their impact on both oral and systemic homeostasis. The first section concludes with “Salivary Neuropeptides and Cytokines: Indicators of Stress and Resilience”, which introduces a psychoneuroimmunological dimension to salivary analysis, highlighting its potential as a biomarker for understanding stress and adaptation processes.

The second section focuses on clinical applications and oral diseases. “Xerostomia and Its Impact on Oral Health” and “Hyposalivation and Xerostomia” examine the effects of reduced salivary flow from both symptomatic and pathophysiological perspectives. “Biomarkers in Saliva to Assess the State of Periodontal Disease” highlights the role of salivary biomarkers in identifying and monitoring periodontal disease, one of the most prevalent conditions worldwide. “Salivary Biomarkers for Screening and Monitoring of Oral Diseases” and “Evaluation of Salivary Biomarkers for Early Diagnosis of Oral Potentially Malignant Disorders” reinforce the idea that saliva can become an essential ally for the early detection of oral diseases, including potentially malignant disorders. This section closes with “Salivary Biomarkers in the Diagnosis and Prognosis of Oral Disease”, which offers an integrative perspective on the current and future value of salivary biomarkers in clinical practice.

The final section addresses therapeutic strategies and emerging technologies. “Management and Treatment of Sialorrhea” focuses on therapeutic approaches to control this condition, which affects the quality of life of many patients. “Perspective Chapter: New Technologies in Salivary Gland Surgery” provides an update on technological advances in salivary gland surgery, opening new possibilities for minimally invasive approaches. Finally, “Nanoparticle-Based Biosensors for Salivary Analysis in Dentistry” presents the potential of nanosensors as highly sensitive diagnostic tools for various oral diseases, highlighting the role of technology in the future of salivary analysis.

Together, these contributions reflect not only the diversity of current applications in salivary analysis but also the interdisciplinary richness of a rapidly expanding field. From molecular biology to clinical dentistry, from biosensor technology to neuroscience, each chapter adds an essential piece to the overall picture.

We are confident that this volume will be valuable for both researchers and clinicians, providing an updated framework to guide new investigations and strengthen the use of saliva as a diagnostic fluid in daily practice. We believe it has immense potential.

We want to express our gratitude to all the authors who contributed their knowledge and dedication, as well as to the editorial team who made this work possible. We hope this book will serve as both an inspiration and a practical tool for those seeking to advance the integration of salivary biomarkers in oral and overall health.

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Section 1

Salivary Biomarkers and Biological Foundations

Salivary Biomarkers in Primary Sjögren's Syndrome: A Systematic Review of Diagnostic and Prognostic Accuracy

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Laura Chaparro and Juan Aitken-Saavedra*

Abstract

Sjögren's syndrome (SS) is a systemic autoimmune disease that remains difficult to diagnose and can be classified as primary (pSS) or secondary, depending on its association with other autoimmune conditions. This study aimed to evaluate the accuracy of salivary biomarkers (SBs) in the diagnosis and progression of pSS. A systematic review was conducted between March and August 2023 following PRISMA guidelines. Literature searches were performed in PubMed, Web of Science, and SciELO using the terms "Sjögren's Syndrome" AND "saliva." Studies published in the last 10 years, involving humans and written in Spanish, English, or Portuguese, were included if they assessed the diagnostic or prognostic accuracy of SBs. The risk of bias was evaluated using the ROBINS-E tool. Thirty-five case-control and cohort studies were included; 25 evaluated the diagnostic use of SBs, highlighting markers such as neutrophil gelatinase-associated lipocalin, interleukin-6, and beta-2 microglobulin, all significantly elevated in pSS patients ($p < 0.05$). Eleven studies assessed disease progression, showing associations between higher SB concentrations and longer disease duration, higher focus score (FoSc), and hyposalivation. Epidermal growth factor (EGF) was the most frequently cited biomarker, decreased in advanced disease and related to epithelial integrity, while the S100A8/A9 protein complex was elevated in pSS patients with lymphoma or high FoSc ($p < 0.05$). These findings suggest that SBs associated with inflammation have potential as the complementary diagnostic tools for pSS, though further validation and standardized methodologies are required to confirm their clinical utility and improve patient outcomes.

Keywords: saliva, Sjögren's syndrome, biomarkers, diagnosis, proteomic

1. Introduction

Sjögren's syndrome (SS) is an autoimmune rheumatic disease that primarily affects postmenopausal women [1]. One of its most relevant clinical manifestations is a marked reduction in salivary flow, which negatively impacts patients' quality

of life [2]. Despite its clinical significance, SS remains underdiagnosed and is often detected in advanced stages, when symptoms are already evident [3].

Over the past decades, several diagnostic criteria for primary Sjögren's syndrome (pSS) have been developed, with the most widely accepted being that established by the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) in 2016 [4]. However, the accuracy of these criteria depends on the examiner's expertise and the patient's cooperation, which may limit their applicability. Furthermore, diagnostic tests are often performed late, when the disease is already clinically established [5].

In this context, the search for less invasive and more objective diagnostic tools has gained considerable interest. Recently, saliva has drawn attention as a source of biomarkers due to its multiple advantages: sample collection is simple, non-invasive, inexpensive, and feasible at different times of the day, unlike other biological fluids such as blood. The detection of salivary biomarkers could contribute not only to the early diagnosis of SS but also to the assessment of disease progression and monitoring of therapeutic response. However, the available literature still shows contradictory results, highlighting the need to validate their clinical utility in large cohorts and under standardized protocols.

Considering the impact of SS on quality of life and the limitations of traditional diagnostic criteria, it is a priority to advance in the identification of salivary biomarkers with high diagnostic accuracy and prognostic value. These would allow for improved early detection of pSS and provide more adequate monitoring of its clinical course.

In this regard, the aim of the present study was to evaluate the diagnostic and prognostic accuracy of salivary biomarkers reported in recent literature, in order to determine their usefulness as complementary tools in the diagnosis and monitoring of primary Sjögren's syndrome progression.

2. Materials and methods

This systematic review was conducted following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The study was structured around the research question using the PICO model: Population (patients diagnosed with primary Sjögren's syndrome), intervention or exposure (evaluation of salivary biomarkers), comparison (patients without a diagnosis of primary Sjögren's syndrome), and outcome (effectiveness of salivary biomarkers in the diagnosis and progression of pSS).

An electronic literature search was conducted in Medline (via PubMed), Web of Science, and SciELO databases. The search strategy was built using combinations of key terms such as "Sjogren's Syndrome," "Syndrome, Sjogren's," "Sicca Syndrome," "Syndrome, Sicca," "Saliva," and "Salival," employing the Boolean operators "OR" for synonyms and "AND" to combine different concepts (**Table 1**).

Filters were applied to restrict the search to studies conducted in humans and published within the last 10 years.

During the initial search and selection phase, articles were retrieved from the three databases using the specified terms. Duplicate records were removed with EndNote (version X7). Subsequently, two independent reviewers (M.C.I. and L.C.R.) screened titles and abstracts using Rayyan® – Intelligent Systematic Review,

Database	Algorithm
PubMed	(((((Saliva[MeSH Terms])) OR (“saliva”[MeSH Terms])) AND (sjogrens syndrome[MeSH Terms])) OR (syndrome, sjogren’s[MeSH Terms])) OR (Sjogren Syndrome[MeSH Terms])) OR (sicca syndrome[MeSH Terms])) OR (syndrome, sicca[MeSH Terms])
Scielo	TS = (sjogrens syndrome OR syndrome, sjogren’s OR Sjogren Syndrome OR sicca syndrome OR syndrome, sicca) AND TS = (saliva OR saliva)
WoS	TS = (sjogrens syndrome OR syndrome, sjogren’s OR Sjogren Syndrome OR sicca syndrome OR syndrome, sicca) AND TS = (saliva OR saliva)

Table 1.
Search algorithm used in the databases.

excluding those not related to the objectives of the review. In case of disagreement, consensus was reached through discussion, with a third reviewer (J.A.S.) intervening when necessary for the final decision. Preselected articles were then downloaded for full-text review.

The included studies comprised human research (case-control, cross-sectional, and cohort studies) evaluating salivary biomarkers in adult patients diagnosed with primary Sjögren’s syndrome. Articles published in Spanish, English, and Portuguese were considered. Studies based on animal models, biomarkers extracted from salivary glands through biopsies or cell cultures, narrative reviews, and clinical case reports were excluded.

Critical appraisal was performed by the lead investigator (M.C.I.) together with the reviewers (L.C.R. and J.P.A.). The following aspects were considered: sample size, adequate description of methodology and techniques used, bias control through training and/or calibration, fulfillment of study objectives, scientific relevance, and conflict of interest declaration. To evaluate the risk of bias in the included studies, we employed the Newcastle-Ottawa Scale (NOS), which is specifically designed for assessing the quality of non-randomized, observational studies. Given that the studies included in this review were primarily observational in nature comprising diagnostic and prognostic investigations the NOS provides a structured and validated framework to assess potential biases across three domains: selection of study groups, comparability of groups, and ascertainment of exposure or outcome.

Relevant data were organized in a Microsoft Excel® spreadsheet designed as a data collection tool. Key characteristics of each study were extracted into a table with four columns: (1) first author, year of publication, and country of origin; (2) study type; (3) population characteristics (case and control groups, number of participants, mean age, and female proportion); and (4) type(s) of salivary biomarkers or ions studied. Two additional tables were created to group biomarkers according to their diagnostic or disease progression utility.

3. Results

The literature search was conducted between March and August 2023. A total of 1071 records were retrieved from MEDLINE/PubMed, Web of Science, and SciELO databases. These were imported into EndNote (version X7), where 68 duplicate

records were removed. The remaining 1003 articles were screened by title and abstract using the Rayyan® – Intelligent Systematic Review platform. Of these, 110 met the eligibility criteria and were selected for full-text review. After detailed evaluation, 51 articles were included and 84 excluded. In total, 1029 studies were excluded, mainly for not meeting the predefined eligibility criteria (**Figure 1**).

Subsequently, 13 additional articles were excluded due to low quality after methodological assessment using the Robins-E guidelines. Following this rigorous process, a total of 38 studies were included in the final synthesis. All selected studies were observational in humans, mainly case-control (34), with a smaller number of cohort (2) and cross-sectional (2) studies. The included articles were published between 2013 and 2023.

Most studies evaluated salivary biomarkers in patients with primary Sjögren's syndrome (pSS), although four articles did not specify the classification of the syndrome. Control groups varied across studies and included healthy individuals, patients with SICCA syndrome (symptoms present but negative biopsy and autoantibody profile), patients with other connective tissue disorders, general control subjects, and patients with secondary Sjögren's syndrome. Regarding sex distribution, nine articles included only female patients with pSS, while the rest had a majority of female participants; 11 studies did not specify sex distribution. The mean age of patients ranged between 45 and 60 years.

Among the 27 studies evaluating salivary biomarkers for the diagnosis of pSS, a total of 54 biomarkers were identified, 36 of which were found to be elevated in patients compared to control groups. The most frequently reported biomarkers included interleukin 6 (IL-6), neutrophil elastase (ELANE), and beta-2 microglobulin (β 2MG), mentioned in four, five, and four studies, respectively. Four biomarkers

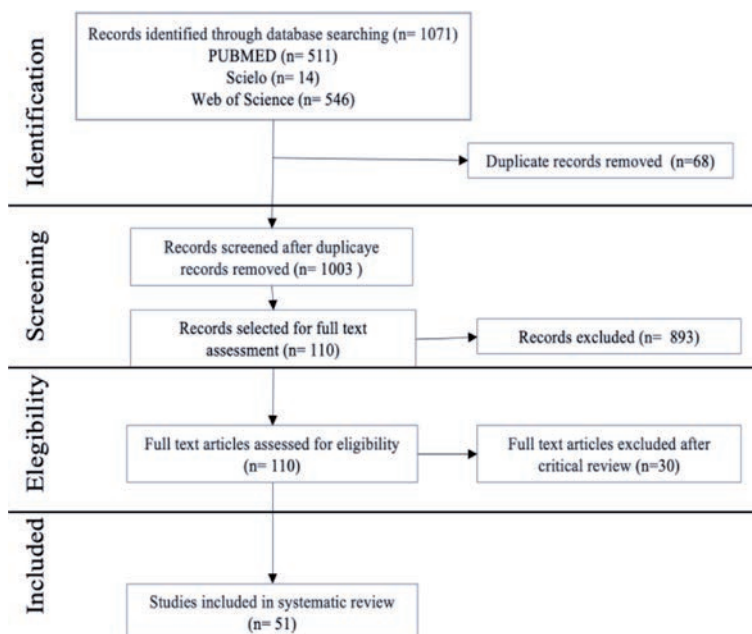


Figure 1. PRISMA flow diagram, presenting the stages of this initial review, from articles identified through database searching to the selection of articles included for the systematic review.

were reported with decreased levels, the most frequent being epidermal growth factor (EGF), cited in three studies. In particular, IL-6 showed significant increases in both saliva and serum of pSS patients compared with controls in multiple studies [6–8], with p-values ranging from 0.012 to 0.0001, and correlated with inflammatory parameters such as erythrocyte sedimentation rate ($r = 0.252$). Interleukin 17A was also significantly elevated in both saliva and serum [6].

β 2MG showed consistent increases across several studies, including significant differences compared with control groups [4, 9–11], and was also reported with diagnostic accuracy metrics, reaching an AUC of up to 0.90, sensitivity of 89%, and specificity of 83% [10]. NGAL was another biomarker frequently elevated in pSS patients [9, 12, 13], with one study highlighting that eight of 11 pSS patients presented increased levels compared to only two of 11 healthy controls. Other proposed biomarkers included TNF- α , with significantly higher levels compared to healthy controls and rheumatoid arthritis patients ([14]; $p < 0.0001$), as well as IgG, which showed relevant differences in one study [3]. In contrast, IgA did not show significant changes ($p > 0.05$). Regarding regulatory proteins, Sembler-Møller et al. [15] reported significant increases in NE, CALR, and TRIM29, whose combined use reached high discriminative capacity between pSS and non-pSS (AUC = 0.97; 95% CI: 0.93–1.00).

With respect to biomarkers with decreased levels, epidermal growth factor (EGF) was the most frequently cited [16], showing significant reductions ($p < 0.05$ and $p = 0.003$, respectively). However, Błochowiak et al. [17] did not find statistically significant differences in either EGF or VEGF.

In total, the pSS groups included 1129 individuals and the control groups 1158, with mean ages ranging from 34 to 62.1 years. In six studies, only women were included in the case groups, while 22 studies included men (always less than 10% of participants), and four studies did not provide this information. The main salivary biomarkers identified as potential diagnostic tools for pSS are summarized in **Table 2**.

In the 11 studies that evaluated the role of salivary biomarkers in monitoring pSS progression, a total of 35 biomarkers were reported, 33 of which showed higher levels in patients compared to controls. The most cited included S100 proteins, specifically S100 A8 and S100 A9, each mentioned in two studies. Epidermal growth factor (EGF) was the most frequently cited biomarker with reduced levels, reported in three studies. These works included a total of 467 pSS patients and 289 controls, with mean ages ranging between 47.5 and 70 years. In three articles, only women were included in the case group, while four included men (less than 10%) and five did not specify sex. Control groups included healthy individuals (without underlying disease), patients with secondary Sjögren's syndrome, patients with SICCA, and individuals with other autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and scleroderma. The salivary biomarkers studied as complementary tools for evaluating pSS progression are detailed in **Table 3**.

Among the most relevant findings in progression, Delaleu et al. [32] reported a significant increase in ELANE ($p < 0.05$), with a direct correlation between this biomarker and the presence of hyposalivation in pSS patients. Consistently, Moreno-Quispe et al. [8] found a correlation between salivary IL-6 levels and stimulated salivary flow rate ($p = 0.02$), in addition to an association with xerostomia ($r = 0.31$; $p = 0.06$).

Regarding EGF, Azuma et al. [16] observed a progressive and significant decrease in this biomarker with advancing disease severity ($p < 0.05$), with initial values of 3652.4 ± 4211.2 pg./10 min dropping to 1640.9 ± 1774.8 pg./10 min at

Author Year Country	Study	Patients						Biological biomarkers or ions		
		Patients			Controls			> High levels	=	< Low levels
		Patients N°	Female (%)	Mean age (years)	Control N°	% female	Mean age (years)			
Jayakanthan et al., 2016. India [18]	CC	43	95.34	40	31	N/S	N/S	L-selectina IL-7		
Agrawi et al., 2017. Norway [12]	CC	27	N/S	52.4	32	N/S	N/S	NGAL PAMP CPNE1		
Benchabane et al., 2016 Algeria [6]	CC	44	90.9	43.9	15	80	41.4	IL-6 IL-17a NO		
Hall et al., 2017. USA [4]	CC	15	N/S	N/S	29	N/S	N/S	β2MG	CAH6 BPIFB2	
Moreno-Quispe et al., 2020 Spain [8]	CC	35	100	56.5	35	100	54.4	IL-6		
Sembler-Møller et al., 2021 Denmark [19]	CC	24	91.6	55	16	67.5	53	TRIM29	Vitronectin CLUS CALR NE	
Jin et al., 2019 China [3]	CC	12	94.1	57.6	24	N/S	N/S	IgG	IgA IgM	
Azuma et al., 2018 Japan [16]	CC	40	92.5	55	23	N/S	N/S			EGF

Author Year Country	Study	Patients						Biological biomarkers or ions		
		Patients			Controls			> High levels	=	< Low levels
		Patients N°	Female (%)	Mean age (years)	Control N°	% female	Mean age (years)			
Yamane et al., 2021 Japan [20]	CC	9	88.9	62.1	7	100	56.7		HNP-1 SPRR2D MPO NE CTSG	
Sembler-Møller et al., 2020 Denmark [15]	CC	24	91.6	55	16	87.5	53	NE CALR TRIM29		
Karabulut et al., 2018 Turkey [14]	CC	43	93	51.1	69	74.3	54.6	TNF- α Caspasa-1	IFN- γ	
Aqrawi et al., 2019 Norway [9]	CC	10	100	55.2	25	100	N/S	MVO NGAL FKBP1A β 2MG SLUR1 CLUS FCN1 ANXA4		
Riega-Torres., 2017 Mexico [11]	T	128	98.9	53.1	64	98.4	45.9	β 2MG		
Azuma et al., 2018 Japan [16]	CC	40	92.5	55.4	23	78.2	56.1			EGF

Author Year Country	Study	Patients						Biological biomarkers or ions		
		Patients			Controls			> High levels	=	< Low levels
		Patients N°	Female (%)	Mean age (years)	Control N°	% female	Mean age (years)			
Sandhya et al., 2017 India [21]	CC	15	86.6	34	15	N/S	34	FLC kappa FLC lambda		
Błochowiak et al., 2018 Poland [17]	CC	36	100	pSS: 28.5 sSS: 56	15	66.6	23		EGF VEGF	
Garreto et al., 2021 Brazil [22]	CC	20	95	pSS: 55.4 sSS: 48.4	20	95	47.7	DPP4/CD26	ELANE CTSG PRTN3	
Chaudhury et al., 2016 Sweden [23]	CC	25	N/S	55	35	N/S	55. 71			MUC5B MUC7
Ohyama et al., 2015 Japan [24]	CC	90	93.3	61.9	86	XND: 83.3 RX: 31.8 Control: 41.6	XND: 69.4 RX: 67.3 Control: 42.4	IL-1beta IL-8 IFN- γ IL-6 IL-12p70 TNF IL-2 IL-4 IL-5 IL-10 IL-17A	IgA	

Author Year Country	Study	Patients						Biological biomarkers or ions		
		Patients			Controls			> High levels	=	< Low levels
		Patients N°	Female (%)	Mean age (years)	Control N°	% female	Mean age (years)			
Hung et al., 2019 Taiwan [7]	CC	138	94.9	56	100	95	55	IL-6	IL-17A TNF- α IgA	
Sandhya et al., 2022 India [25]	T	78	94.9	42.8	138	Healthy: 97.3 Control: 88.7	Healthy: 43.7 Control: 43.9	FLC lambda IgG IgM		
Asashima et al., 2013 Japan [10]	CC	71	97.1	60	179	sSS: 98 non-SS: 79.6 Healthy: 57.3	sSS: 55.8 non-SS: 60 Healthy: 50.7	β 2MG Na+ K+	Cl-	
Kamounah et al., 2023 USA [26]	CC	34	100	54	35	88.6	57.8	SSA/Ro-52		
Choudhury et al., 2023 India [27]	CC	10	100	41.5	42	Healthy: 72 RA: 88	Healthy: 35 RA: 41.8		DMBT1	
Delaleu et al., 2015 Norway [28]	CC	48	100	N/S	24	N/S	N/S	IL-4 IL-5 CLUS		FGF4
Mandal et al., 2018 India [29]	CC	45	N/S	40	42	92.5	35.5		CXCL13	

Author Year Country	Study	Patients						Biological biomarkers or ions		
		Patients			Controls			> High levels	=	< Low levels
		Patients N°	Female (%)	Mean age (years)	Control N°	% female	Mean age (years)			
Agrawi et al., 2020 Norway [13]	CC	25	84.6	N/S	18	100	N/S	NGAL		

CC, Case-control study; pSS, primary Sjögren's syndrome; sSS, secondary Sjögren's syndrome; N/S, not specified; ss/SSc, systemic sclerosis and Sicca; ss/SLE, systemic lupus erythematosus and Sicca; ss/RA, rheumatoid arthritis and Sicca; T, cross-sectional study; SAPHO, SAPHO syndrome; XND, Xerostomia associated with neuropsychiatric disorder or drug; RX, associated with radiation-induced xerostomia; noSS(CTD), connective tissue disease other than Sjögren's syndrome; IL, interleukin; PAMP, adipocyte plasma membrane-associated proteins; CPNE1, copine-1; NO, nitric oxide; CCL26, chemokine ligand 26; MIA, melanoma inhibitory activity; CCL1, chemokine ligand 1; GDF2, growth differentiation factor 2; ULBP2, UL16 binding protein 2; CCL8, chemokine ligand 8; IL2RA, interleukin-2 receptor alpha chain; B2MG, beta-2 microglobulin; CAH6, carbonic anhydrase 6; BPIFB2, BPI fold containing family B member 2; TRIM29, tripartite motif containing 29; CALR, calreticulin; TNF- α , tumor necrosis factor-alpha; IFN- γ , interferon-gamma; NGAL/ LCN2, neutrophil gelatinase-associated lipocalin; FKBP1A, FK506 binding protein 1A; SLUR1, secreted Ly-6/uPAR-related protein 1; CLUS, clusterin; FCN-1, ficolin-1; ANXA4, annexin A4; EGF, epidermal growth factor; FLC kappa, kappa free light chains; FLC lambda, lambda free light chains; VEGF, vascular endothelial growth factor; DPP4/ CD26, dipeptidyl peptidase-4/CD26 serine protease; ELANE, NE, neutrophil elastase; CTSG, cathepsin G; PRTN3, proteinase 3; MUC7, mucin 7; MUC5B, mucin 5B; SSA/ RO-52, Anti-Ro52 antibodies; DMBT1, deleted in malignant brain tumors 1 protein; FGF4, fibroblast growth factor 4; CXCL13, chemokine ligand 13; SPRR2D, small proline-rich protein 2D; MPO, myeloperoxidase; HNP-1, human neutrophil peptide-1; MVO, major vault protein; anti-ENO1, anti-alpha-enolase antibody; Na⁺, sodium; Cl⁻, chloride; K⁺, potassium.

Table 2.

Characterization of studies included in the systematic review and type of salivary biomarker as a diagnostic tool.

Author Year Country	Study	Patients						Biological biomarkers or ions		
		Case			Control			>High levels	=	<Low levels
		Cases N°	% female	Mean Age (years)	Control N°	% female	Mean Age (years)			
Jazzar et al., 2018 England [30]	CC	70	N/S	SS-HR: 51.9 SS-LR: 51.7 SS subgroup: 51.5	SNOX: 14 Healthy: 18	N/S	SNOX: 62.7 Healthy: 50.7	S100A8/A9		
Azuma et al., 2015 Japan [31]	Cohort	23	pSS: 85.7 sSS: 100	pSS: 61,4 sSS: 56.4	SNOX: 14	71.40	59.9			EGF
Delaleu et al., 2016 Switzerland [32]	Cohort	48	N/S	Range 41–52	No control group			ACE ADIPOQ APCS APOH AREG CD40 CEACAM5 CSF1 IL-18 IL-1A IL-1RN ELANE MMP3 MUC16 SOD1 TF TFF3 TGFA TTR		SPP1 UMOD
Chatzis et al., 2021 Greece [33]	CC	45	sSS: 90 SSL: 93.3	sSS: 80 SSL: 70	37	Healthy: 100 Sicca: 81 NHL: 33.3	Healthy: 48 Sicca: 50 NHL: 59.5	CXCL13		
Azuma et al., 2018 Japan [16]	CC	40	pSS: 92.5 sSS: 100	pSS: 55 sSS: 56	23	N/S	N/S			EGF
Lee et al., 2022 Rep. of Korea [34]	CC	70	100	49.5	35	100	47.4			SDC-1

Author Year Country	Study	Patients						Biological biomarkers or ions		
		Case			Control			>High levels	=	<Low levels
		Cases N°	% female	Mean Age (years)	Control N°	% female	Mean Age (years)			
Moreno-Quispe et al., 2020 Spain [8]	CC	36	100	56.5	35	100	54.4	IL-6		
Cecchetti et al., 2019 Italy [35]	CC	20	N/S	pSS High FoSc/ normal flow: 41 SSp Alto FoSc/ low flow: 66 SSp Bajo FoSc/ high flow: 55	20	Same sex	Same age	S100 A2/ A7/ A8/ A9/A11/A12 CTSG		
Martini et al., 2017 Italy [36]	CC	5	N/S	47.5	20	100	same age	Cistatina-S miR-126 miR-335-5p		
Cui et al., 2017 USA [37]	CC	70	N/S	N/S	50	N/S	N/S	CFL1 ENO1 RGI2		
Azuma et al., 2018 Japan [16]	CC	40	92,5	Severe SS: 61,5 Moderate SS: 52,7	23	78,2	56,1			EGF

CC, Case-control study; pSS, primary Sjögren's syndrome; sSS, secondary Sjögren's syndrome; N/S, not specified; SS-M, primary Sjögren's syndrome patients diagnosed with MALT lymphoma; SS-HR, primary Sjögren's syndrome patients with high risk of developing MALT lymphoma; SS-LR, primary Sjögren's syndrome patients with low risk of developing MALT lymphoma; SS sub-group, primary Sjögren's syndrome patients diagnosed without MALT lymphoma risk; SNOX, patients with autoimmune disease other than pSS; SGF, salivary gland function; FoSc, focus score; SSL, Sjögren's syndrome associated with non-Hodgkin lymphoma; NHL, non-Hodgkin lymphoma; Sicca, Sicca symptoms/group; non-SS, does not have Sjögren's syndrome / control without Sjögren's syndrome; S100/A8, calgranulin A; S100/A9, calgranulin B; EGF, epidermal growth factor; ACE, angiotensin-converting enzyme; ADIPOQ, adiponectin; APCS, serum amyloid P-component; APOH, apolipoprotein H; AREG, amphiregulin; CD40, CD40 receptor; CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; CSF1, colony stimulating factor 1; IL-18, interleukin 18; IL-1^a, interleukin 1^a; IL-1RN, interleukin 1RN; NGAL, Neutrophil gelatinase-associated lipocalin; MMP3, matrix metalloproteinase 3; MUC16, mucin 16; SOD1, superoxide dismutase 1; TF, transferrin; TFF3, trefoil factor 3; TGFA, transforming growth factor alpha; TTR, transthyretin; CXCL13, chemokine ligand 13; SDC-1, syndecan 1; IL-6, interleukin 6; S100/A2/A7, psoriasina; miR-126, microRNA-126; miR-335-5p, microRNA-335-5p; CFL1, cofilin-1; ENO1, alpha-enolase 1; RGI2, Rho GDP dissociation inhibitor 2; CTSG, cathepsin G.

Table 3.

Characterization of studies included in the systematic review and identification of salivary biomarkers for the progression of pSS.

reevaluation. Previously, Azuma et al. [16] had reported that EGF reduction correlated significantly with low scores on the OHIP-14 questionnaire ($r = -0.847$; $p = 0.008$), reflecting a direct impact on oral health-related quality of life in pSS patients.

Throughout the systematic review, certain salivary biomarkers were more frequently reported across studies. **Figure 2** presents a classification of these biomarkers according to their effectiveness in diagnosis or disease progression. Among the most cited was IL-17A, elevated in two studies. NGAL levels were reported as increased both in diagnosis (three studies) and progression (one study), while IL-6 levels were elevated in both contexts (five studies). IgA was not significantly changed (three studies). ELANE/NE and TNF- α levels were found elevated in two studies, although three studies did not report significant differences in ELANE/NE concentrations. CTSG was evaluated for diagnostic utility, with one study reporting increased levels and another no changes; one study also reported increased CTSG in disease progression. β 2MG was assessed exclusively for diagnostic value, with four studies reporting significantly elevated levels in pSS patients. Finally, the only biomarker consistently reported as decreased was EGF, both in diagnosis (two studies) and in progression (three studies). A quantitative synthesis (meta-analysis) was not performed due to the substantial methodological heterogeneity across the included studies.

As shown in **Figure 3**, the included studies were evaluated separately according to their design. Diagnostic studies generally showed a low to high risk of bias, with most concerns related to comparability and control of confounding factors. In contrast, progression studies (**Figure 4**) exhibited a range, from low to moderate, particularly in the domains of selection of participants and outcome measurement. These findings indicate that while the overall quality of evidence for diagnostic studies is acceptable, results from diagnosis studies with high risk of bias should be interpreted with caution.

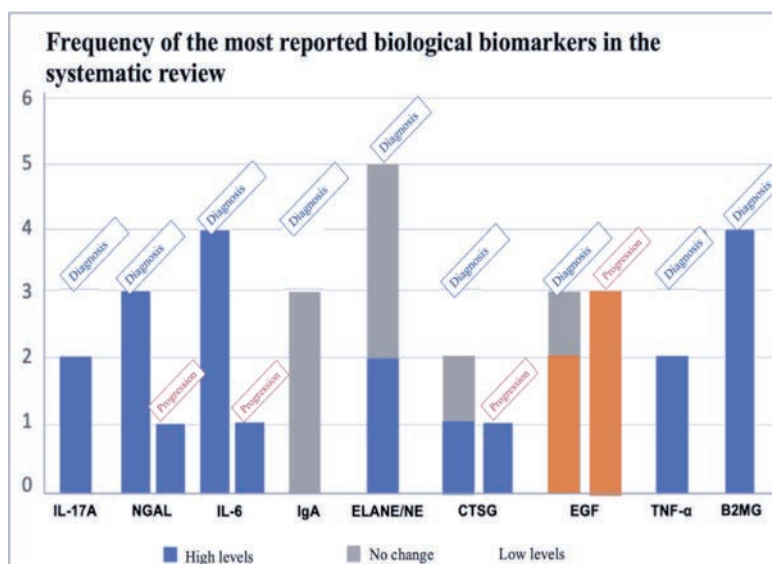


Figure 2.
Frequency of the most reported biological biomarkers in the systematic review.

	Risk of bias			
	D1	D2	D3	Overall
Jayakanthan et al. [10]				
Aqrawi et al. [18]				
Benchabane et al. [9]				
Hall et al. [11]				
Moreno-Quispe et al. [16]				
Sembler-Møller et al. [19]				
Jin et al. [20]				
Azuma et al., 2014				
Yamane et al. [21]				
Sembler-Møller et al. [22]				
Karabulut et al. [23]				
Aqrawi et al. [5]				
Riega-Torres [24]				
Azuma et al. [4]				
Sandhya et al., 2017				
Błochowiak et al. [25]				
Garreto et al. [26]				
Chaudhury et al. [27]				
Ohyama et al. [3]				
Hung et al. [28]				
Sandhya et al. [29]				
Asashima et al. [17]				
Kamounah et al. [30]				
Choudhury et al. [1]				
Delaleu et al. [7]				
Mandal et al. [31]				
Aqrawi et al. [32]				

D1: Selection
D2: Comparability
D3: Outcome

Judgement
 High
 Unclear
 Low

Figure 3.
Risk of bias in diagnostic studies assessed using the Newcastle-Ottawa scale (NOS).

Study	Risk of bias			
	D1	D2	D3	Overall
Jazzar et al. [2]				
Azuma et al. [33]				
Delaleu et al. [34]				
Chatzis et al. [35]				
Azuma et al., 2014				
Lee et al. [15]				
Moreno-Quispe et al. [16]				
Cecchetti et al. [14]				
Martini et al. [36]				
Cui et al. [37]				
Azuma et al. [4]				

D1: Selection
D2: Comparability
D3: Outcome

Judgement
 High
 Unclear
 Low

Figure 4.
Risk of bias in progression studies assessed using the Newcastle-Ottawa scale (NOS).

4. Discussion

Primary Sjögren's syndrome (pSS) is a chronic autoimmune disease that primarily affects the exocrine glands, with a significant impact on patients' quality of life. Early identification is crucial to prevent complications, which justifies the search for complementary diagnostic tools. In this context, salivary biomarkers (SBs) have attracted considerable interest due to the accessibility, low cost, and non-invasive nature of saliva [38].

Although pSS has an autoimmune origin, its etiology is multifactorial, involving genetic factors, innate and adaptive immune responses, and the influence of sex hormones, among others [39]. The latter is particularly relevant, as most of the studies included focused on women in their fourth and fifth decades of life. Estrogen may protect glandular cells while also promoting autoantibody production even after menopause. This contributes to the accumulation of immune complexes, activation of inflammasomes, increased interferons (IFNs), and consequently, glandular dysfunction [39]. Such hormonal differences partly explain the higher prevalence of the syndrome in women, as also reflected in the analyzed populations.

Inflammatory damage in salivary glands induced by pSS can be detected through SBs. One of the most notable is β 2-microglobulin (β 2MG), regulated by IFN and associated with mononuclear cell infiltration, reflecting disease activity [10]. Several studies report significantly elevated β 2MG levels in pSS patients, reinforcing its diagnostic value [4, 9, 11]. Cytokines have also been widely studied, and pSS progression

is commonly described in three phases: initial infiltration of CD4⁺ Th cells and B cells into salivary glands, extraglandular infiltration with pseudolymphomas, and eventual evolution to B-cell lymphoma [24]. Th1 and Th17 cytokines are associated with inflammation, while Th2 cytokines are involved in B-cell activation and immunoglobulin production. Interleukin-6 (IL-6) has been reported at elevated levels in pSS patients [7]; however, its overexpression in saliva does not appear to be exclusive to this disease, limiting its specificity.

Another relevant proinflammatory marker is TNF- α , implicated in glandular cell apoptosis and in the activation of metalloproteinases such as MMP-9, contributing to tissue damage [7]. It also facilitates the formation of nuclear autoantigens [40], and its elevation in saliva during early stages suggests a key role in innate immunity and disease onset [14, 40].

Epidermal growth factor (EGF), synthesized in salivary glands, contributes to oral mucosal integrity and tissue repair. Its reduction in saliva has been associated with inflammatory conditions [41], and in pSS patients, sustained depletion compromises tissue regeneration [31]. This suggests that EGF could be useful for both diagnosis and monitoring of progression, although available evidence remains heterogeneous and limited in sample size.

Other biomarkers such as neutrophil gelatinase-associated lipocalin (NGAL), associated with the histological focus score (FoSc), reinforce the concept of active inflammation in early stages of the disease [13]. Proteases such as ELANE and CTSG have also been investigated, being implicated in extracellular matrix degradation and the regulation of neutrophil migration [20, 42]. Overexpression of the S100A8/A9 complex has also been observed in the saliva of pSS patients, especially in those with lymphoma or high FoSc, suggesting its potential value in identifying patient subgroups and monitoring progression [35], although studies with larger sample sizes are still needed.

A central challenge is the marked heterogeneity of the studies, both in patient characteristics (healthy controls vs. other autoimmune diseases), pharmacological treatments, and saliva collection methodologies. The difference between stimulated and unstimulated saliva also affects the results; some studies suggest that stimulated saliva provides a more consistent proteomic profile, which is particularly relevant in pSS patients who frequently experience hyposalivation [43, 44]. These methodological discrepancies prevented the performance of a meta-analysis, leading instead to a narrative analysis. It should also be noted that the protocol was not registered in PROSPERO, which constitutes a methodological limitation of this study.

The stage of the disease may also influence the usefulness of biomarkers, as certain markers appear to be more informative in early phases than in advanced stages [45]. In this regard, SBs could complement traditional serum biomarkers (anti-SSA/Ro, anti-SSB/La, CXCL13) and the ACR/EULAR classification criteria, particularly in seronegative patients or those in early stages. However, most SBs reflect general inflammatory processes rather than pSS-specific mechanisms, which limits their diagnostic specificity.

Overall, the available evidence supports that SBs are more robust as complementary diagnostic tools than as markers for monitoring pSS progression. Clinical implementation could be strengthened by the use of combined biomarker panels, which may improve diagnostic accuracy. To advance their applicability, multicenter studies with larger cohorts, homogeneous inclusion criteria, and standardized methodologies are required, also incorporating the evaluation of stimulated saliva.

In conclusion, salivary biomarkers show potential as complementary diagnostic tools in pSS but still lack sufficient validation for monitoring progression. Future research should focus on validating combined panels, directly comparing SBs with serum biomarkers, and evaluating their integration into diagnostic algorithms capable of achieving sensitivities above 90%.

5. Conclusions

Salivary biomarkers (SBs) associated with the inflammatory process of primary Sjögren's syndrome (pSS) show potential as complementary diagnostic tools, but their clinical utility remains to be validated. Future research should focus on multi-center studies with larger sample sizes, standardized saliva collection protocols, and robust statistical analyses of diagnostic accuracy (sensitivity, specificity, AUC, and likelihood ratios). The evaluation of SB panels may improve diagnostic performance and facilitate their integration into existing diagnostic pathways, ultimately contributing to earlier detection and better patient outcomes.

Conflict of interest

The authors declare no conflict of interest.

Notes/thanks/other declarations

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Chapter 2

Role of Antioxidants and Oxidants in Saliva

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Abstract

This chapter explores the role of salivary antioxidants (AO) and oxidants as biomarkers in periodontal and systemic health. It delves into the dynamic balance between oxidative stress and antioxidant defense in saliva, highlighting their significance in diagnosing periodontal disease. The chapter discusses key salivary markers, including reactive oxygen species (ROS) and enzymatic/nonenzymatic antioxidants, and their correlation with periodontal inflammation, tissue destruction, and healing. Additionally, it examines their potential to reflect systemic conditions like diabetes and cardiovascular diseases. By emphasizing noninvasive diagnostic strategies, this chapter underscores the emerging importance of salivary biomarkers in personalized medicine and early disease detection. It also gives reference to some studies done by the author themselves on this subject.

Keywords: antioxidants, oxidants, oxidant stress, biomarker, saliva

1. Introduction

Oxidants, also known as reactive oxygen species (ROS), are produced in human body cells as part of the routine biochemical pathway occurring in our bodies. They play an important role in communicating with cells by acting as signaling molecules. Their overproduction can cause damage to the cells and tissues, and to counteract this, antioxidants (AO) are also present naturally. These prevent or delay oxidation of substances. In case the balance between ROS and AO gets disrupted and goes in favor of ROS, it results in oxidant stress (OS). Many chronic inflammatory diseases have OS as a common factor in the pathogenesis of their disease process. Some of them include diabetes, periodontitis, heart disease, and rheumatoid arthritis [1]. ROS has the ability to damage cells and tissues of the body by acting against the basic building blocks of the body, such as DNA,

lipids, and proteins [2]. This occurs by a process called peroxidation, or oxidation of the molecules. The effect of OS on the main building blocks, DNA, proteins, and lipids can be understood diagrammatically by **Figures 1–3**, respectively.

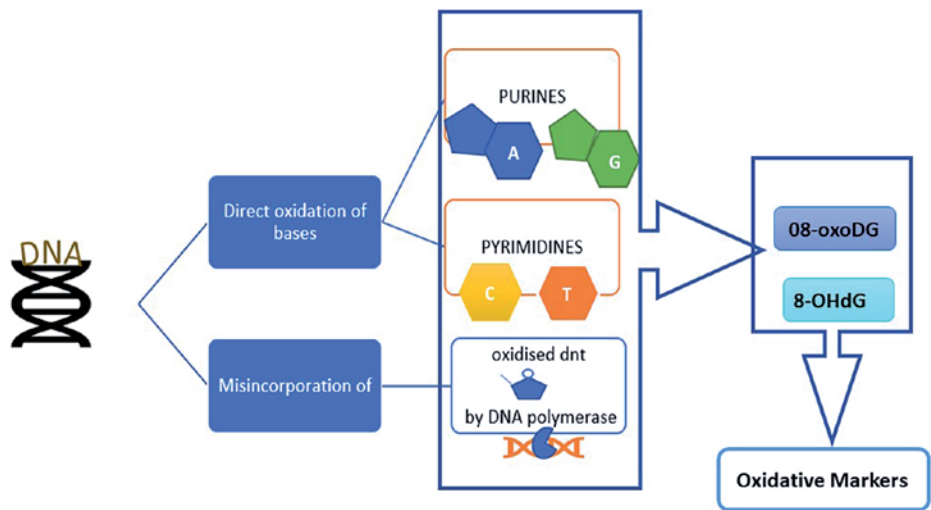


Figure 1. Direct and indirect effect of oxidant stress on DNA by oxidation of purines/pyrimidines and misincorporation of oxidized deoxynucleoside triphosphate (dnt) into DNA by DNA polymerase leading to formation of by products, o8-oxoDG (8-Oxo-2'-deoxyguanosine) and 8-OHdG (8-hydroxy-2'deoxyguanosine) which serve as oxidant stress markers for DNA [3].

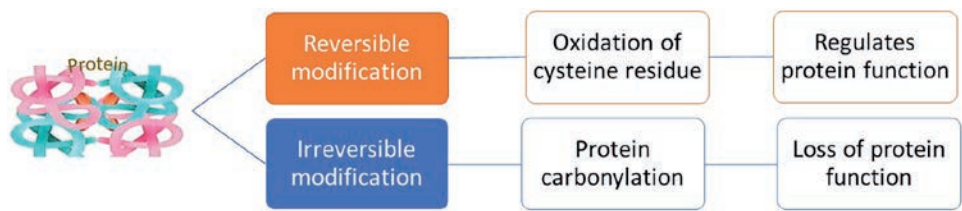


Figure 2. Two methods of modification of proteins by oxidant stress [3].

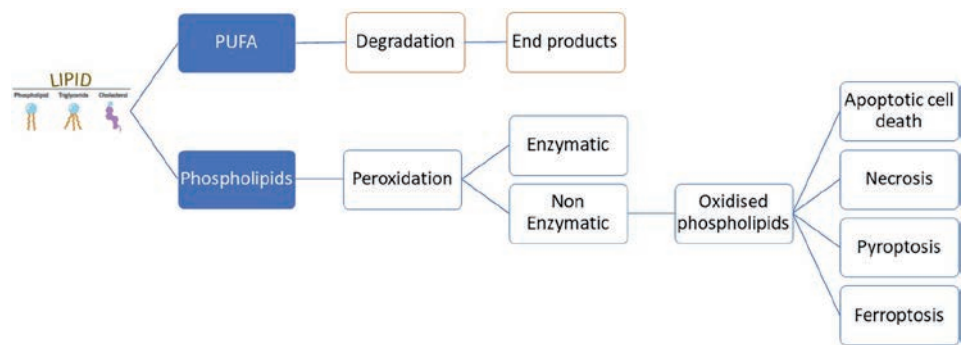


Figure 3. Effect of Ros on varied lipid components such as polyunsaturated fatty acids (PUFA) and phosphor lipids resulting in its degradation and peroxidation respectively [3].

2. Source of oxidants and their generation pathway

The excess ROS (e.g., superoxide anion O_2^- , hydroxyl radicals $OH^\bullet$, hydrogen peroxide H_2O_2) produced in our bodies have the ability to damage lipids, proteins, and DNA. The damages that occur are observed in the form of lipid peroxidation, protein carbonylation, and DNA strand breakage, which result in cellular apoptosis, mitochondrial dysfunction, and tissue degeneration.

Oxidants, or free radicals, are unstable molecules, atoms, or ions owing to the presence of unpaired electrons and ultimately making them highly reactive. To achieve stability, attach or acquire electrons from other molecules, which in turn leads to the oxidation of the molecule. This process itself generates a free radical, making it a continuous process called a chain reaction. The detailed mechanism is shown in **Figure 4**. Free radicals include ROS and reactive nitrogen species (RNS).

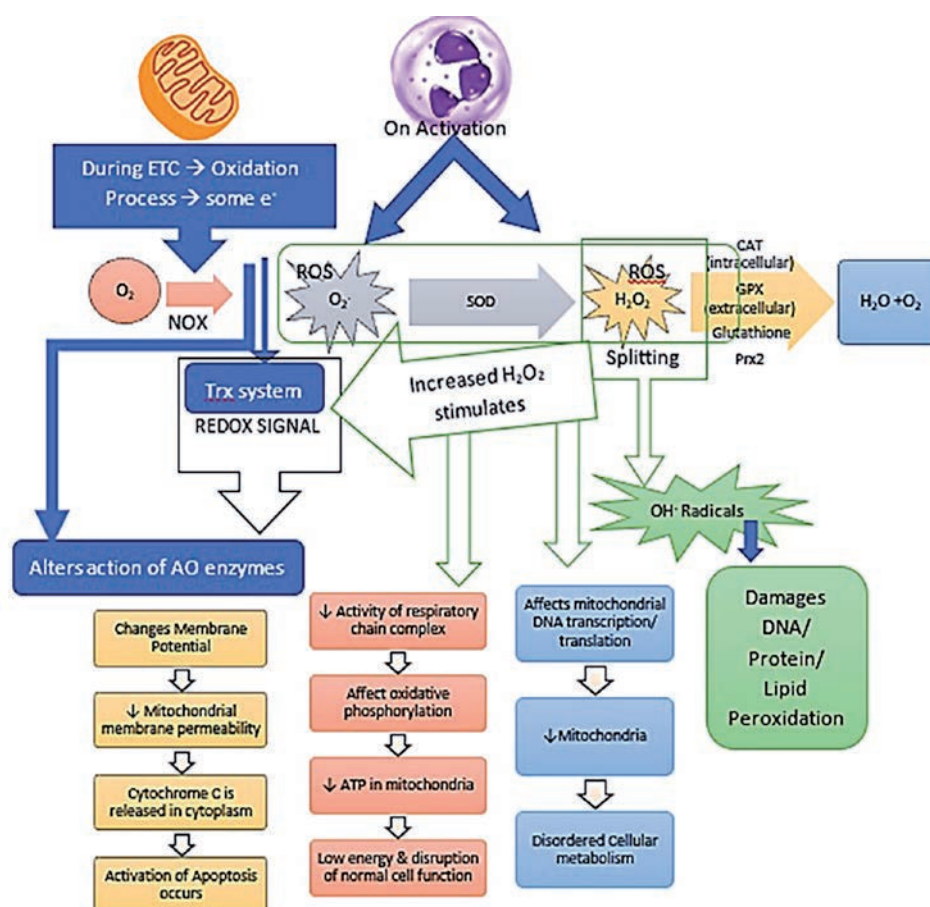


Figure 4. Biochemical reaction involving reactive oxygen species (Ros) generation and the effect it has on the molecules. The flow chart shows how oxygen is split into superoxide (O_2^-) by NADPH Oxidase (NOX) and as a byproduct of oxidation during electron transport chain (ETC). Superoxide, is further converted into hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD). H_2O_2 is normally converted to water and oxygen intracellularly by catalase (CAT), extracellularly by glutathione peroxidase (Gpx), glutathione and peroxiredoxin2 (Prx2). In case of absence of antioxidants, H_2O_2 can split into hydroxyl ions (OH^-). O_2^- , OH^- and H_2O_2 are ROS, which stimulate the thioredoxin system (Trx) and alter activity of antioxidant (AO) and individually cause damage to the cell and its building blocks. ROS are produced by polymorphonuclear neutrophil (PMNs) [4, 5].

RNS are produced when nitrous oxide (NO) combines with O_2^- to form peroxynitrate ($ONOO^-$), which attacks lipids and also activates nuclear factor kappa beta ($Nf\kappa\beta$) and activated protein-1 (AP-1). The various free radicals can be classified, as shown in **Figure 5**. The effect of ROS is dependent upon its dose and context of exposure. In low concentrations, ROS act as secondary messengers by playing a role in signal transduction cascades intracellularly, facilitating physiological functions like cell growth, autophagy, and immune and inflammatory responses. The endogenous sources of ROS can be endogenous or toxic exogenous products, as shown in **Figure 6** [6]. ROS are short-lived, which makes their detection difficult, and ROS-related degraded products

ROS		RNS	
Radical	Non Radical	Radical	Non Radical
• Molecular oxygen O_2^{**} • Superoxide O_2^{*-} • Hydroxyl OH^* • Alkoxy RO^* • Peroxyl • Hydroperoxyl	• Hydrogen Peroxide H_2O_2 • Peroxide $ROOR$ • Singlet oxygen O_2^* • Ozone O_3 • Hydroxyl ion $^*OH^-$ • Peroxynitrite $^*ONOO^-$	• Nitric Oxide NO • Nitrogen dioxide NO_2	• Peroxynitrite $ONOO^-$ • Alkyl peroxynitrite $ROONO$ • Nitronium cation NO^{2+} • Nitroxyl cation NO^+ • Nitroxyl Anion NO^- • Nitrogen oxides N_xO_x

Figure 5.
Classification of free radicals.

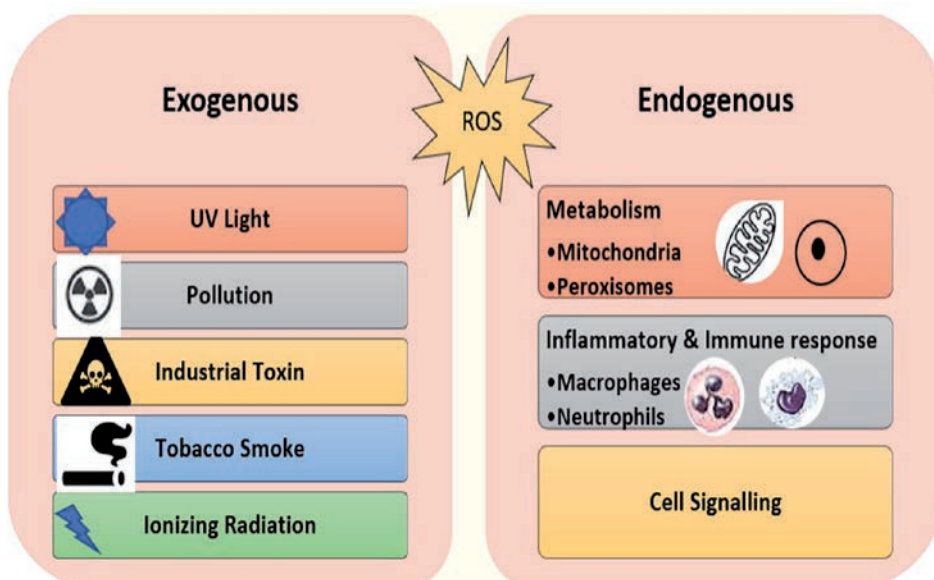


Figure 6.
Different sources of Ros exogenous and endogenous [6].

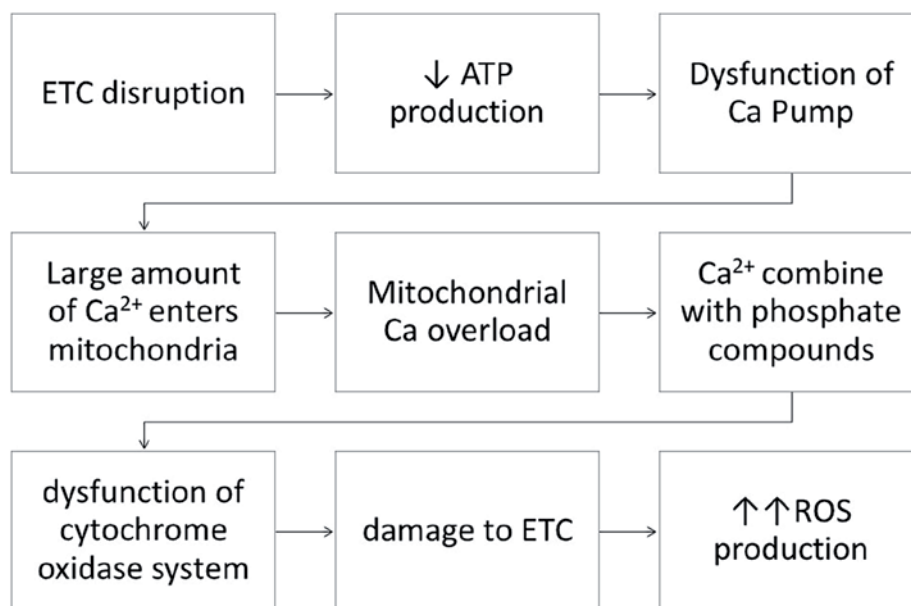


Figure 7.
 Further processes which increase the ROS production due to disruption of the electron transport chain (ETC) as a result of calcium (Ca) overload [4].

are used to assess their activity [3]. Furthermore, within the mitochondria, during the biological oxidation process of the electron transport chain (ETC), disruption of the normal detoxification mechanism of reactive oxygen species (ROS), such as the conversion of H₂O₂ into water, leads to the events outlined in the flowchart (**Figure 7**).

3. Salivary oxidants: Role in periodontal and systemic disease

Saliva serves as a diagnostic mirror of oral and systemic health. Among its many constituents, salivary oxidants—particularly reactive oxygen species (ROS)—play a critical role in inflammatory responses, especially in periodontal disease. In periodontium, oxidants are generated as natural by-products of cellular metabolism, particularly through the activity of neutrophils, monocytes, and macrophages during host immune responses to bacterial pathogens such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* [7]. While low levels of ROS are essential for cell signaling and immune defense, an excess leads to oxidative stress, resulting in tissue destruction and dysregulation of repair mechanisms.

In periodontal disease, the balance between oxidants and antioxidants is disrupted, resulting in oxidative stress, which contributes to the pathogenesis and progression of periodontitis. Elevated levels of oxidative biomarkers such as malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), and nitric oxide have been consistently found in the saliva of individuals with periodontal disease [8, 9]. These molecules indicate lipid peroxidation, DNA damage, and nitrosative stress, respectively, all of which contribute to periodontal tissue degradation. According to recent studies by Sui et al. [10], ROS can cause apoptosis of human periodontal ligament stem cells (hPDLSCs).

Moreover, oxidative stress originating from periodontitis is not limited to local effects. ROS and their inflammatory mediators can enter systemic circulation, contributing to endothelial dysfunction, insulin resistance, and systemic inflammation. Studies have linked elevated salivary ROS to systemic diseases, including cardiovascular disease, diabetes mellitus, and chronic kidney disease [11–13]. For example, oxidative stress has been shown to promote atherosclerotic plaque formation and destabilization, bridging the pathophysiology between periodontitis and cardiovascular disease [14].

Furthermore, salivary oxidants can influence host immune responses by modulating cytokine production, such as tumor necrosis factor-alpha (TNF- α) and interleukins like IL-1 β and IL-6, which perpetuate inflammation in both periodontal and systemic tissues [8]. A recent study demonstrated that oxidative markers in saliva could serve as reliable indicators for systemic oxidative stress levels, especially in conditions such as diabetes, where glycation end products exacerbate ROS production [15].

Further effects of ROS in relation to periodontium are summarized in **Figure 8**.

Taken together, these findings highlight the dual impact of salivary oxidants: they act as pathogenic agents in periodontal tissue breakdown and as messengers that amplify systemic inflammatory responses. Thus, monitoring salivary oxidants may aid in the early detection and management of both periodontal and systemic diseases, presenting opportunities for personalized treatment strategies.

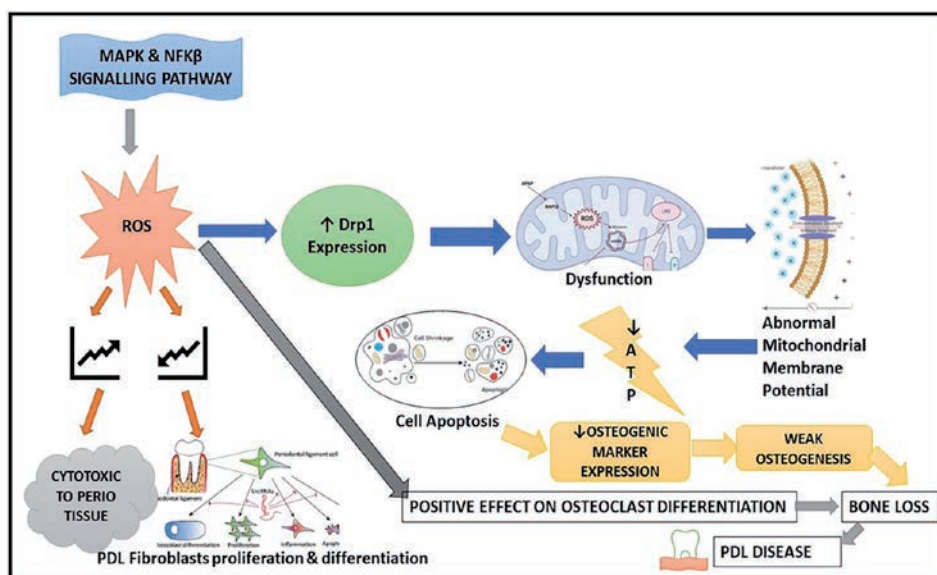


Figure 8.

How Ros can cause increased dynamin related protein (Drp1) expression which is a key regulator of mitochondrial fission and can lead to dysfunction of mitochondria and abnormal mitochondrial membrane potential and decreased ATP production leading to apoptosis. Apoptotic signal decreases osteogenic marker expression and weakens osteogenesis, while the mitogen activated protein kinase (MAPK) & nuclear factor kappa beta (Nfkb) mediated signaling pathway show positive effects of ROS on osteoclast differentiation leading to periodontal bone loss. It also shows that high Ros levels are cytotoxic to periodontal tissues and low concentration stimulates proliferation and differentiation of periodontal ligament (PDL) fibroblasts [3, 4].

4. Antioxidants in saliva: Protective mechanisms

While oxidants contribute to tissue destruction, *antioxidants* (AO) play a vital role by acting as protective agents that neutralize reactive oxygen species (ROS) and maintain redox homeostasis. Saliva, a readily available diagnostic fluid, contains a dynamic mix of enzymatic and nonenzymatic antioxidants that form the first line of defense against oxidative stress in the oral cavity.

Halliwell and Gutteridge define AO as substances that, when present in low concentration compared to ROS, significantly delay or inhibit the oxidation of that substrate. AO defenses include directly scavenging ROS (nonenzymatic) or by action of the enzymes (enzymatic). AO enzymes are regulated by nuclear factor erythroid 2-related factor 2 (Nrf2), which is a transcription factor. The classification is shown below [6]. The primary AO are the enzymatic, while the nonenzymatic are considered as the secondary mechanisms to remove ROS as they are taken from the diet [16].

4.1 Types of salivary antioxidants

The salivary antioxidants can be classified into enzymatic and nonenzymatic. The list of these, along with their functions, is summarized in **Table 1** [17, 18] and diagrammatically presented in **Figure 9** [5, 6].

4.2 Mechanisms of protection

AO provide protective function in the body by the following mechanisms:

1. *Neutralization of ROS*: Superoxide dismutase (SOD) converts superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2). Following this, catalase (CAT) and glutathione peroxidase (GPx) detoxify H_2O_2 into water and oxygen.

Type	Examples	Function
Enzymatic	Superoxide dismutase (SOD),	Converts superoxide radicals into hydrogen peroxide.
	Glutathione peroxidase (GPx),	Reduces hydrogen peroxide and lipid peroxides using glutathione.
	Catalase	Breaks down hydrogen peroxide into water and oxygen, reducing its harmful potential.
Non-enzymatic	Uric acid	Accounts for more than 70% of the total antioxidant activity in saliva [17].
	Ascorbic acid (Vitamin C)	A powerful water-soluble antioxidant known for its role in collagen synthesis and immune modulation. It gets oxidized to dehydro-ascorbic acid (DHA) [18]
	Vitamin E and Glutathione	Present in low quantities but contribute to the overall redox balance.
	Albumin	Direct scavenging of free radicals, metal ion chelation
Trace Elements	Zinc, Selenium	Act as cofactors for antioxidant enzymes

Table 1.
Classifies the salivary antioxidants.

SOD • Present in cytosol and mitochondria of all aerobic cell. • It carries out simultaneous oxidation and reduction reaction which calls dismutation. • It has metal ion co factor of Cu/Zn/Mn/Fe and forms different isoenzymes namely, • SOD1 which contains Cu/Zn • SOD2 which contains Mn • SOD3 which is present in extracellular compartment.
CAT • Present in all eukaryotic cells in mitochondria and peroxisomes
GPx • Uses glutathione as cofactor for oxidation. • $\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2\text{H}_2\text{O}$ • Detoxification - part of phase II enzymes which are responsible for conjugation with xenobiotics with peptides and sugar • Gpx1- cytosol & mitochondria • Gpx2- intestine - extracellular • Gpx3 - extracellular • Gpx4- prefer lipid peroxides • Gpx5-8- not well identified
PON2 (Paraonase 2) • It has a dual role of acting as lactonase and Ao enzyme. It prevents oxidation of low density lipoprotein (LDL) and lipid peroxidation in plasma membrane. • PON2 interacts with ETC in the inner mitochondrial membrane and decrease the production of superoxide (O_2^-)
GSH (Glutathione) • It is a tripeptide of three amino acids (cysteine, glycine, glutamic acid). • It is the most abundant and low molecular weight Ao synthesized in eukaryotic and prokaryotic cells.

Figure 9.
Details of the salivary antioxidants.

Uric acid and glutathione have the ability to directly scavenge hydroxyl radicals.

2. Inhibition of inflammatory mediators

Antioxidants have the ability to suppress the release of cytokines like interleukin-1 beta ($\text{IL-1}\beta$) and tumor necrosis factor alpha ($\text{TNF-}\alpha$), which reduce inflammation-induced tissue damage [7].

3. Protection of structural biomolecules

Antioxidants prevent lipid peroxidation, protein carbonylation, and DNA oxidation, thus preserving cellular integrity.

4. Maintenance of oral tissue homeostasis

Antioxidants balance the oxidants. A balanced redox environment promotes wound healing and periodontal regeneration and supports healthy microbiota [19]. Protective mechanisms are further depicted in **Figures 10–12** [3, 20].

4.3 Factors affecting antioxidant levels in saliva

The oral cavity is an open system. This makes its content, including the saliva, be in a dynamic state and be affected by multiple factors. The factors that play a role in affecting the AO levels are mentioned briefly in **Table 2** [19, 21–26].

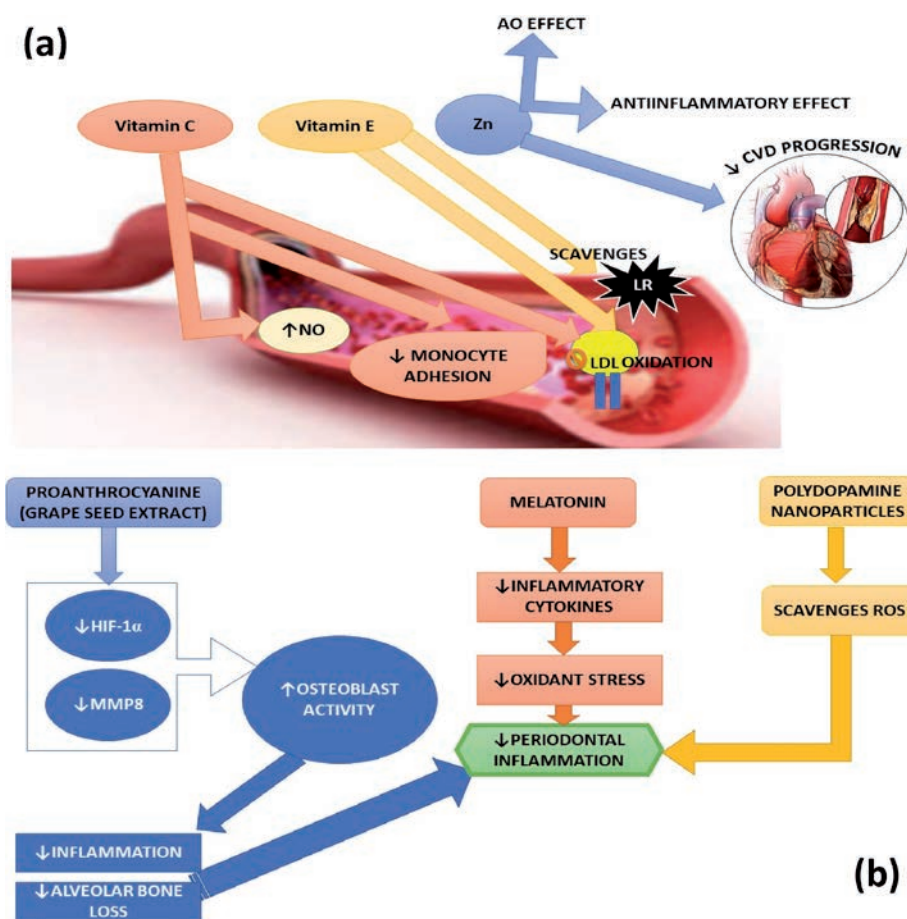


Figure 10.
 (a) Illustration of how antioxidants like vitamin c, vitamin e and zinc (Zn) decrease cardiovascular disease progression and (b) Illustrates how the additional antioxidants like proanthocyanine, melatonin and polydopamine nanoparticles decrease periodontal inflammation by scavenging Ros and increased osteoblast activity [3, 20].

4.4 Role of phase II enzymes in antioxidant effects

The phase II enzymes are part of the xenobiotic metabolism, which has phases of oxidation (phase I), conjugation/detoxification (phase II), and elimination (phase III). The phase II enzymes include glutathione peroxidase (Gpx), GSH (glutathione), UGT (UDP-glucuronosyltransferase), Srx (sulfiredoxin), Trx (thioredoxin), TrxR (thioredoxin reductase), HO-1 (heme oxygenase), and NQO1 (NADPH quinone oxidoreductase-1). The phase II enzymes are important in the cellular response to oxidative stress [6].

4.5 Antioxidant response element (ARE)/electrophile response element (EpRE)

ARE is a cis-acting enhancer sequence found in the promoter region of many cyto-protective AO and phase II enzyme genes. It has a core sequence of 5'-TGACnmGC-3' and participates in the expression of genes in response to oxidant stress. The induction of ARE is mediated by protein transcription factor Nrf2 by reacting with small musculoaponeurotic fibrosarcoma (sMaf) proteins to form a heterodimer [6].

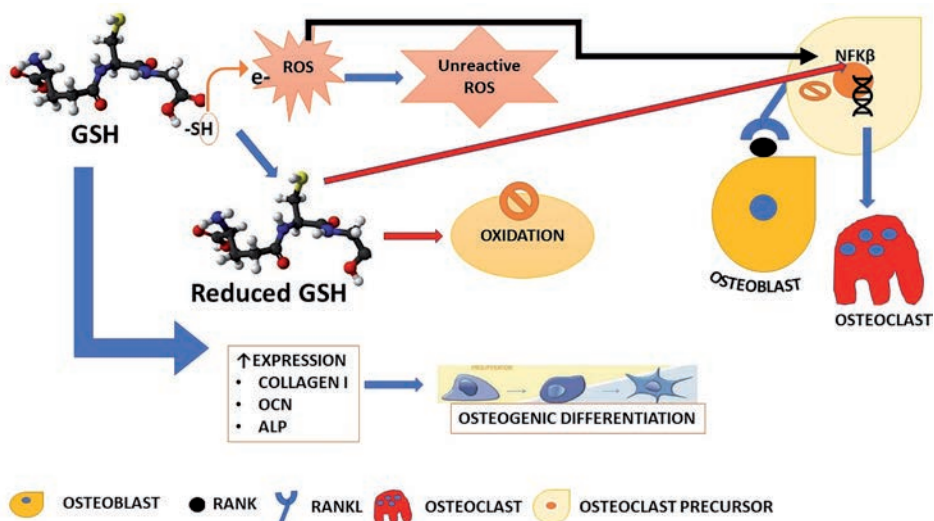


Figure 11.

How sulfhydryl (-SH) part of glutathione (GSH) gets reduced when it combines with unpaired electron of Ros resulting in unreactive Ros which is unable to give rise to osteoclast formation via the Ros mediated receptor activator of nuclear factor (RANK)/RANK Ligand (RANKL)/nuclear factor kappa beta (NFκβ) pathway. The reduced GSH also inhibits the osteoclast formation and oxidation of the osteoblasts. GSH increases type 1 collagen, osteocalcin (OCN) and alkaline phosphatase (ALP) expression resulting in differentiation of osteoblasts cells facilitating bone formation. In this way GSH prevents oxidant effect of Ros and acts as AO.

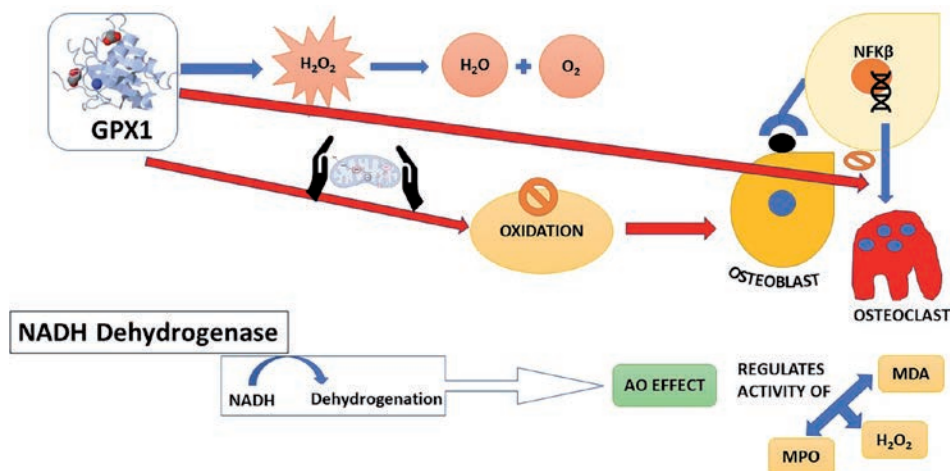


Figure 12.

How glutathione peroxidase (GPx1) as an AO converts H₂O₂ to water and also protects mitochondria and osteoblasts from oxidation. Additionally, it inhibits the nuclear factor kappa beta (NFκβ) pathway of osteoclast formation. NADH Dehydrogenase exerts its AO effect by dehydrogenation of NADH and regulates activity of myeloperoxidase (MPO), H₂O₂ and Malondialdehyde (MDA).

5. Pathway linking periodontitis, systemic inflammation, and oxidative stress

Oxidant stress is a state when the effects of ROS and AO are unbalanced and oxidize the body, resulting in aging and diseases in the body. It is also referred to as a

Influencing Factor	Effect on Antioxidant Activity
Periodontal inflammation	Decreases total antioxidant capacity (TAC) due to consumption by ROS [19]
Smoking	Reduces salivary Vitamin C and glutathione levels [21]
Age & Gender	Elderly show reduced enzymatic antioxidant levels; some gender-based differences reported [22]
Systemic Diseases	Diabetes and cardiovascular disease may reduce salivary antioxidant potential [23, 24]

Table 2.
Describes the factors which affect the antioxidant activity.

common pathological state [4]. The oxidant stress (OS) effect on the cell is described by its ability to cause the cell cycle arrest in the Go phase (quiescent, nondividing) and inhibit DNA synthesis of the cell (**Figure 13**).

Table 3 describes the pathological events of the pathway that links periodontal disease, systemic inflammation, and oxidative stress (**Figure 14**).

5.1 Oxidative stress as a mechanistic link between periodontitis and systemic diseases

In the absence of AO, ROS/RNS/free radicals have the ability to combine with all biomolecules. Proteins, owing to their susceptibility to being a common target for posttranslational modifications, are easily affected by this mechanism [18]. **Table 4** and Figures 15–19 describe the role of ROS and AO in linking periodontal disease and systemic diseases [4, 6, 27, 29, 30]. Oral lichen planus (OLP) has also been linked with ROS in its pathogenesis. T-cell infiltration seen in OLP stimulates ROS production, which leads to apoptosis of cells seen in OLP [31].

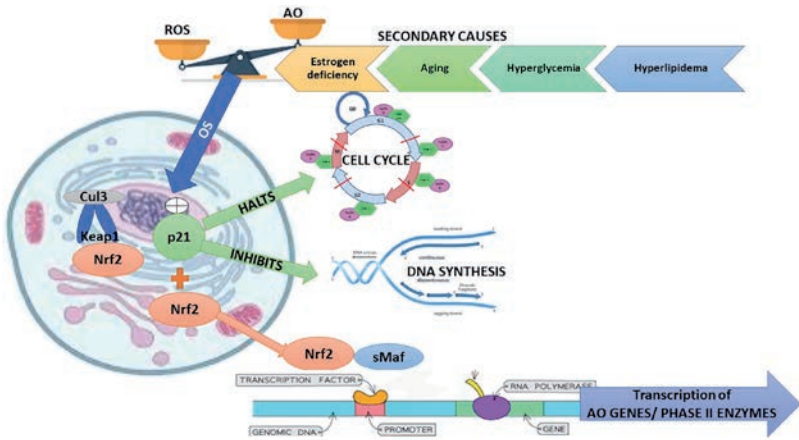


Figure 13.
How oxidant stress (OS) can occur secondary to aging, estrogen deficiency etc. and by imbalance in AO and ROS. OS stimulates p21 (p53 regulated cyclin dependent kinase inhibitor) which inhibits DNA synthesis and can combine with Nrf2 (nuclear factor erythroid -2 related factor). The combination under OS causes its stabilization and it can activate the transcription of AO enzymes. In absence of OS, Nrf2 is combined with Keap1 (kelch like ECH associated protein) and Cul3 (cullin 3 E3 ubiquitin ligase) which causes easy degradation of it by proteases to prevent unnecessary activation [4, 6].

Step	Pathological event	Description	Consequence
1	Bacterial invasion of periodontal tissues	Periodontal pathogens (e.g., <i>P. gingivalis</i> , <i>T. forsythia</i>) infiltrate gingival tissues	Initiates local immune response
2	Activation of innate immune system	Neutrophils, macrophages, and dendritic cells respond by releasing inflammatory cytokines (IL-1 β , TNF- α , IL-6)	Leads to local inflammation
3	ROS production by immune cells	Neutrophils produce excess <i>reactive oxygen species (ROS)</i> to kill bacteria	ROS leads to oxidative damage of local tissues
4	Breakdown of epithelial barrier	Continuous inflammation and oxidative damage compromise the epithelial lining	Facilitates bacterial entry into bloodstream
5	Dissemination of bacteria and inflammatory mediators	Pathogens, endotoxins (e.g., LPS), and cytokines enter systemic circulation	Triggers <i>systemic inflammation</i>
6	Systemic oxidative stress	Excess ROS production systemically + reduced antioxidant defenses ($\downarrow$ GSH, $\downarrow$ SOD)	Causes oxidative damage to distant organs
7	Endothelial dysfunction	Oxidative stress impairs nitric oxide (NO) signaling in blood vessels	Promotes atherosclerosis and hypertension
8	Chronic low-grade inflammation	Sustained systemic inflammation alters immune responses and metabolism	Contributes to insulin resistance, liver damage, neuroinflammation
9	Disease manifestation	Target organs affected: heart, pancreas, liver, brain, kidneys, placenta	Links to CVD, <i>diabetes</i> , CKD, <i>Alzheimer's</i> , <i>adverse pregnancy outcomes</i>

Table 3.

Briefly describes the steps of the pathway which links periodontal disease, systemic inflammation and oxidative stress.

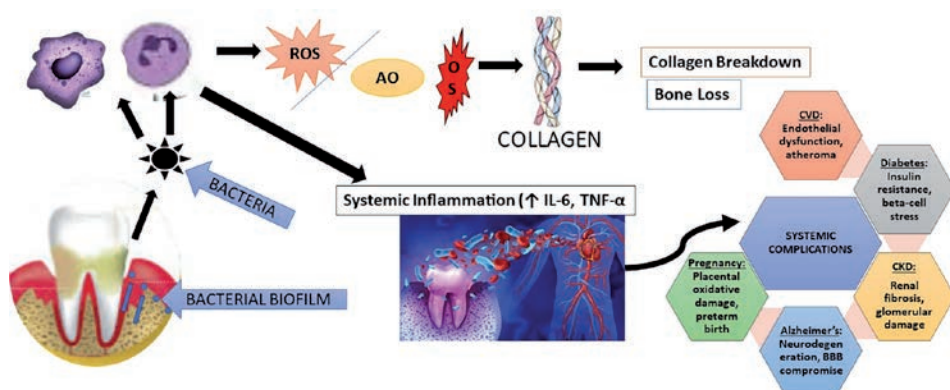


Figure 14.

Summarized view of how periodontal infection can lead to oxidative stress in different target organs via systemic spread.

Systemic condition	Role of oxidative stress in periodontitis	Mechanism of link to systemic disease
Cardiovascular Disease (CVD)	Periodontal inflammation increases ROS levels in circulation	ROS contribute to <i>endothelial dysfunction</i> , <i>oxidation of LDL</i> , and <i>plaque instability</i> , triggering <i>atherosclerosis</i> and <i>CVD progression</i> .
Diabetes Mellitus	Oxidative stress impairs insulin signaling	Enhanced <i>oxidative burden</i> worsens <i>insulin resistance</i> , and high glucose levels further promote ROS generation, creating a <i>vicious cycle</i> .
Chronic Kidney Disease (CKD)	ROS promote systemic inflammation and endothelial injury	ROS-mediated inflammation affects <i>glomerular function</i> , contributing to <i>renal fibrosis</i> and <i>CKD progression</i> .
Alzheimer's Disease	Periodontitis-induced ROS cross the blood–brain barrier	ROS contribute to <i>neuroinflammation</i> , <i>amyloid-beta accumulation</i> , and <i>neuronal damage</i> , worsening cognitive decline.
Adverse Pregnancy Outcomes	ROS impair placental function	Increased oxidative stress leads to <i>placental inflammation</i> , <i>reduced fetal perfusion</i> , and increased risk of <i>preterm birth</i> or <i>low birth weight</i> .

Table 4.
Describes the oxidative stress as the connecting link of periodontal disease and systemic disease.

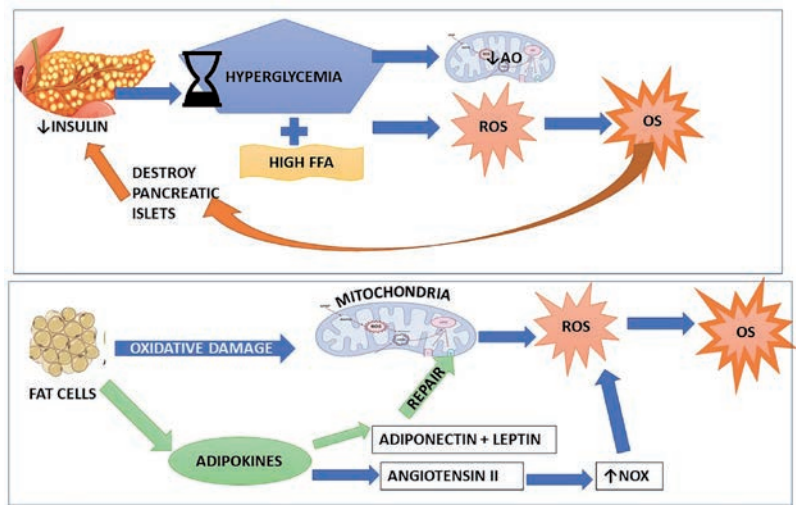


Figure 15.
How in diabetes, decreased insulin resulting in hyperglycemic state can caused decreases AO in mitochondria. High free fatty acids (FFA) and hyperglycemic state can cause generation of Ros and lead to state of oxidant stress (OS). OS in turn leads to destruction of the islet cells in pancreas resulting in decreased insulin production, making it a vicious cycle [4].

5.2 Oxidant-antioxidant imbalance and disease progression

Periodontal disease is characterized by a chronic inflammatory response triggered by microbial biofilms, which initiates a cascade of immune responses and oxidative stress. The key event in disease progression is the imbalance between *reactive oxygen species (ROS)* and the *body's antioxidant defense mechanisms*, leading to tissue destruction.

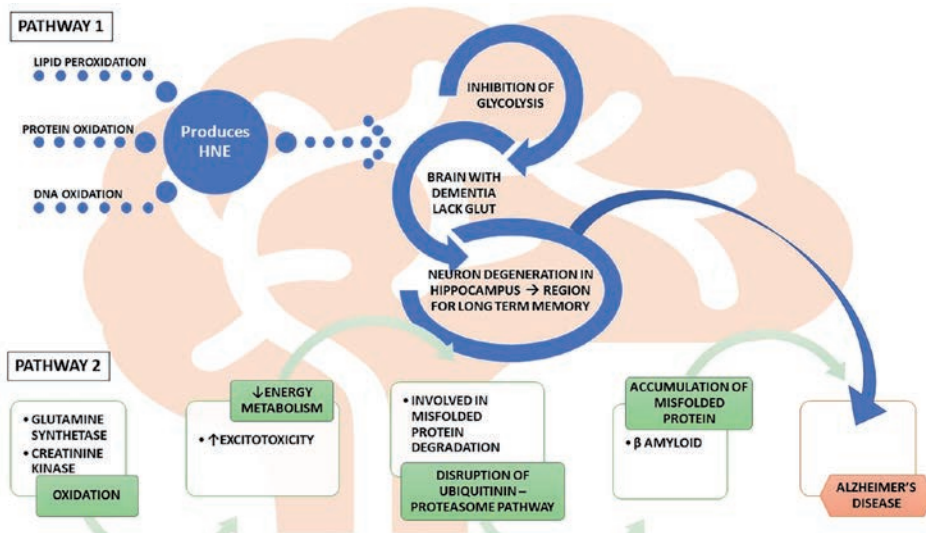


Figure 16.

Two pathways in the Brain by which oxidant stress and ROS are implicated in the pathogenesis of Alzheimer's disease. The first involves formation of 4-Hydroxynonenal (HNE) from lipid/protein peroxidation and DNA oxidation. HNE, is a highly reactive aldehyde which inhibit glycolysis. Additionally, in dementia the patients lack glucose transporter (GLUT) leading to neuron degeneration in Hippocampus. As per the second pathway oxidation of enzymes glutamine synthetase and creatinine kinase decreases energy metabolism and increases excitotoxicity which is required for degradation of misfolded protein. Thus accumulation of misfolded protein like β amyloid continues and together with the first pathway results in Alzheimer's disease [27].

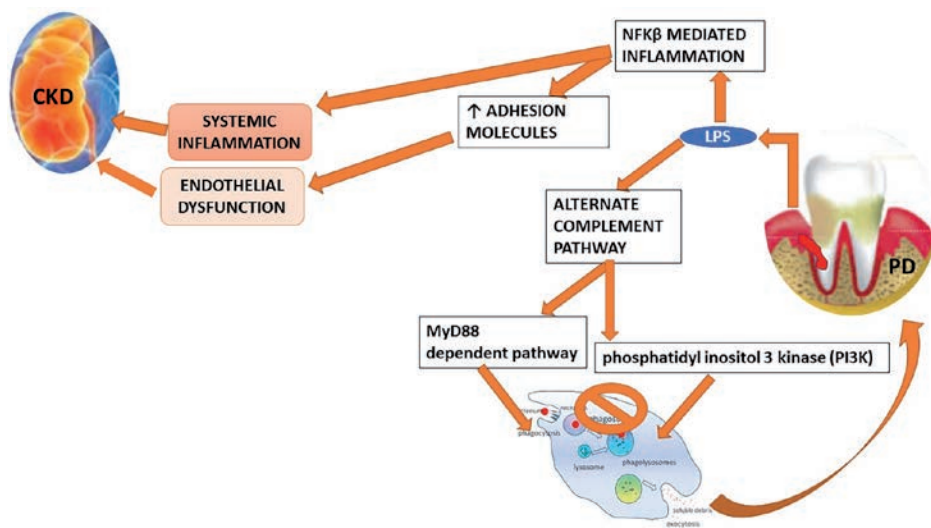


Figure 17.

How the periodontal disease (PD) can result in chronic kidney disease (CKD). The red complex bacteria from the periodontal pocket have lipopolysaccharide (LPS) which initiates inflammation via NFKB pathway which can spread systemically and reach the kidneys and also increase the adhesion molecule expression in the endothelium of the kidneys causing endothelial dysfunction. Alternatively, LPS can trigger alternate complement system which can prevent phagolysosome action in immune cells resulting in decreased immune response and a risk factor for periodontal disease [28].

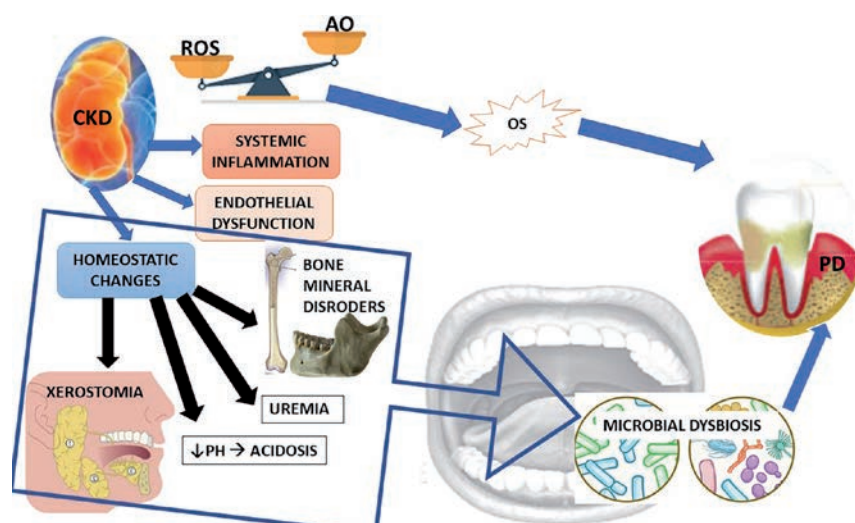


Figure 18.
 How CKD patients have systemic inflammation and endothelial dysfunction. An imbalance of AO and ROS can lead to oxidant stress resulting in periodontal disease. Additionally homeostatic changes occur in CKD which can result in bone mineral disorders, xerostomia, decrease in pH leading to acidosis and uremia which can reach salivary glands and cause dysbiosis in oral cavity predisposing to periodontal disease [28].

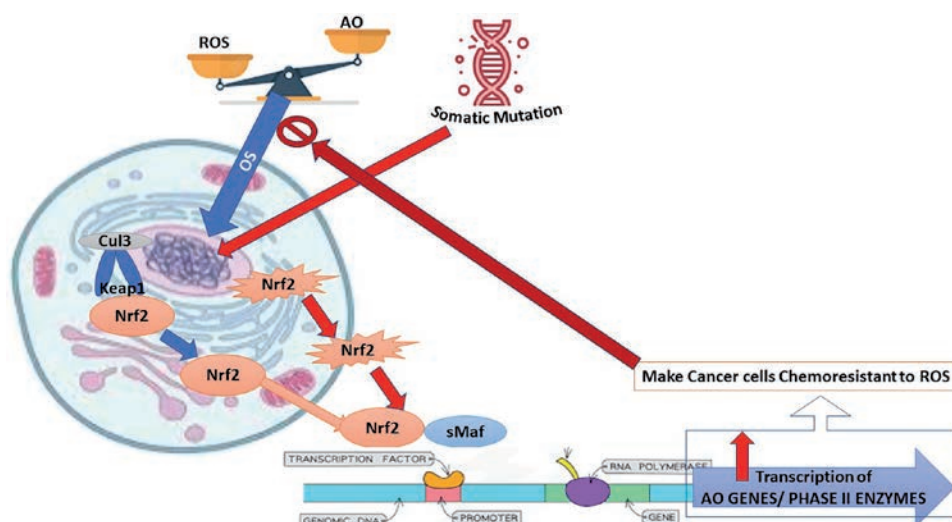


Figure 19.
 Nrf2 acts as a protector against Ros damage but in case of any somatic mutation it results in creation of hyperactive Nrf2 which increases the transcription of antioxidant genes which although prevent Ros action but also make cancer cells immune to Ros action. This ironically increases the surviving capacity of cancer cells and make them chemo-resistant [6].

5.3 Mechanism of oxidative imbalance in periodontitis

Continuous oxidative damage perpetuates inflammation in the tissues, leading to a vicious cycle of *periodontal tissue breakdown* (Table 5) [7, 29]. Additionally, oxidative

Stage	Pathophysiological Process
Bacterial challenge	Pathogens like <i>Porphyromonas gingivalis</i> stimulate immune cells
ROS production	Activated neutrophils release ROS (superoxide, hydrogen peroxide, hydroxyl radicals)
Antioxidant depletion	Excess ROS overwhelms enzymatic (e.g., SOD, GPx) and non-enzymatic (e.g., uric acid, vitamin C) systems
Lipid, DNA, and protein damage	ROS induce lipid peroxidation, DNA damage, and enzyme dysfunction
Tissue destruction	Collagen degradation, alveolar bone resorption, and periodontal attachment loss

Table 5.
Lists the stages resulting in oxidative imbalance in periodontal disease.

stress is also implicated in peri-implant disease. Peri-implant disease is an infectious inflammation that occurs in the soft and hard tissues around implants, including peri-implant mucositis and peri-implantitis [28]. The role of ROS in dental caries has also been implicated. Caries is also considered multifactorial, and literature suggests that teeth become susceptible to bacterial acids due to downregulation of parotid hormone from hypothalamic signaling. This occurs due to high sucrose levels, which inhibit dentinal fluid movement [32].

5.4 Markers of oxidative stress and imbalance

In saliva, the ROS markers and AO marker levels can give an indication of the oxidative stress and imbalance in the body. Key indicators of this imbalance include (**Table 6**):

ROS markers: *Malondialdehyde (MDA)* is a lipid peroxidation marker elevated in the saliva of periodontitis patients. *8-hydroxydeoxyguanosine (8-OHdG)* is a DNA oxidative damage marker. Protein damage from oxidative stress can be assessed by *advanced oxidation protein products (AOPPs)*. *Thiobarbituric acid reactive substance (TBARS)* is a marker for lipid oxidation in saliva and is related to periodontal disease. *Advanced glycation end (AGE) products* can be used as an end-product biomarker for assessing carbohydrate damage in the body.

Biomarker	Type	Clinical Relevance
Reactive oxygen species (ROS)	Oxidant	Increased in active periodontitis, indicating oxidative stress
Malondialdehyde (MDA)	Lipid peroxidation byproduct	Marker of tissue damage
8-hydroxydeoxyguanosine (8-OHdG)	DNA oxidation marker	Correlates with cellular damage in periodontal tissues
Total antioxidant capacity (TAC)	Antioxidant marker	Decreases in disease state
Myeloperoxidase (MPO)	Enzymatic oxidant	Elevated in periodontal inflammation
Cytokines (IL-1 β , TNF- α)	Pro-inflammatory	Drive soft tissue and bone destruction in periodontitis

Table 6.
Lists the different biomarkers, their type and clinical relevance.

AO markers: Reduced total antioxidant capacity (TAC) is common in patients with advanced disease and can be used to assess both caries and periodontal disease. Ferric reducing antioxidant power (FRAP) is also an important marker for AO activity [28, 32].

6. Salivary biomarkers: Diagnostic potential in periodontitis and systemic health

Saliva, an easily accessible and noninvasive diagnostic fluid, has emerged as a promising medium for detecting biomarkers associated with both *oral* and *systemic diseases*. Its use in point-of-care diagnostics is increasingly being explored due to advances in proteomics, metabolomics, and artificial intelligence (AI)-driven data analysis. Saliva as a diagnostic fluid has great potential to be used in large clinical trials. Its accessibility can make self-management of disease in epidemiological studies possible. Saliva also contains gingival crevicular flow (GCF) volume, thus proving not only the systemic information but also the local microenvironment information of the periodontal tissues. It contains molecular and cellular components of host response and the microbes, which can serve as biomarkers to detect disease. The mouth is a site to detect systemic infection, as it acts as a repository for infectious pathogens that have entered elsewhere [33].

6.1 Salivary biomarkers in periodontal disease

Salivary biomarkers provide real-time insights into the inflammatory and oxidative status of periodontal tissues. Several oxidative and inflammatory markers in saliva have been associated with disease presence and severity. They are listed in **Table 5**.

These biomarkers can serve not only for *diagnosis* but also for *monitoring disease progression* and *evaluating response to therapy* [29, 34].

6.2 Salivary biomarkers and systemic diseases

Periodontitis, being a chronic inflammatory condition, contributes to systemic inflammation through the release of oxidative byproducts and cytokines into circulation. Studies have found elevated salivary oxidative stress markers in *diabetes mellitus*, wherein an increase in MDA and a decrease in TAC have been noted [35]. In *cardio-vascular disease*, high levels of inflammatory cytokines and oxidative metabolites have been reported [36]. *Rheumatoid arthritis and autoimmune conditions* have shown similar inflammatory salivary profiles as periodontitis [37]. This *systemic spillover effect* makes saliva a potential mirror of overall inflammatory burden and metabolic status. Saliva acts as a diagnostic window to the body by being used as a chair-side diagnostic tool in detecting systemic inflammation by using AI-assisted analysis and smart chip analysis of the disease biomarkers. Increased levels of IL-1- β , ROS, and MDA in saliva are suggestive of oral inflammation, while increased levels of C-reactive protein (CRP) and TNF- α in saliva suggest systemic inflammation.

6.3 Systemic repercussions of oxidative stress in periodontitis

The local oxidative environment contributes to systemic inflammatory burden by releasing *pro-inflammatory cytokines (IL-6, TNF- α)*, *reactive metabolites*, *bacterial endotoxins*, and *oxidized proteins and lipids* into circulation.

This systemic dissemination contributes to comorbidities such as *diabetes mellitus*, which occurs as a result of inflammation-induced insulin resistance. *Cardiovascular disease develops as a result of atherosclerosis and endothelial dysfunction. Apart from these, systemic repercussions of oxidative stress can result in adverse pregnancy outcomes such as preterm birth and low birth weight, chronic kidney disease, and neurodegeneration (e.g., Alzheimer's)* [38].

6.4 Advantages of salivary diagnostics

The main benefit of using saliva for diagnostics is its noninvasive mode of collection, which requires no needle pricking and seldom requires additional equipment when compared with blood or serum as a diagnostic fluid. This noninvasive method of collection makes it easy to collect and is even comfortable for the patient. The possibility of chairside tests and point-of-care devices can be developed based on this premise. Easy collection can also help in early detection and monitoring for diseases or conditions.

6.5 Clinical implications

- *Diagnostic potential:* Studies have shown that the *total antioxidant capacity (TAC)* of saliva is significantly lower in patients with periodontitis compared to healthy controls [25].
- *Therapeutic monitoring:* Post-scaling and root planing, antioxidant levels in saliva improve, indicating healing and reduced oxidative stress.
- *Adjunctive therapy:* Antioxidant-rich diets, supplements, or local antioxidant applications (e.g., coenzyme Q10 gel) have shown positive effects on periodontal outcomes [26].
- *Therapeutic modulation:* Adjunctive use of antioxidants like coenzyme Q10, green tea extract, and lycopene may aid periodontal therapy. Nicotinamide mononucleotide and nicotinamide ribose, when taken as supplements, can improve endothelial function under conditions of oxidative stress [32].
- *Monitoring progression:* Salivary oxidative markers may help assess *risk, disease activity, and treatment response*.
- *Systemic risk screening:* Oxidative stress profiles may serve as a warning sign for associated systemic diseases.

7. Emerging trends: Salivary diagnostics and personalized medicine

The noninvasive, easily accessible nature of saliva makes it an ideal diagnostic fluid. In recent years, significant advances have been made in utilizing *salivary biomarkers*—especially oxidants and antioxidants—for *early detection, risk assessment, and personalized treatment planning* in periodontology and systemic health. Salivary diagnostics have gained substantial traction as a promising, noninvasive modality for the detection and monitoring of both oral and systemic diseases. The interplay between oxidative stress and antioxidant defense mechanisms in saliva offers a unique

window into the pathophysiology of periodontal conditions. Recent technological advances, including biosensors, salivaomics, and artificial intelligence (AI), have transformed the landscape of salivary biomarker detection, facilitating a shift toward *personalized periodontal care*.

7.1 Advances in salivary diagnostic technologies

These are mentioned briefly in **Table 7** [39–43].

7.2 Role in personalized periodontal care

The use of *salivary oxidant-antioxidant profiling* allows clinicians to:

- Stratify patients based on oxidative stress status
- Predict *periodontal disease progression*
- Monitor response to antioxidant-based adjunctive therapies
- Customize treatment frequency and preventive care

Example: A patient with low total antioxidant capacity and high MDA levels may benefit from *antioxidant supplementation* and more frequent periodontal maintenance visits [43, 44].

7.3 Integration into systemic health monitoring

Due to its reflective nature, saliva can also serve as a *surrogate fluid for systemic diseases* such as:

- *Diabetes mellitus:* Increased salivary ROS and decreased TAC are common.
- *Cardiovascular disease:* Elevated salivary MDA and AOPPs in patients with hypertension or atherosclerosis.
- *Neurodegenerative disorders:* Salivary oxidative stress markers linked to cognitive decline in Alzheimer’s disease [45, 46].

Tool/Platform	Function
Lab-on-chip microfluidic devices	Detect oxidative stress biomarkers from small salivary volumes [39, 40]
Salivaomics	Integration of proteomics, transcriptomics, metabolomics, and microbiomics. Allow multi-omic profiling to stratify patients by risk and response to treatment [41]
AI-powered analysis platforms	Interpret complex salivary biomarker profiles to predict disease risk [42]
Biosensors & electrochemical assays	Real-time monitoring of ROS, antioxidants (e.g., uric acid, glutathione) [43]

Table 7.
Lists the tools used in advanced diagnostic techniques in saliva.

7.4 Challenges and future directions

Clinical application and use of AO and ROS are not without their challenges but are still important factors to be researched. The points have been summarized in **Table 8**.

7.5 Vision for precision periodontal medicine

The convergence of *salivary diagnostics*, *AI*, and *genomics* is paving the way for *precision periodontology*, creating a concept where *presymptomatic disease* can be detected early and therapy will be guided by *individual biochemical profiles*. *The patient outcomes* will be optimized with *real-time feedback loops*.

The incorporation of salivary oxidative stress profiling into clinical workflows enables early identification of high-risk individuals, tailored interventions, including antioxidant supplementation, and monitoring of treatment response and compliance. Stratification of recall intervals based on individual risk profiles.

For instance, individuals presenting with elevated malondialdehyde (MDA) and reduced total antioxidant capacity (TAC) have been shown to exhibit a heightened inflammatory response and may require more intensive periodontal maintenance therapy [47, 48].

7.6 Author’s contribution and relevant studies

The primary author has contributed to the field of antioxidants and oxidants by carrying out cross-sectional studies comparing the salivary and serum AO and ROS in healthy, chronic periodontitis, and ischemic heart disease patients. In both studies, ROS and AO status were assessed, respectively, in healthy chronic periodontitis patients and patients with ischemic heart disease using both serum and saliva samples [24, 49]. This was done to assess the change in AO or ROS activity in diseased individuals when compared with healthy individuals. Additionally, the comparison between serum and saliva was done to assess whether saliva itself could be used as a primary diagnostic fluid to assess AO and ROS activity and whether it could be used as a biomarker (**Table 9**). Another study is under publication related to oxidant stress. Other relevant studies are described briefly in **Tables 10** and **11** [7, 21, 29, 50–56].

Challenge	Strategy
Inter-individual variability in saliva	Standardization of collection and storage protocols
Low biomarker concentrations	Enhanced sensitivity using nanotechnology-based biosensors
Clinical adoption	Integration with chairside kits and electronic health records (EHR). Development of consensus-driven diagnostic thresholds
Cost and infrastructure	Integration with digital platforms and scalable point-of-care devices

Table 8.
Describes the challenges and associated strategy to counteract in saliva diagnostics.

Author	Study	Conclusion
Pampani et al., 2021 [50]	To compare serum & salivary oxidant levels in healthy, chronic periodontitis (CP), and ischemic heart disease (IHD) and find any correlation	The oxidant levels are significantly hampered in periodontitis and IHD subjects as compared to healthy subjects. The oxidants, whether serum or salivary, did not always show the proportional change as a result of change in oxidant stress
Punj et al., 2017 [24]	To investigate relationship between periodontitis and ischemic heart disease by estimation of total antioxidant status in saliva and serum	Antioxidant capacity is significantly hampered in chronic periodontitis and ischemic heart disease patients with or without periodontitis as compared to healthy controls. The salivary and serum antioxidants may not follow the same increase or decrease as a result of increased oxidant stress due to disease.

Table 9.
Studies conducted by authors on salivary Ao and Ros.

Author	Methodology	Study focus	Key findings
Tóthová et al. [50]	Case-control study	Oxidative stress markers in saliva of periodontal patients - MDA, SOD, TAC	Periodontal patients showed higher oxidative markers and reduced antioxidants compared to healthy controls
Tsai et al. [51]	Antioxidant capacity assay in unstimulated saliva	Salivary antioxidant activity in CP	Decreased TAC in patients with CP
Akalin et al. [52]	Longitudinal study (pre-post scaling)	Effects of nonsurgical therapy on salivary oxidants	Decrease in MDA levels and increase in SOD and catalase after periodontal therapy
Chapple et al. [7]	Literature review	Role of ROS in periodontal tissue destruction	ROS from neutrophils contributes to periodontal damage; antioxidants help counterbalance
Moore et al. [21]	Biochemical Analysis	GCF antioxidants in gingivitis and periodontitis	Reduced glutathione and uric acid levels in patients with active periodontal disease

Table 10.
Lists studies related to Ao and Ros and their clinical significance.

Author(s), year	Study design	Key findings	Relevance
Battino et al. [53]	Review	Saliva plays a central role in oral redox homeostasis.	Introduced concept of oral cavity as a redox-active environment.
D'Aiuto et al. [54]	Periodontitis vs. healthy	Higher salivary ROS in periodontal patients.	Direct link between oxidants and periodontal disease.
Tóthová & Celec, [29]	Review	Salivary glutathione and uric acid levels act as biomarkers.	Emphasizes non-enzymatic antioxidants in diagnostics.
Buduneli & Kinane [55]	Review	Diabetes increases oxidative load in saliva.	Links systemic inflammation with salivary biomarkers.
Kononen et al. [56]	Literature review	Cardiovascular disease and salivary oxidative changes coexist.	Adds interdisciplinary relevance.

Table 11.
Gives an overview of literature related to oxidative stress, antioxidants, oxidants and saliva.

8. Conclusion

The role of salivary antioxidants and oxidants is seldom limited to serving as a biomarker to detect oxidant stress. It serves as a tool and a connecting link to find a correlation between chronic systemic disease and periodontal diseases. It can be of great use in real-time estimation of disease status using a noninvasive, easily available sample from the patient, i.e., saliva. The omics technology coupled with AI makes the clinical application more feasible and accurate in today's day and age. Monitoring of chronic diseases like diabetes mellitus using wearable technology is a reality today; soon lab-on-chip devices to monitor oral diseases like periodontal disease will also become a reality.

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Conflict of interest

The authors declare no conflict of interest.

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Salivary Neuropeptides and Cytokines: Indicators of Stress and Resilience

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Abstract

Stress is a fundamental physiological and psychological response to environmental challenges, regulated by the interplay between the hypothalamic-pituitary-adrenal (HPA) axis, the autonomic nervous system, and immune-inflammatory pathways. Resilience, the ability to adapt and recover from stress, is increasingly recognized as a key factor in maintaining homeostasis and preventing stress-related pathology. Traditional biomarker assessment methods, such as blood sampling, pose limitations due to their invasiveness and inability to capture real-time physiological changes. Saliva has emerged as an optimal diagnostic fluid due to its non-invasive nature and its capacity to reflect dynamic neuroendocrine and immune responses. This chapter explores the use of salivary neuropeptides, hormones, and cytokines as biomarkers of stress and resilience, detailing their physiological roles and measurement methodologies. Key biomarkers including various hormones, immune modulating neuropeptides along with cytokines and chemokines, are discussed. We discuss their relevance in distinguishing between acute and chronic stress responses and their potential utility in identifying individuals in early stages or at risk for stress-related disorders. Emerging evidence supports the integration of salivary biomarker analysis in clinical and research settings, with advancements in immunoassays and mass spectrometry improving sensitivity and specificity. Future research should focus on systems approaches and long-term biomarker profiling to enhance predictive modeling of stress resilience. The application of salivary biomarkers in stress research holds significant promise for personalized diagnostics and early interventions aimed at mitigating the long-term effects of chronic stress.

Keywords: salivary biomarkers, stress resilience, hypothalamic-pituitary-adrenal (HPA) axis, neuropeptides, cytokines

1. Introduction

1.1 The physiological basis of stress and resilience in human health

Stress is a universal experience, eliciting a range of physiological and psychological responses that enable humans to adapt to challenges. At its core, stress is a survival mechanism designed to help the body respond to immediate threats. However, the

same mechanisms that promote short-term adaptation can have deleterious effects when activated persistently. Chronic stress, unlike acute stress, disrupts homeostasis and has been implicated in the development of numerous health conditions, including cardiovascular disease, immune dysfunction, and mental health disorders [1].

Resilience, on the other hand, is the ability to withstand, adapt, and recover from stress. Resilience is not merely the absence of stress responses but rather a complex interplay of biological, psychological, and environmental factors that modulate how individuals experience and recover from stressors [2]. Advances in biomarker research have provided a lens through which resilience can be quantitatively measured, offering insights into how physiological processes underpin adaptive capacity. This chapter explores the role of biomarkers, particularly those found in saliva, in understanding the dynamics of stress and resilience.

1.2 Importance of non-invasive biomarkers in studying stress responses

The identification and monitoring of biomarkers have revolutionized our understanding of stress and its impact on human health. Biomarkers are measurable indicators of biological processes which can serve as crucial tools in elucidating the physiological pathways activated by stress and resilience [3]. Traditional methods of biomarker collection, such as blood sampling, are invasive, costly, and often impractical in non-clinical settings.

Saliva, as a diagnostic fluid, overcomes these limitations. Non-invasive, cost-effective, and readily accessible, saliva offers a unique medium for capturing dynamic changes in stress-related biomarkers. Unlike other bodily fluids, saliva reflects real-time physiological responses, providing a snapshot of neuroendocrine and immune activity [4]. The ease of collection, coupled with advancements in assay technologies, has positioned saliva at the forefront of biomarker research, enabling a deeper understanding of the physiological basis of stress and resilience [5].

2. The role of saliva in biomarker research

2.1 Why saliva is an ideal diagnostic fluid

Saliva has emerged as a valuable diagnostic fluid in biomedical research and clinical practice due to its unique properties and advantages over traditional biofluids like blood and urine. One of the primary benefits of saliva is the ease of collection. Salivary sampling is non-invasive, painless, and does not require specialized medical personnel or equipment. This makes it particularly suitable for repeated measurements, large-scale screenings, and use in populations where blood draws are impractical, such as with children, the elderly, or individuals with needle phobia [6].

Another significant advantage is that saliva reflects real time systemic responses [7]. Salivary glands are highly vascularized, allowing for the rapid transfer of hormones, peptides, and other molecules from the bloodstream into saliva [8]. This close association means that changes in physiological or pathological states can be quickly detected in saliva, providing timely insights into an individual's health status.

Additionally, saliva collection reduces the risk of complications associated with blood sampling, such as infection or hematoma, and eliminates the need for biohazardous waste disposal. The simplicity and safety of saliva collection facilitate its use in various settings, including remote locations, field studies, and at-home monitoring [9].

2.2 Salivary biomarkers as indicators of stress

Saliva contains a complex mixture of enzymes, hormones, antibodies, cytokines, and other biomolecules that mirror the body's physiological condition [10]. As such, it serves as a rich source of biomarkers for assessing stress responses. The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic-adrenal-medullary (SAM) axis, both integral to the stress response, release several hormones and neuropeptides that can be detected in saliva [11, 12].

The use of salivary biomarkers allows researchers and clinicians to monitor stress non-invasively and in real-time. This is particularly important for understanding the dynamic nature of stress and its impact on health. Salivary biomarkers can provide insights into both acute stress reactions and chronic stress states, aiding in the assessment of how individuals respond to and recover from stressors.

2.3 Hormones

Hormones are central to the body's regulation of stress and overall physiological balance, extending beyond the well-studied stress hormones cortisol and CRH. Thyroid hormones, as well as sex hormones like estrogen, progesterone, and testosterone, also influence how the body responds to stress and maintain homeostasis. The ability to measure these hormones non-invasively in saliva has opened new opportunities for exploring their roles in health and disease.

Research has increasingly recognized the multifaceted roles hormones play in stress responses and homeostasis. The non-invasive measurement of these hormones in saliva has been validated and utilized in numerous studies, providing valuable insights into their dynamics during stress [13].

2.3.1 Cortisol: The stress hormone

Cortisol, often referred to as the “stress hormone,” is synthesized in the adrenal cortex as part of the hypothalamic–pituitary–adrenal (HPA) axis. Its primary function is to regulate the body's response to stress by mobilizing energy, modulating immune activity, and maintaining cardiovascular function [14]. Cortisol levels increase rapidly in response to acute stress, enabling the body to cope with immediate challenges.

Saliva provides a reliable, non-invasive medium for measuring cortisol levels. Salivary cortisol reflects the biologically active, unbound fraction of cortisol in the bloodstream, making it a direct indicator of HPA axis activity [15]. Cortisol follows a diurnal rhythm, with peak levels in the morning that decline throughout the day. Disruptions to this rhythm, such as elevated evening cortisol or a flattened daily pattern, are often indicative of chronic stress or HPA axis dysregulation [16]. Advances in salivary assays allow researchers to capture these fluctuations, providing insights into both acute stress responses and longer-term patterns of stress exposure.

2.3.2 Corticotropin-releasing hormone (CRH): The HPA Axis initiator

CRH, a peptide hormone secreted by the hypothalamus, serves as the key initiator of the HPA axis. In response to a stressor, CRH stimulates the anterior pituitary to release adrenocorticotrophic hormone (ACTH), which in turn prompts cortisol production in the adrenal glands [17]. While more challenging to measure due to its

lower concentrations in saliva, CRH remains an important biomarker for studying the early stages of the stress response.

Emerging research has highlighted the role of CRH not only in HPA axis activation but also in modulating inflammation. CRH receptors are expressed on immune cells, allowing it to influence cytokine production and immune function during stress [18]. Advances in assay sensitivity have improved the ability to measure salivary CRH, offering a promising avenue for exploring its contributions to stress physiology.

2.3.3 Dehydroepiandrosterone (DHEA): A counterbalance to cortisol

In addition to cortisol and CRH, hormones such as dehydroepiandrosterone (DHEA) have been explored in relation to stress. DHEA, an adrenal hormone, is involved in counterbalancing the effects of cortisol. Elevated DHEA levels are associated with resilience and adaptive stress responses, while low levels are linked to chronic stress and burnout.

Studies have shown that higher salivary DHEA levels correlate with better mood states and lower perceived stress [19]. Morgan et al. found that individuals exposed to acute stress exhibited increases in DHEA, suggesting its role in mitigating stress effects and promoting psychological well-being [20].

2.3.4 Thyroid hormones: Regulators of metabolism and stress sensitivity

Thyroid hormones, including thyroxine (T4) and triiodothyronine (T3), play critical roles in regulating metabolism, energy production, and overall physiological stability and brain health [21]. These hormones also influence how the body copes with stress. Hypothyroidism (low thyroid activity) is often associated with fatigue, depression, and a blunted stress response, while hyperthyroidism (excess thyroid activity) can heighten sensitivity to stress and anxiety [21]. Thyroid hormone levels can fluctuate during stress, influenced by interactions between the hypothalamic–pituitary–thyroid (HPT) axis and the HPA axis. Stress can impact thyroid function, potentially leading to alterations in hormone levels [22]. While salivary thyroid hormone measurements are less common, emerging techniques are enabling more accurate detection of free T3 and T4 in saliva, offering potential for studying how thyroid function modulates resilience and stress adaptation [23].

2.3.5 Sex hormones: Estrogen, progesterone, and testosterone

Sex hormones play multifaceted roles in stress physiology, influencing both the neuroendocrine and immune systems. Salivary measurements of these hormones allow researchers to explore their contributions to stress responses without invasive procedures.

Estrogen has anti-inflammatory properties and is known to modulate HPA axis activity. During stress, estrogen can attenuate the cortisol response, reducing the physiological burden of stress [24]. Fluctuations in estrogen levels—such as during menopause or the menstrual cycle—can alter stress sensitivity. Low estrogen levels are linked to increased inflammation and heightened stress reactivity, contributing to conditions like anxiety and depression [25].

Progesterone works synergistically with estrogen to regulate the stress response. It has calming effects on the nervous system, often referred to as a neuroprotective

hormone. Progesterone modulates immune responses, helping to prevent excessive inflammation during stress [26]. Variations in salivary progesterone levels during the menstrual cycle and pregnancy provide insights into how hormonal shifts influence stress resilience.

Testosterone plays an essential role in regulating aggression, motivation, and energy levels during stress. Higher levels of testosterone are associated with a more robust stress response, particularly in physical challenges [27]. Salivary testosterone levels fluctuate with acute stress and have been linked to resilience and recovery. Imbalances in testosterone—such as those occurring with aging—can exacerbate the negative effects of stress [28].

The interplay between thyroid and sex hormones with cortisol and CRH highlights the complexity of the body's stress regulation systems. For example, thyroid dysfunction can amplify cortisol dysregulation [29], while hormonal shifts during menopause or andropause may exacerbate inflammation and stress sensitivity [30]. Understanding these relationships is crucial for identifying at-risk populations and developing targeted interventions.

2.4 Neuropeptides

Neuropeptides are small, protein-like signaling molecules that play critical roles in the nervous system by regulating stress responses, emotional states, and immune functions. Unlike classical neurotransmitters, neuropeptides often have longer-lasting effects, binding to specific receptors to initiate or modulate physiological changes across multiple systems [31]. They are key mediators in the body's adaptive mechanisms to both acute and chronic stress and are increasingly recognized as biomarkers of resilience. Recent advancements have enabled the non-invasive measurement of neuropeptides in saliva, an accessible medium that reflects dynamic changes in the neuroendocrine system [32]. Salivary neuropeptide analysis offers insights into the complex interplay between the nervous, endocrine, and immune systems, making it a powerful tool for understanding stress responses and resilience mechanisms [33].

Neuropeptide Y (NPY) is one of the most extensively studied neuropeptides in the context of stress. Released from sympathetic nerve endings during activation of the hypothalamic-pituitary-adrenal (HPA) axis, NPY regulates cardiovascular function, appetite, and emotional behaviors while mitigating excessive stress responses [34]. Elevated levels of salivary NPY are associated with acute stress, reflecting activation of the sympathetic nervous system. Morgan et al. (2002) found that higher NPY levels in Special Forces soldiers undergoing extreme survival training correlated with improved stress tolerance and cognitive performance, highlighting its role in stress resilience [35]. Mechanistically, NPY reduces inflammation by downregulating pro-inflammatory cytokines and modulates emotional responses through its actions on Y1 and Y2 receptors in brain regions like the amygdala [34].

Substance P (SP) is another neuropeptide closely linked to stress responses, immune function, and pain perception. Released by sensory neurons, SP is a pro-inflammatory mediator that exacerbates immune responses during chronic stress [36]. Salivary SP levels rise under stress conditions, correlating with immune activation and inflammatory processes. It has been demonstrated that elevated SP levels triggered inflammation and behavioral changes in chronic stress models [37]. SP exerts its effects by binding to the neurokinin-1 receptor (NK1R), which amplifies cytokine release, linking psychological stress to inflammatory diseases such as arthritis and inflammatory bowel disease (IBD) [36].

Endorphins, often referred to as the body's "natural opioids," are neuropeptides released in response to stress, exercise, and pain. They bind to opioid receptors in the brain to reduce pain perception and produce a sense of euphoria, often described as a "runner's high" [38]. Elevated salivary endorphin levels have been detected following physical activity and acute stress exposure, reflecting their role in promoting stress resilience [39]. Mechanistically, endorphins modulate the HPA axis by inhibiting excessive cortisol release, thereby reducing the physiological burden of stress [39]. Their ability to alleviate emotional distress and enhance mood makes them essential mediators in stress recovery.

Neurotensin is a neuropeptide that acts as both a neurotransmitter and neuromodulator, playing roles in stress adaptation, emotional regulation, and immune response [40]. Salivary neurotensin levels have been found to rise during acute stress, reflecting its role in modulating the stress response. Neurotensin interacts with dopamine pathways in the brain, contributing to emotional balance and resilience [41]. Additionally, neurotensin reduces inflammation by inhibiting the release of pro-inflammatory cytokines, positioning it as a protective factor against stress-induced immune dysregulation [42]. Further studies are needed to fully elucidate the role of salivary neurotensin in chronic stress and its potential as a biomarker for emotional regulation.

2.5 Cytokines

Cytokines are small proteins released by cells, particularly those of the immune system, and play a pivotal role in cell signaling. Pro-inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) are integral to the body's response to stress and inflammation [43].

Stress can modulate immune function by influencing cytokine production. Elevated levels of IL-6 and TNF- α in saliva have been associated with acute stress, reflecting activation of inflammatory pathways. These cytokines contribute to the development of stress-related symptoms and are implicated in the pathophysiology of various disorders, including cardiovascular disease, depression, and autoimmune conditions [44].

Measuring salivary cytokines provides a window into the neuroimmune interactions during stress. It allows for the assessment of how psychological factors can influence immune function and contribute to disease processes. Understanding these interactions is crucial for developing interventions aimed at mitigating the negative health effects of stress and increasing resilience [45].

In summary, saliva serves as an excellent medium for biomarker research due to its non-invasive collection methods and its ability to reflect real-time physiological changes. Neuropeptides, hormones along with cytokines such as IL-6 and TNF- α , are key salivary biomarkers that provide valuable insights into the body's stress response and inflammatory processes. The study of these biomarkers enhances our understanding of the mechanisms underlying stress and resilience, ultimately contributing to better health outcomes.

3. Stress, neuropeptides, and cytokines: Mechanistic insights

3.1 Correlation between cortisol levels and cytokine fluctuations during acute stress

The body's response to acute stress involves an intricate coordination between the neuroendocrine and immune systems. A key feature of this response is the activation

of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in the release of cortisol, a central stress hormone. Elevated cortisol levels have been consistently linked with fluctuations in pro-inflammatory cytokines, particularly interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) [46, 47].

Research has demonstrated that acute psychological stress induces a transient spike in salivary cortisol, which closely mirrors cytokine fluctuations, suggesting a tightly regulated immune-endocrine interaction [11, 48]. Higher IL-6 and TNF- α levels observed during acute stress suggest a temporary inflammatory response designed to enhance immune surveillance [49, 50]. This interplay highlights cortisol's dual role as both a modulator of immune function and a key biomarker of stress intensity.

3.2 Association between cytokine profiles and resilience to stress

While acute stress responses can be adaptive, chronic or maladaptive stress leads to cytokine dysregulation, which can compromise stress resilience [51, 52]. Resilience, or the ability to recover from stress, has been associated with anti-inflammatory cytokines, such as interleukin-10 (IL-10), which counteracts the effects of pro-inflammatory markers like IL-6 and TNF- α [53, 54].

Individuals with higher resilience tend to maintain a balanced cytokine profile, marked by lower levels of chronic inflammation [55, 56]. This equilibrium is crucial for preventing long-term stress-related pathologies, including autoimmune diseases and metabolic disorders [57]. Emerging evidence suggests that cytokine ratios, particularly IL-10 to IL-6, may serve as predictive biomarkers for an individual's resilience capacity [58, 59].

3.3 Acute vs. chronic stress impacts

The physiological effects of acute vs. chronic stress differ significantly, particularly in neuropeptide and cytokine dynamics [49, 50]. Acute stress leads to a temporary increase in cortisol and pro-inflammatory cytokines, which helps mobilize the body to respond to challenges [51]. However, chronic stress results in persistent HPA axis activation, leading to sustained elevations in IL-6 and TNF- α , which are implicated in cardiovascular disease, depression, and neurodegeneration [52, 53]. Prolonged stress exposure disrupts cortisol's diurnal rhythm, causing immune dysregulation and increasing susceptibility to chronic inflammation [54, 55]. Distinguishing between acute and chronic stress responses is essential in clinical and research settings to design targeted interventions [56].

3.4 Variations in biomarker responses

Not all individuals respond to stress the same way. Biomarker responses vary due to age, sex, genetic predisposition, and past trauma [46, 47]. For example, research indicates that individuals with a history of early-life trauma exhibit heightened cytokine responses during acute stress, suggesting a priming effect on the immune system [11, 48]. Genetic variations in glucocorticoid receptors (NR3C1) and cytokine-signaling pathways further contribute to individual differences in stress susceptibility and resilience [60, 61]. These findings reinforce the need for personalized approaches in biomarker research and stress-related healthcare [62].

3.5 Long-term health implications of cytokine dysregulation

Chronic cytokine dysregulation has far-reaching implications for physical and mental health. Prolonged elevations in IL-6 and TNF- α are associated with metabolic syndrome, autoimmune diseases, and cancer progression [57, 63]. For example, Zhang et al. [64] demonstrated that chronic inflammation drives insulin resistance, a hallmark of metabolic dysfunction [58]. Similarly, Dinarello [65] highlighted that sustained cytokine elevation can disrupt immune homeostasis, contributing to autoimmune disorders [59]. Additionally, inflammation is implicated in tumor progression, with evidence showing that cytokine-driven changes in the tumor microenvironment facilitate cancer development [49].

In psychiatric research, Dantzer et al. [56] showed that elevated IL-6 and CRP levels are associated with depressive symptoms, supporting a neuroimmune link in mood disorders [50]. Similarly, Hori and Kim [66] reviewed inflammatory markers in PTSD, demonstrating that chronic inflammation exacerbates both physiological and psychological symptoms [51].

The interplay between stress, neuropeptides, and cytokines is a rapidly evolving field with critical implications for understanding disease mechanisms and resilience-building strategies [52, 53]. Future research should explore personalized interventions, such as anti-inflammatory therapies, cognitive-behavioral strategies, and lifestyle modifications, to mitigate the long-term effects of chronic stress [47, 54].

4. Interplay between trauma, stress, and salivary biomarkers

4.1 How previous trauma influences biomarker levels

Trauma, whether experienced in childhood or adulthood, has profound and lasting effects on stress response systems. Exposure to trauma can lead to dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis and the immune system, which are critical in the body’s response to stress [49, 66]. Salivary biomarkers, including cortisol and pro-inflammatory cytokines, provide valuable insights into these changes, offering a measurable link between past trauma and stress-related physiological adaptations [53]. Research has consistently demonstrated that individuals with a history of significant trauma exhibit elevated levels of salivary interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) following stress exposure [52, 67]. This exaggerated inflammatory response may stem from the “priming” of the immune system, where previous trauma sensitizes immune cells to future stressors [68].

Supporting this, Hori and Kim [66] reviewed the connection between PTSD and inflammation, showing that individuals with post-traumatic stress disorder (PTSD) consistently exhibit higher levels of IL-6, C-reactive protein (CRP), and other inflammatory markers [48]. Their findings suggest that the heightened inflammatory state in PTSD contributes to comorbidities such as cardiovascular and metabolic disorders [62]. Similarly, Pace et al. [67] explored trauma-induced immune dysregulation in women with PTSD, finding significantly higher basal levels of pro-inflammatory cytokines (TNF- α , IL-6) compared to controls [52]. These cytokines were further elevated after acute stress, confirming the role of prior trauma in immune system priming [49].

In a landmark study, Danese et al. [52] examined the long-term effects of childhood maltreatment on inflammation, revealing that adults with early life adversity

had persistently elevated CRP levels, which is a key marker of systemic inflammation [68]. Their results reinforce the idea that trauma during critical developmental periods has lasting effects on immune pathways, increasing vulnerability to stress-related diseases later in life [57]. Moreover, Slavich and Irwin [49] investigated biobehavioral pathways linking stress and inflammation, emphasizing how social stressors (e.g., conflict, rejection) activate the HPA axis and the sympathetic nervous system. This activation leads to increased IL-6 and TNF- α , exacerbating chronic inflammatory states in individuals with a history of trauma [58]. Finally, Miller and Cole (2012) found that early-life stress predisposes individuals to a chronic pro-inflammatory phenotype, characterized by exaggerated cytokine responses to future social stressors [59]. These findings suggest that prior trauma alters immune function, leading to a heightened inflammatory reactivity in response to everyday stressors [49].

Together, these studies highlight a consistent pattern: trauma, particularly when experienced early or repeatedly, primes the immune system, resulting in exaggerated inflammatory responses to later stressors. This chronic inflammatory state contributes not only to immediate physiological effects but also to long-term health risks, including depression, anxiety, autoimmune diseases, and cardiovascular disorders [50, 51].

4.2 Connections to stress resilience and vulnerability

Not all individuals with a history of trauma exhibit maladaptive stress responses, underscoring the critical role of resilience in shaping physiological outcomes [52]. Resilience acts as a buffer against the negative effects of stress, influencing the regulation of salivary biomarkers, including cortisol and cytokines [53]. Studies have shown that resilient individuals tend to maintain a balanced cytokine profile, even when exposed to acute stress [54]. For example, higher ratios of anti-inflammatory cytokines (IL-10) to pro-inflammatory cytokines (IL-6) are commonly observed in those with greater psychological resilience [55]. This balance mitigates the harmful effects of inflammation, supporting faster recovery from stress [56].

Conversely, individuals with low resilience or high vulnerability often exhibit persistently elevated pro-inflammatory biomarkers, reflecting a chronic state of immune activation that worsens health outcomes [46]. Understanding these differences in biological resilience mechanisms may inform targeted interventions to improve stress adaptation [47].

4.3 Exploration of cytokine-mediated inflammatory responses in stress contexts

Cytokines play a central role in mediating stress-induced inflammation. Acute stress activates the HPA axis and the sympathetic nervous system, leading to cortisol and catecholamine release, which subsequently influence cytokine production [48]. While this response is adaptive in the short term, chronic stress or unresolved trauma can lead to sustained cytokine dysregulation [11].

Neuroimmune research has established that elevated salivary IL-6 and TNF- α are not just markers of stress, but also contributors to its negative physiological and psychological effects [60]. These cytokines have been strongly implicated in the development of mood disorders (depression, anxiety) and chronic health conditions (cardiovascular disease, autoimmune disorders) [61]. A notable finding in resilience research is the dynamic nature of cytokine responses. While IL-6 levels typically peak after stress exposure, resilient individuals show a faster return to baseline compared

to their vulnerable counterparts [62]. This rapid resolution of inflammation is a hallmark of adaptive stress responses, underscoring the importance of maintaining cytokine balance for long-term health [63].

The interplay between trauma, stress, and salivary biomarkers highlights the complex interactions between the neuroendocrine and immune systems. Trauma sensitizes the body's stress response mechanisms, often resulting in exaggerated inflammatory reactivity to future stressors [57]. However, resilience can buffer these effects, promoting a balanced cytokine profile and healthier stress responses [64]. Salivary biomarkers serve as powerful tools for assessing the long-term impacts of trauma and stress, offering objective measures of stress resilience [65]. Future research should focus on targeted interventions, such as stress management strategies, anti-inflammatory therapies, and behavioral interventions, to mitigate the long-term effects of chronic stress and trauma.

5. Methodological framework for salivary research

5.1 Protocols for saliva collection and storage

In resilience-based research, salivary monitoring unlocks a new potential for deeper analysis with a highly accurate and quantifiable measurement of the stress response. Growing interest in this field has driven the development of diverse and innovative methodologies to evaluate the molecular and microbial breakdown of saliva [8]. These developments unlock a whole new landscape with applications in both research and clinical sectors.

To begin any saliva-based project, researchers must determine how to collect the saliva sample. Spitting, drooling, absorption, and suction-based collection methods seem to be the most utilized with a spitting collection method prevailing, possibly due to ease of collection [69, 70]. This can then be further augmented by salivary stimulation if desired. As a combination of both parasympathetic (production) and sympathetic (release) nerve activity, salivation can be stimulated through various pathways [71]. While one method has not been shown superior over another, the most used ones include oral-based paraffin wax or sugar-free gum, and smell-based stimulation [70]. One study found that biomarkers such as DHEA, testosterone, estradiol, progesterone, and sIgA produced statistically significant differences ($p < 0.005$) when using cotton-based absorption methods compared to cotton-free methods. Cortisol, however, was not affected, suggesting that a selected collection method may introduce a significant source of unsystematic error [72]. Therefore, it is highly recommended to follow one collection method throughout a study and participants are given proper guidance to minimize error.

Samples may be stored at room temperature for a maximum of 30–90 minutes if analysis is to be performed immediately [73]. As expected though, storage at lower temperatures, for shorter periods of time seems to best preserve accurate salivary biomarker concentrations. Thus, samples should be refrigerated or put on ice immediately after collection [9]. For long term storage, temperatures should be kept in ultra-low ranges, as most analytes have been shown to have increased stability at temperatures less than -80°C [74, 75]. While the maximum length of storage is highly biomarker dependent, storage for less than one month is recommended as the standard until further analysis can offer more solute-specific guidance [75]. Repeated cycles of thawing and re-freezing should not raise concern with salivary cortisol

samples as it has been shown to have no effect on concentration levels, however, efforts should be made with other biomarkers, such as cytokines, which demonstrate a necessity to minimize freezing and thawing cycles [76, 77].

5.2 Assay methods for cortisol and cytokine measurement

As salivary research becomes more prevalent, so has the need for a cost effective, high-throughput analysis platform. Methods of measurement generally fall into two categories: Immunoassays and Liquid Chromatography-Mass Spectrometry (LC-MS/MS).

Radioimmunoassay (RIA), Enzyme immunoassay (EIA), and Enzyme-linked immunosorbent assay (ELISA) are considered gold standard due to their high sensitivity, high throughput, and low-cost [78]. Immunoassays utilize antigen-antibody interactions to detect target molecules, even at ultra-low concentrations. They are commonly used to measure salivary cortisol due to their high sensitivity, allowing quantification even at the nadir of the diurnal rhythm [79]. However, they are not without compromise. Most notably, the analytical specificity may be limited by antibody cross-reactivity with other steroids and even matrix effects.

LC-MS/MS serves the most accurate method of choice for measuring biological samples with a highly sensitive and specific analysis, not plagued by cross-reactivity concerns. Its use, however, is limited due to numerous practical constraints including high cost and limited availability [80].

When determining which assay to use there is a balance between analytical precision and diagnostic utility that must be considered. For example, from an analytical perspective mass spectrometry offers the best method to measure the true value of cortisol in a sample [79]. From a diagnostic value, however, the concerns of cross-reactivity of cortisol metabolites may be an advantage for detection of overall glucocorticoid production [81]. This nuance highlights the importance of understanding specific biomarker interrelationships when setting research design. These methods may also be used to analyze cytokine and neuropeptide salivary concentrations, however, due to differences in specificity it has been recommended that an analyte-specific approach be considered to optimize precision and accuracy [77].

In recent years immunosensors have gained traction exhibiting advantages of broader application performing outside the lab by nonexperts. Working similarly to immunoassays – binding antibody and a corresponding antigen – immunosensors build off immunoassays by forming the immunocomplex on the same platform [82]. Distribution of an easy to use, immediate measurement device shows promise to improve analysis pathways simplifying research methods and consequently, reducing chances for error. While still a novel and immature platform, future studies and clinical applications may benefit from this new technology [78].

PH may also be used as an inexpensive means to assess physiological response to stressors and be measured using digital pH meters or pH test strips, depending on research requirements [83].

5.3 Addressing variability in salivary biomarker studies

The relationship between biomarkers and their respective concentrations in saliva is complex and not well understood. While the concentration of a biomarker in saliva is often correlated with levels in the bloodstream, the degree of correlation is specific for each biomarker and influenced by both physiological and methodological factors

such as molecular size, protein binding, and the mechanism of transfer. Additionally, salivary flow rate can dilute or concentrate biomarker levels, affecting the reliability and reproduction of studies.

Fat soluble molecule concentrations, including steroids like cortisol, have been shown to be influenced by different collection methods even when controlling for individual variations. This is then further confounded by the time of collection where a significant variation in salivary cortisol levels is present throughout the day [84, 85].

Despite increased saliva sample size with stimulation, care must be taken to account for the effects stimulation on salivary samples. With regards to the gold-standard of the human stress response, cortisol diffuses passively into saliva independent of salivary flow rate, thus stimulation is not necessary [86]. On the other hand, a stimulated collection has been shown to influence the microbiota composition which may alter the hosts' stress response leading to downstream cortisol influence [87, 88]. It should also be noted that eating, drinking, and teeth brushing immediately before collection may cause micro-abrasions that should also be controlled for and recommended against [89]. While an optimal method of collection has not yet been established, maintaining a consistent collection method with any saliva-based research will offer the most consistent results [90]. This is especially true when using multi-panel assays and conducting research with multiple analytes as different biomarkers may reflect true plasma/serum at different rates, especially in the context of salivary excretion.

6. Applications and case studies

6.1 Implications for clinical diagnostics: Using salivary biomarkers to predict stress resilience

Applications for the measurement of stress resilience extend beyond just the research landscape. Higher levels of resilience have been associated with increased health, social and emotional intelligence, and a higher outlook on life which ultimately prevents the onset of disease [91]. Identifying individuals with poor coping mechanisms and an inability to process stress is paramount in the prevention of downstream effects. Currently, the most utilized and reliable method to assess resilience is self-report methods, but not without bias and challenges. Sociological factors, such as prior life trauma, have been proven to be associated with a poor stress response and offer some insight, but the stress response is highly individualized [92, 93]. Consequently, these questionnaires offer little or no predictive utility [94].

The insight to be gained by a direct, quantifiable measurement in the assessment of resilience can lead to improvements in a person's quality of life [91, 95]. As a non-invasive collection, saliva-based assays may be distributed widely to offer new point-of-care solutions to evaluate stress resilience.

Salivary cortisol, shown to be equitable to blood cortisol, serves as a hallmark marker for the establishment of the stress response [89]. The response is highly individualized, and different approaches have been presented in attempt to model this response [96]. In the application of stress, cortisol levels increase in response to stress and decrease during times of recovery. This allows for quantification of the stress response in an individual through repeat measurements. Just the ability to quantify this response and provide information has shown to provide placebo for inducing a lower cortisol response, a quality associated with higher resilience [97].

As a known regulator in the acute stress response, cytokines and growth factors serve roles in cell signaling including modulation of the inflammatory response. Both IL-6, a pro-inflammatory, and IL-10, an anti-inflammatory, have been successfully used to predict therapeutic success in individuals with PTSD [98]. IFN-gamma levels have also been identified as a potential predictive biomarker for early-onset post stroke depression (PSD) and linked to increased activation of the HPA axis and further downstream effects of chronic inflammation [99]. Other growth factor molecules have also been linked with individual resilience. Salivary epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF)-AA have been shown to be significantly correlated with resilience scores. As predictive factors, decreases in EGF and PDGFAA during acute stress and increases in VEGF during recovery after the acute stress response are all protective for resilience [45].

Neuropeptides also serve to regulate the stress response and have been shown to predictive resilience. Polymorphisms in the neuropeptide Y gene are linked with impaired stress processing and exogenous neuropeptide Y prevents the negative consequences of stress [100]. Oxytocin, generally known as the love hormone, responsible for social bonding, may also offer predictive value. In prolonged repeated stress, oxytocin seems to precede increases in cortisol in response to anticipated stress [101]. Further studies have shown that oxytocin serves a critical process regulating the stress response, consequently elevated baseline levels display protective effects during stress events [102]. Further neuropeptides, such as alpha-MSH, Beta-Endorphin, Neurotensin, and Substance P also offer insight to resilience as they are highly correlated with oxytocin across the acute stress response [12].

6.2 Potential for identifying individuals at risk for chronic stress-related conditions

The development of the stress response is ever-evolving, beginning in the prenatal period extending throughout life [103, 104]. The stress response is recalibrated during puberty to match current environmental conditions and may recalibrate later in life again [105]. From an epigenetic perspective, early-life stressors produce long-lasting memory with impact on organismal resilience and longevity [106]. Evidence shows focus on resilience and coping methods early on have positive impacts on children's ability to manage daily stressors [107]. Attending to this often-overlooked aspect of personal development may lead to an increased quality of life and longevity, as well [108, 109].

In acute stress, activation of the HPA axis leads to the downstream release of many protective biomarkers. Chronic stress exposure, however, combined with suboptimal stress regulation and attenuation may predispose individuals to chronic stress-related conditions due to the disruption of the natural balance between hormones, neuropeptides, cytokines, neurotransmitters, and other markers in the body [96, 110]. This places those exposed to extended stressors and those with poor resilience at increased risk for developing chronic stress-related conditions.

Prolonged elevation of cortisol levels, as seen in chronic stress states, have been associated with an increased risk of cardiovascular disease and stroke, as glucocorticoids play a critical role in regulating inflammatory, proliferative, and remodeling responses to injury and vascular occlusion [111]. Notably, sustained cortisol elevations in the months preceding an acute myocardial infarction have been observed, suggesting a potential role for cortisol as a predictive risk factor for MI [112]. The

effects of a chronic elevation in cortisol levels extend to dental health as well. Elevated cortisol has been recognized as a significant predictor of poor dental health, with associations found between heightened cortisol levels and increased plaque accumulation and gingivitis [113]. Beyond these physiological effects, higher cortisol levels have been significantly linked to suicidal outcomes, further highlighting the broad impact of dysregulated stress responses on both physical and mental health [114].

Salivary lysozyme, α -amylase (sAA), chromogranin A (CgA), immunoglobulin-A (sIgA) also show promise as markers of stress with strong relationships to physiological stress [115, 116]. These have been associated with systemic autoimmune diseases as well, including Sjogren's, myasthenia gravis, autoimmune pancreatitis, and Diabetes Type 2 [117–119]. Their altered expression in response to chronic stress suggests a potential role in early disease detection, as persistent dysregulation may contribute to immune dysfunction and exacerbate inflammatory processes. Additionally, variations in these biomarkers may help differentiate between acute and chronic stress responses, offering insights into disease progression and individualized treatment strategies.

Inflammatory markers play a crucial role in the stress response, though prolonged elevation has been linked with chronic, systemic autoimmune disorders. IL-1 β , TNF- α , and IL-6, have been reliably determined from saliva and have increased significantly in response to stress across multiple studies [120]. Dysregulation of pro-inflammatory cytokines, such as IL-6, have been linked to numerous pathological roles in the development for cancers, inflammatory and autoimmune diseases. Developing later in life, individuals are at increased risk for neuroinflammatory diseases including multiple sclerosis, amyotrophic lateral sclerosis, Alzheimer's disease, neuromyelitis optica, and autoimmune encephalitis [121, 122]. TNF- α is a pro-inflammatory cytokine that has been implicated in the pathogenesis of autoimmune diseases including inflammatory bowel disease, psoriatic arthritis, and rheumatoid arthritis [123, 124]. IL-10, serving as an anti-inflammatory, displays an increase in risk for autoimmune disease, especially inflammatory bowel disease, especially in chronic dysregulation [125].

7. Conclusion

The study of salivary biomarkers has emerged as a critical avenue for advancing our understanding of the physiological mechanisms underlying stress and resilience. As a non-invasive and dynamic biological fluid, saliva provides a unique window into neuroendocrine and immune processes, allowing for the real-time assessment of molecular responses to acute and chronic stress and provides a snapshot of an individual's nervous system tone. The integration of salivary neuropeptides, hormones, and cytokines in stress research has facilitated the identification of key regulatory pathways involved in stress adaptation, focusing on the intricate interplay between the hypothalamic-pituitary-adrenal (HPA) axis, autonomic nervous system, and immune function (See **Table 1**. for a summary of biomarkers discussed in this chapter).

The findings presented in this chapter highlight the utility of salivary biomarkers in distinguishing between adaptive and maladaptive stress responses. Hormones such as cortisol, dehydroepiandrosterone (DHEA), and corticotropin-releasing hormone (CRH) serve as robust indicators of HPA axis activity, while neuropeptides such as

Biomarker	Primary function	Type of stress response	Clinical implications	Measurement method
Cortisol	HPA axis regulation, energy mobilization	Acute & chronic; adaptive/maladaptive	Anxiety, depression, cardiovascular risk	ELISA, LC-MS/MS
DHEA	Counteracts cortisol; promotes resilience	Acute & adaptive	Burnout, mood regulation, psychological resilience	Immunoassay, LC-MS/MS
CRH	Initiates HPA axis response	Acute	Inflammation modulation, PTSD, anxiety	High-sensitivity immunoassay
IL-6	Pro-inflammatory cytokine	Acute & chronic	Depression, CVD, autoimmune diseases	ELISA, multiplex cytokine panels
TNF- α	Pro-inflammatory cytokine	Acute & chronic	Chronic inflammation, autoimmune diseases	ELISA, multiplex cytokine panels
IL-10	Anti-inflammatory cytokine	Adaptive	Predictor of stress resilience and recovery	ELISA, multiplex cytokine panels
Neuropeptide Y	Modulates sympathetic tone, emotional regulation	Acute & adaptive	Resilience, stress tolerance, PTSD resilience	Immunoassay, LC-MS/MS
Substance P	Pain perception, immune activation	Chronic; maladaptive	Linked to inflammation, IBD, arthritis	Immunoassay
Oxytocin	Social bonding, stress buffering	Acute & adaptive	Protective in chronic stress, predictive of resilience	ELISA, emerging immunoassays
Endorphins	Pain relief, mood elevation	Acute & adaptive	Emotional recovery, resilience	ELISA, immunoassays
Neurotensin	Emotional regulation, anti-inflammatory	Acute	Inflammation modulation, emotional balance	Immunoassay
Testosterone	Aggression, motivation, energy	Acute	Stress adaptation, aging-related decline	Immunoassay, LC-MS/MS
Estrogen	HPA modulation, anti-inflammatory	Adaptive	Menopause-related stress, anxiety, depression	Immunoassay, LC-MS/MS
Progesterone	Neuroprotection, immune modulation	Adaptive	Hormonal resilience, immune balance	Immunoassay, LC-MS/MS
Thyroid Hormones (T3/T4)	Metabolic regulation, brain function	Chronic stress modulation	Fatigue, depression, metabolic syndrome	LC-MS/MS
sIgA, α -amylase	Immune defense, sympathetic activity indicator	Acute	Autoimmune risk, chronic stress exposure	ELISA, point-of-care biosensors

Table 1.
Summary of key salivary biomarkers in stress and resilience.

substance P, oxytocin and endorphins contribute to the modulation of emotional and physiological resilience. Additionally, cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) provide insights into the inflammatory consequences of stress exposure, reinforcing the concept that chronic stress may drive immune dysregulation and subsequent disease pathogenesis.

Beyond their role in elucidating the mechanistic basis of stress and resilience, salivary biomarkers hold considerable promise for translational applications in clinical diagnostics and personalized medicine. Their non-invasive nature and real-time sensitivity to stress-induced physiological changes make them particularly well-suited for longitudinal studies and early detection and monitoring of individuals at risk or in early stages of stress-related pathologies. Additionally, the refinement of salivary assay methodologies, including high-sensitivity immunoassays and mass spectrometry-based approaches, will further enhance the specificity and reliability of these biomarkers in research and clinical settings.

Future research should aim to integrate multi-omics approaches, incorporating genomics, proteomics, and metabolomics to construct a more comprehensive biomarker signature of stress resilience and stress induced system breakdown. Furthermore, repeated measures studies are needed to elucidate the long-term health implications of salivary biomarker fluctuations and their potential role in predicting susceptibility to stress-related disorders. Through advances in our understanding of salivary neuropeptides and cytokines, this field continues to bridge the gap between molecular stress-based research and clinical intervention, offering novel insights into resilience mechanisms and strategies for mitigating the unfortunate adverse effects of chronic stress.

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Section 2

Clinical Applications and Oral Diseases

Xerostomia and Its Impact on Oral Health

Younjung Park

Abstract

Xerostomia, known as dry mouth, has significant implications for systemic and oral health. It is frequently associated with conditions such as Sjögren's syndrome, medication side effects, radiation therapy, and various systemic diseases. These factors contribute to salivary dysfunction, leading to oral discomfort and an increased risk of infections, including oral candidiasis. Reduced saliva flow can result in dental caries, mucosal dryness, altered taste, and difficulties with swallowing, all of which can substantially diminish a patient's quality of life. This chapter explores xerostomia's systemic and oral health impacts, focusing on conditions like Sjögren's syndrome, where autoimmune-mediated glandular dysfunction results in persistent dryness and secondary complications. Diagnostic evaluation involves subjective assessments, salivary flow testing, imaging, and laboratory analysis to differentiate true hyposalivation from perceived dryness. Management strategies emphasize early identification, tailored interventions, preventive care, incorporating saliva substitutes, sialogogues, antifungal therapy, and interdisciplinary collaboration. This chapter aims to enhance clinical understanding of xerostomia's far-reaching effects by addressing fungal infections and other consequences of salivary dysfunction. A comprehensive, patient-specific approach is essential to improving treatment outcomes and long-term oral health.

Keywords: xerostomia, dry mouth, salivary dysfunction, oral candidiasis, Sjögren's syndrome

1. Introduction

Xerostomia refers to the subjective sensation of dry mouth, while hyposalivation is an objectively measured decrease in salivary flow. Although often related, only about 37% of xerostomia patients exhibit actual salivary reduction [1]. Hyposalivation is diagnosed through sialometry, whereas xerostomia is assessed via patient-reported symptoms. Xerostomia affects 0.9–64.8% of the general population, with a higher prevalence in older adults (30% over 65, 40% over 80) [2, 3]. It is commonly linked to chronic diseases (uncontrolled diabetes, hyper- and hypothyroidism, autoimmune disorders), medication use (anticholinergics, antidepressants, antihypertensives), and radiation therapy.

Saliva protects oral tissues, aids digestion, prevents infections, and facilitates remineralization [4]. Reduced saliva flow leads to caries, periodontal disease, oral infections, dysphagia, and impaired healing, significantly affecting quality of life.

Xerostomia is a key concern in oral medicine, increasing risks for dental decay, candidiasis, speech/swallowing difficulties, impaired sense of taste, and chronic discomfort. Early diagnosis and multidisciplinary management are crucial to improving patient outcomes.

2. Etiology and pathophysiology of xerostomia

2.1 Diseases causing oral dryness

Several systemic diseases contribute to xerostomia by directly or indirectly impairing salivary gland function. Autoimmune disorders, particularly Sjögren's syndrome, are among the leading causes. In Sjögren's syndrome, lymphocytic infiltration progressively destroys the salivary glands, leading to severe and often irreversible dry mouth [5]. Other autoimmune conditions, such as systemic lupus erythematosus and rheumatoid arthritis, also contribute to chronic inflammation that may affect glandular function and reduce saliva production [6, 7].

Metabolic diseases can also lead to xerostomia through various mechanisms. Diabetes mellitus is commonly associated with dry mouth due to autonomic neuropathy affecting salivary gland regulation and systemic dehydration from increased urinary fluid loss. Chronic kidney disease further exacerbates fluid imbalance, reducing salivary secretion and increasing oral dryness.

Neurological disorders, especially those impacting autonomic nervous system regulation, can impair salivary reflexes and secretion. Conditions like Parkinson's and Alzheimer's disease are known to reduce salivary output, resulting in increased oral dryness and discomfort. In addition, stroke patients often experience salivary gland dysfunction due to neurological damage, which disrupts the normal control of reflexive saliva production.

Psychological conditions such as anxiety and depression are frequently linked to xerostomia, either due to autonomic dysregulation of salivary secretion or as a side effect of psychotropic medications. Chronic stress and prolonged activation of the hypothalamic-pituitary-adrenal (HPA) axis may reduce saliva flow and worsen xerostomia. A comprehensive medical evaluation is essential to identify the underlying causes and inform effective management strategies [8].

2.2 Medication-induced dry mouth

Over 500 medications are linked to xerostomia, primarily due to their impact on salivary gland function and autonomic regulation. A major contributor to this condition is the anticholinergic class of drugs, which includes antidepressants (such as tricyclics, SSRIs, and SNRIs), antipsychotics, and antihistamines. These medications inhibit muscarinic receptors in the salivary glands, resulting in decreased secretion and a persistent sensation of dry mouth [9, 10].

Additionally, certain antihypertensive medications, particularly beta-blockers and diuretics, can contribute to xerostomia by impacting fluid balance and the autonomic regulation of salivation. Diuretics increase urinary fluid loss, which can lead to systemic dehydration, while beta-blockers disrupt the regulation of the autonomic nervous system, contributing to reduced saliva production.

Opioids and muscle relaxants, which are frequently prescribed for chronic pain and neurological conditions, suppress salivary gland activity through central nervous

system depression. This effect can exacerbate oral dryness, particularly in patients already experiencing underlying salivary dysfunction.

Polypharmacy is a major risk factor for medication-induced xerostomia, especially in elderly patients who often take multiple drugs that cumulatively impair salivary gland function [11]. The concomitant use of antihypertensives, psychotropic medications, and analgesics can diminish salivary flow, contributing to oral discomfort, dysphagia, and increased dental complications. A thorough medication review may mitigate these symptoms and enhance salivary function.

2.3 Cancer and cancer treatment-related salivary gland dysfunction

Cancer and its treatments can lead to severe and often persistent xerostomia, primarily due to direct damage to salivary glands and systemic treatment effects. Among the most significant causes is head and neck radiation therapy, which can irreversibly impair salivary gland function. The severity of radiation-induced hyposalivation depends on the total radiation dose, fractionation schedule, and field of exposure, with higher doses often resulting in permanent glandular atrophy and severe dry mouth [12, 13].

While systemic in nature, chemotherapy can also affect salivary function, particularly by targeting rapidly dividing cells, including salivary gland acini. Unlike radiation-induced xerostomia, chemotherapy-related salivary dysfunction is generally reversible after treatment cessation, though some patients may experience temporary dry mouth, altered taste, or mucosal irritation during therapy [14].

Bone marrow transplantation presents an additional risk factor for long-term xerostomia, especially in patients who develop graft-versus-host disease (GVHD). In these instances, the immune system targets the salivary glands, causing chronic inflammation, fibrosis, and progressive dysfunction of the glands. This immune-mediated damage can lead to persistent oral dryness, heightening the risk of secondary infections, causing mucosal discomfort, and resulting in difficulties with speaking or swallowing.

3. Clinical manifestations and oral health implications

3.1 Direct oral effects of xerostomia

Xerostomia significantly affects oral function and comfort, leading to multiple complications. Mucosal dryness often causes a sticky, rough, or burning sensation, making speaking and swallowing difficult. Patients may experience irritation or soreness, particularly when eating spicy or acidic foods, especially in the presence of fissured tongue (**Figure 1**).

A lack of salivary buffering capacity increases the risk of dental caries, as saliva is essential for neutralizing acids and remineralizing enamel. With reduced salivary flow, cervical and root caries progress rapidly, posing a serious threat to dental health.

Saliva also plays a crucial role in taste perception, and its absence can result in dysgeusia, where patients report a metallic or altered taste. This disruption can diminish the enjoyment of food and lead to changes in dietary habits.

Additionally, xerostomia contributes to halitosis (bad breath) due to the proliferation of anaerobic bacteria, which produce volatile sulfur compounds (VSCs). Bacterial accumulation worsens without adequate saliva to cleanse the oral cavity, exacerbating oral malodor and further impacting patient confidence and quality of life.



Figure 1.
Clinical presentation of a tongue with severe xerostomia, showing a fissured and dry surface due to significantly reduced salivary flow.

3.2 Increased susceptibility to oral infections

Oral candidiasis is among the most common opportunistic infections in xerostomia patients, particularly in dentate individuals and those wearing dentures. Candida overgrowth can present in various forms, including pseudomembranous candidiasis (white plaques), erythematous candidiasis (atrophic changes with mucosal redness), and angular cheilitis (cracking and inflammation at the corners of the mouth) (Figure 2).

Reducing salivary clearance and antimicrobial function also increases the risk of periodontal disease. Plaque accumulates rapidly without adequate saliva to wash away food debris and buffer acids, leading to gingival inflammation, periodontal pocket formation, and accelerated bone loss. Patients with xerostomia and pre-existing periodontal disease often experience faster disease progression and more incredible difficulty managing symptoms.

Additionally, xerostomia increases the likelihood of secondary bacterial infections, as a dry environment allows opportunistic pathogens to thrive. This can lead to recurrent stomatitis, bacterial sialadenitis, and painful mucosal ulcerations, further complicating oral health. Preventive measures, early detection, and targeted antimicrobial therapies are essential to minimize these risks and maintain oral microbial balance in xerostomic patients.

3.3 Functional and systemic consequences

Xerostomia affects daily function and overall well-being, leading to difficulty swallowing (dysphagia), nutritional deficiencies, and weight loss due to impaired bolus formation. Speech difficulties arise as saliva is essential for articulation, making pronunciation and communication challenging.

A dry oral environment also contributes to mucosal fragility and delayed wound healing. The absence of salivary protective factors makes the mucosa more susceptible to irritation, ulceration, and infections, particularly in denture wearers and post-surgical patients. Wounds in the oral cavity may take longer to heal, increasing the risk of secondary complications and discomfort.

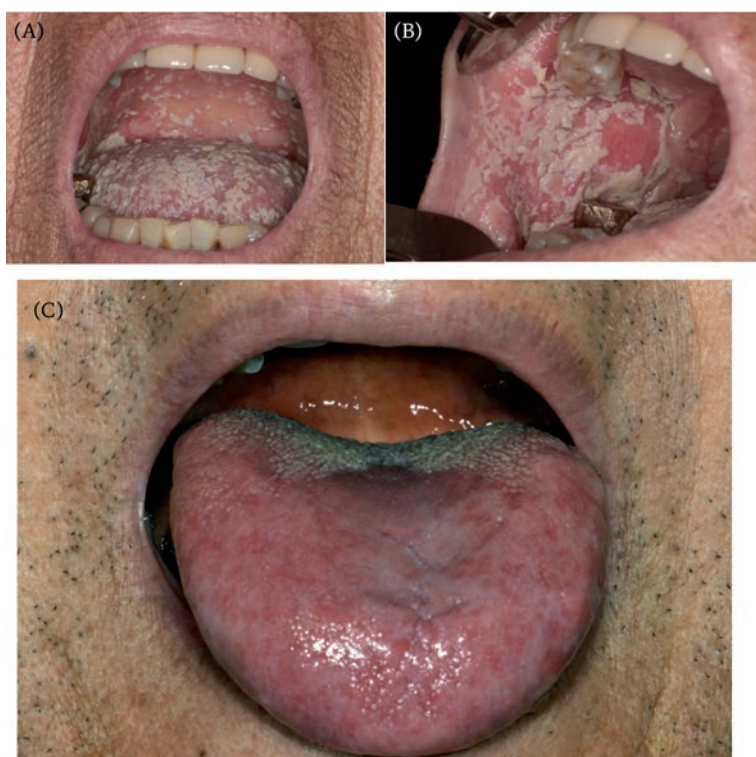


Figure 2.
(A and B) Clinical presentation of pseudomembranous candidiasis, characterized by white plaques that can be wiped off, leaving an erythematous base. (C) Erythematous candidiasis, showing diffuse redness and atrophy, commonly associated with chronic oral mucosal irritation or immunosuppression.

Beyond physical symptoms, chronic dry mouth impacts self-esteem and social interactions, often contributing to anxiety and depression, highlighting the need for comprehensive management [15].

4. Diagnostic approaches

4.1 Subjective assessment

Patient-reported symptoms are essential in diagnosing xerostomia, as subjective dryness does not always correlate with measured salivary flow rates. Standardized questionnaires and clinical interviews help assess symptom severity and its impact on daily life. Validated tools include the Xerostomia Inventory (XI) for evaluating dryness-related symptoms, the Visual Analog Scale (VAS) for dry mouth for quantifying symptom intensity, and the Challacombe Scale, which grades clinical signs of oral dryness.

Key clinical questions, based on the American-European Consensus Group (AECG) criteria for Sjögren's syndrome (2002) [16], include: "Have you had symptoms of dry mouth for at least 3 months?", "Have you experienced recurrent or persistently swollen salivary glands?", and "Do you need to drink liquids to help swallow dry foods?". These targeted questions help identify potential autoimmune causes of xerostomia. Since subjective complaints alone are insufficient to confirm

hyposalivation, objective testing is required to differentiate between true salivary gland dysfunction and perceived dryness.

4.2 Objective salivary flow testing

Measuring salivary output is essential to differentiate true hyposalivation from perceived dryness. Unstimulated whole salivary flow rate (UWS) is assessed after patients refrain from eating, drinking, or smoking for at least 1 h. A normal UWS is ≥ 0.3 mL/min, while hyposalivation is defined as ≤ 0.1 mL/min [17].

Stimulated whole salivary flow rate (SWS) is measured after chewing paraffin wax or using citric acid stimulation. A normal SWS is ≥ 1.0 mL/min, whereas hyposalivation is diagnosed when the flow is ≤ 0.7 mL/min [18].

A significant reduction in both UWS and SWS suggests salivary gland dysfunction. In contrast, normal salivary flow with persistent dry mouth symptoms may indicate neuropathic pain or psychological factors, necessitating further evaluation.

4.3 Imaging and functional assessment of salivary glands

Salivary gland imaging is valuable when systemic or autoimmune diseases are suspected as underlying causes of xerostomia. Sialography evaluates ductal structures and salivary flow obstruction, making it useful for detecting sialolithiasis and ductal abnormalities in Sjögren's syndrome (**Figure 3**). Salivary gland ultrasonography is a non-invasive tool that helps identify glandular atrophy, hypoechogenic areas, and inflammatory changes, particularly in Sjögren's syndrome.

Scintigraphy (salivary gland nuclear imaging) assesses glandular function and secretion dynamics by monitoring the uptake and excretion of technetium-99m pertechnetate, providing insights into functional impairment of salivary flow (**Figure 4**) [19, 20]. Magnetic Resonance Sialography (MR Sialography) offers high-resolution imaging of salivary ducts without requiring contrast injection, making it a useful alternative for evaluating ductal abnormalities in cases where traditional sialography is contraindicated (**Figure 5**) [21]. These imaging modalities help differentiate between functional and structural salivary gland disorders, guiding appropriate diagnosis and treatment planning.

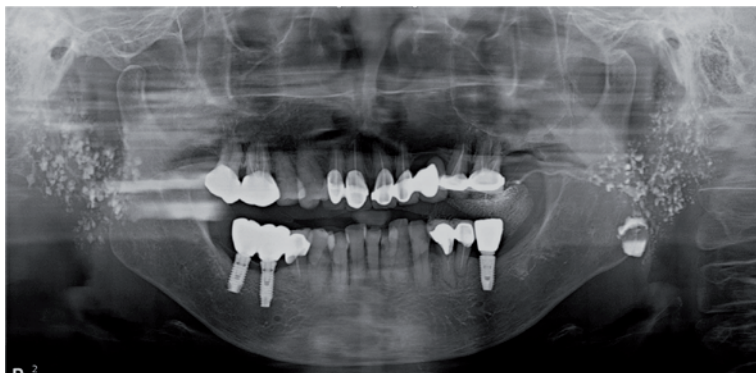


Figure 3. Panoramic radiograph taken after sialography, showing globular sialectasis in both parotid glands, a characteristic finding of sialectasis observed in Sjögren's syndrome.

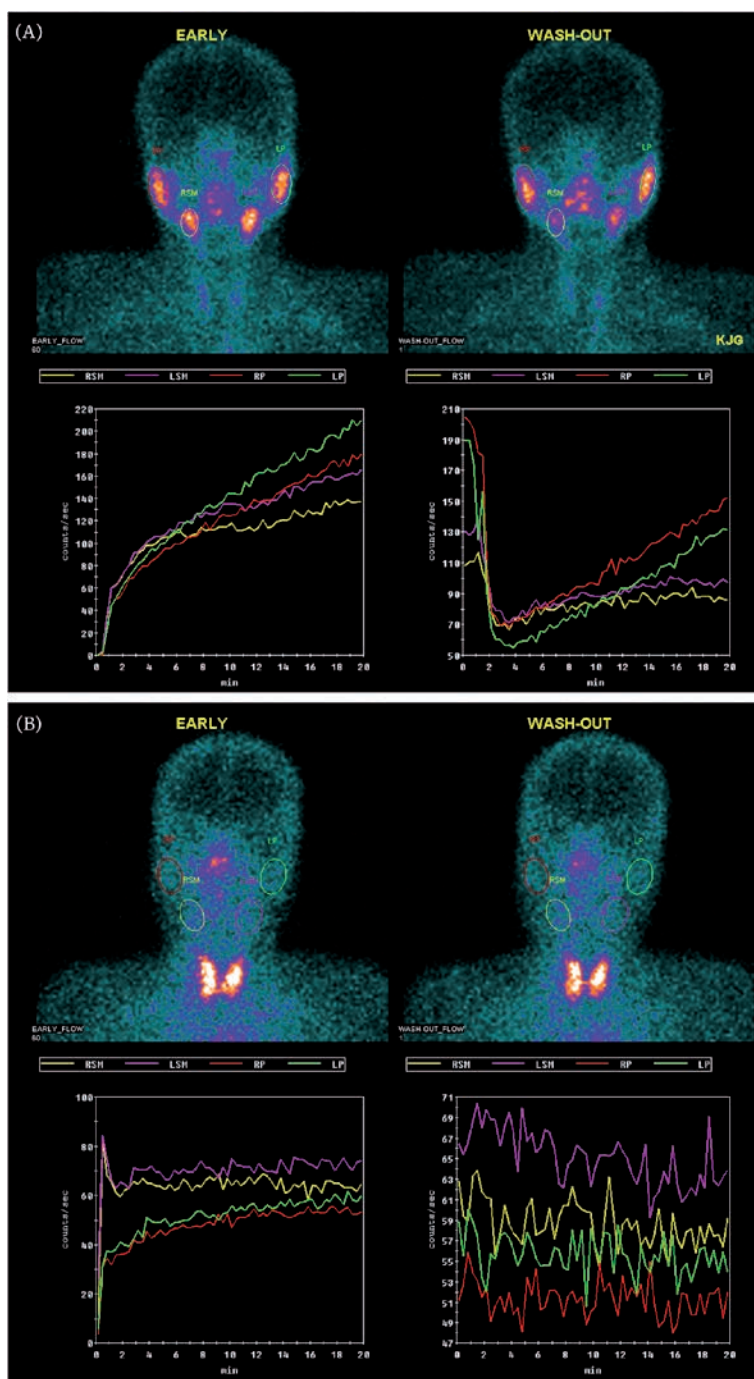


Figure 4.
 (A) Salivary scintigraphy showing normal salivary gland function: Early uptake and washout phases are clearly distinguishable. The hot spots demonstrate symmetrical activity, with reduced intensity during the washout phase, as confirmed by consistent graph patterns. (B) Salivary scintigraphy indicating severely impaired salivary gland function: No definite uptake is observed in both submandibular and parotid glands during the uptake and washout phases. The graph reflects significantly diminished radiotracer activity and atypical patterns, reflecting severe dysfunction. RSM (yellow), right submandibular gland; LSM (pink), left submandibular gland; RP (red), right parotid gland; LP (green), left parotid gland.

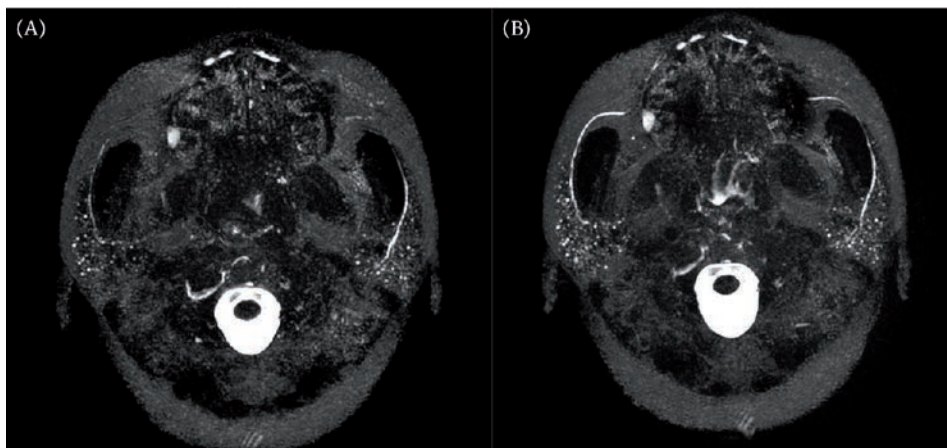


Figure 5. Magnetic resonance sialography images demonstrate punctate and globular sialectasis in both parotid glands, findings commonly observed in Sjögren's syndrome. (A) Pre-stimulation image (Video 1, <https://intech-files.s3.amazonaws.com/ao4Tco0000ARko4IAD/ao9Tco00001sAHxIAM/Videos%20%28Full%20Chapter%29-Xerostomia%20and%20Its%20Impact%20on%20Oral%20Health%20%282025-02-07%2019%3A49%3A24%29.zip>). (B) Post-stimulation image with clearer visualization of parotid ducts (Video 2, <https://intech-files.s3.amazonaws.com/ao4Tco0000ARko4IAD/ao9Tco00001sAHxIAM/Videos%20%28Full%20Chapter%29-Xerostomia%20and%20Its%20Impact%20on%20Oral%20Health%20%282025-02-07%2019%3A49%3A24%29.zip>).

4.4 Laboratory testing for systemic causes

When autoimmune or systemic etiologies are suspected in the development of xerostomia, laboratory evaluations become paramount. Autoantibody assays—most notably anti-SSA/Ro and anti-SSB/La—serve as primary markers for Sjögren's syndrome, while rheumatoid factor (RF) and antinuclear antibodies (ANA) indicate broader autoimmune involvement [16, 22].

Inflammatory markers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) may be elevated in autoimmune-related xerostomia, indicating ongoing systemic inflammation. Salivary biomarkers, including β 2-microglobulin, lactoferrin, and cytokines, can provide additional insight into immune-mediated salivary dysfunction.

A minor salivary gland biopsy is often performed to diagnose Sjögren's syndrome definitively. This biopsy reveals lymphocytic infiltration and glandular destruction [16, 22, 23]. These laboratory tests, in combination with clinical findings and imaging, help confirm systemic causes of xerostomia and guide appropriate treatment strategies.

4.5 Emerging diagnostic tools, such as salivary biomarkers

Salivary diagnostics have emerged as a promising, non-invasive approach for identifying biomarkers associated with xerostomia-related conditions, particularly primary Sjögren's syndrome (pSS). Several studies have demonstrated that specific salivary proteins and mRNA markers are significantly elevated in patients with pSS compared to healthy controls and individuals with other autoimmune diseases. Verified biomarkers such as cathepsin D, α -enolase, and β 2-microglobulin, as well as mRNA markers including MNDA and GIP2, have shown potential to differentiate pSS from similar clinical entities [24].

Proteomic analyses have further identified salivary proteins such as α -amylase precursor, carbonic anhydrase VI, and epidermal fatty acid binding protein as key indicators of pSS, supporting the role of saliva as a diagnostic fluid reflecting both local glandular dysfunction and systemic autoimmune activity [25]. Moreover, comprehensive proteomic profiling has demonstrated that salivary protein alterations in pSS mirror underlying immunopathological processes, including B cell hyperactivity, macrophage differentiation, and T cell chemotaxis [26]. These findings underscore the utility of salivary biomarkers in detecting and monitoring the immunological landscape of pSS.

In addition to autoimmune diseases, salivary analysis is crucial in assessing caries risk, which is highly relevant in xerostomia patients. Saliva plays a critical role in the mechanical clearance of food debris, buffering of acids, and antimicrobial defense. Alterations in salivary flow rate, buffering capacity, and microbial composition, particularly increased levels of mutans streptococci and *Candida albicans*, are associated with heightened caries prevalence in individuals with reduced salivary function [27]. Therefore, comprehensive



Figure 6. This case highlights sialolithiasis as a potential local salivary gland obstruction to consider in the differential diagnosis of xerostomia. (A) Axial CT image of the mandible showing an opaque lesion medial to the left mandibular bone. (B) Coronal CT image showing multiple high-attenuated materials in the left submandibular space and severe atrophy of the left submandibular gland. These findings are consistent with sialolithiasis. (C) Robot-assisted submandibular glandectomy was performed, extracting two calculi, each approximately 5 mm in size.

salivary assessment may contribute to the diagnosis of underlying systemic disorders and caries risk evaluation, enabling the implementation of preventive strategies tailored to xerostomic patients.

The potential application of salivary biomarkers in clinical practice continues to be the subject of ongoing research. Although standardizing collection methods and validating across large cohorts are ongoing challenges, integrating salivary diagnostics into routine evaluation protocols may enhance the accuracy of xerostomia assessment and facilitate precision medicine approaches in oral healthcare.

4.6 Differential diagnosis in oral medicine

True hyposalivation must be distinguished from neuropathic xerostomia, where patients perceive dryness despite normal salivary function. Objective sialometry and imaging help identify actual salivary gland dysfunction, while neuropathic dryness (e.g., burning mouth syndrome, central neuropathic pain) often presents without measurable salivary flow reduction.

Medication-induced xerostomia must be distinguished from systemic causes. A thorough medication history review is essential, as discontinuing or adjusting a xerogenic drug may lead to symptom improvement, whereas systemic conditions like Sjögren's syndrome require further medical intervention.

Local salivary gland obstructions, such as sialolithiasis or mucocoeles, should also be considered (**Figure 6**). Sialography and ultrasonography are useful in identifying obstructive causes, which may require surgical intervention or conservative management. Careful clinical evaluation, salivary testing, and imaging ensure the correct diagnosis and guide appropriate treatment strategies.

5. Management strategies

5.1 General strategies for symptom relief

For patients with mild xerostomia or subjective dryness, symptom relief is the primary focus of management. Hydration and lifestyle modifications play a crucial role in minimizing discomfort. Encouraging frequent water intake throughout the day can help maintain oral moisture, while using humidifiers in dry environments may prevent further dehydration of the oral mucosa, particularly during sleep. Caffeine, alcohol, and tobacco should all be avoided by patients since they dehydrate them and make their symptoms of dry mouth worse.

Saliva substitutes and lubricants provide short-term relief for xerostomia and are particularly useful in cases where salivary gland function is irreversibly compromised. By improving oral lubrication and lowering friction on the mucosa, artificial saliva products—which usually contain carboxymethylcellulose, xylitol, or mucin—help ease discomfort during speaking and swallowing. Patients who suffer from chronic dryness are more likely to choose lipid-based sprays because they provide longer-lasting moisture retention than water-based alternatives.

Dietary modifications also play an important role in symptom relief. Sugar-free chewing gum and lozenges containing citric or malic acid can stimulate residual salivary gland function, though their use should be limited in patients with dental erosion or hypersensitivity. Soft, moist foods are recommended to ease chewing and minimize mucosal irritation, particularly in cases of severe dryness.

In addition to conventional therapies, complementary approaches have been explored to support xerostomia management. Chamomile (*Matricaria chamomilla*) and linseed (*Linum usitatissimum*) saliva substitutes have demonstrated superior symptom relief in elderly patients with xerostomia compared to conventional substitutes, offering benefits such as reduced dry mouth sensation and improved swallowing comfort [28]. Aloe vera has been shown to have moisturizing and wound-healing properties, effectively decreasing xerostomia severity in patients with type 2 diabetes [29]. Similarly, green tea (*Camellia sinensis*) catechins have been associated with increased salivary output and potential modulation of salivary gland function [30].

Although these complementary interventions present promising adjunctive benefits, the overall evidence base remains limited, and their use should supplement, not replace, established treatments. Systematic reviews highlight the need for further high-quality clinical trials to determine their efficacy and long-term safety in xerostomia management [31].

5.2 Pharmacological and non-pharmacological interventions

For patients with moderate to severe xerostomia, symptom relief alone is insufficient, and targeted therapies are necessary to enhance salivary gland function, prevent complications, and maintain oral health. Pharmacological interventions primarily focus on stimulating residual salivary secretion. At the same time, adjunctive treatments aim to mitigate the secondary effects of chronic dry mouth, such as oral infections, dental caries, and periodontal disease [32].

5.2.1 Sialogogues (saliva-stimulating medications)

By stimulating muscarinic receptors, sialogogues can enhance natural saliva production in patients with residual salivary gland function. Pilocarpine, a cholinergic agonist, is commonly administered at 5 mg, three to four times daily, and has shown effectiveness in managing xerostomia associated with radiation therapy and Sjögren's syndrome. However, systemic cholinergic side effects, such as sweating and gastrointestinal discomfort, require careful monitoring. Cevimeline, a selective muscarinic agonist prescribed at 30 mg three times daily, may offer longer-lasting effects with fewer side effects. Bethanechol is occasionally considered for medication-induced xerostomia when discontinuation of the causative agent is not feasible. Nevertheless, sialogogues provide only symptomatic relief and are generally ineffective in cases of complete salivary gland atrophy following advanced autoimmune disease or high-dose radiation therapy.

However, long-term management of salivary gland dysfunction remains challenging. Pharmacologic agents such as pilocarpine show limited sustained efficacy, particularly in patients with radiation-induced salivary gland dysfunction and Sjögren's syndrome. In a large, randomized trial (RTOG 97-09), pilocarpine improved unstimulated salivary flow during radiotherapy, but this did not consistently result in lasting symptom relief or improved quality of life [33]. Systematic reviews also report that approximately half of patients respond to parasympathomimetics, though discontinuation due to side effects is common [34]. In Sjögren's syndrome, management guidelines emphasize objective salivary assessments and stepwise treatment, but durable restoration of gland function is rarely achieved, and therapies remain largely palliative [32]. Similarly, for radiation-induced salivary gland dysfunction, tissue-sparing radiation modalities are the most effective preventive strategy, while

available therapeutic options provide only partial symptomatic relief without fully restoring glandular function [35].

5.2.2 Management of secondary oral complications

Xerostomia increases the risk of oral infections, dental decay, and periodontal disease, requiring proactive management. Oral candidiasis commonly manifests when diminished salivary secretion compromises mucosal defenses, allowing *Candida albicans* to proliferate unchecked. Under these circumstances, *Candida* may transition from a relatively benign yeast form to an invasive hyphal state, bolstering its adherence capacity, tissue penetration, and immune evasion. This morphological shift facilitates biofilm formation and confers resistance to antifungal agents, resulting in persistent infections and mucosal irritation and rendering effective treatment more challenging [36].

High-risk patients, such as those with immunological dysfunction, insufficient salivary flow, or long-term denture wear, require early management. Mild cases respond well to topical antifungals like nystatin suspension, clotrimazole troches, or miconazole gel. Persistent cases may require systemic fluconazole (100–200 mg/day for 7–14 days), while resistant infections might need itraconazole or posaconazole. Addressing xerostomia-related complications early helps mitigate oral candidiasis and improve patient outcomes.

The lack of salivary buffering capacity in xerostomia also predisposes patients to rapidly progressing dental caries, particularly along cervical margins and exposed root surfaces. Preventive measures include high-fluoride toothpaste (5000 ppm fluoride), professionally applied fluoride varnishes, and remineralizing agents such as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) to enhance enamel resistance. Additionally, xerostomia can accelerate plaque accumulation and gingival inflammation, increasing the risk of periodontal disease and gingivitis. Regular professional cleanings, along with the use of chlorhexidine mouth rinses, help control plaque levels and maintain periodontal health.

5.3 Oral medicine perspective on treatment planning

Polypharmacy significantly contributes to xerostomia, particularly in elderly patients and those with chronic conditions. Reviewing medication history helps identify xerogenic drugs, such as anticholinergics, antidepressants, and antihypertensives. Adjusting dosages or switching to alternatives with fewer drying effects should be considered. Collaboration with prescribing physicians and pharmacists ensures medication modifications balance symptom management with overall health needs.

A multidisciplinary approach is essential for patients with severe xerostomia due to autoimmune disease, cancer therapy, or neurological disorders. Rheumatologists manage Sjögren's syndrome with systemic immunomodulatory therapies to reduce inflammation. This autoimmune disease leads to chronic xerostomia, joint pain, and systemic complications like pulmonary fibrosis, lymphoma, and liver disease. Routine follow-ups with rheumatologists, ophthalmologists, and dentists help prevent complications [37]. Given its progressive nature, long-term management should focus on saliva preservation, inflammation control, and secondary complication prevention. Notably, chronic dryness in Sjögren's syndrome is closely linked to diminished health-related quality of life (HRQoL), with patients often experiencing fatigue, pain, and psychological distress, highlighting the need for more effective, long-term management strategies [38].

Oncologists and radiation oncologists manage post-radiation xerostomia with salivary gland-sparing strategies and palliative options. High-dose radiation often results in permanent hyposalivation, mucosal atrophy, and increased risks of dental caries and osteoradionecrosis. Management strategies include intensity-modulated radiation therapy (IMRT) and salivary stimulants like pilocarpine and cevimeline. In patients with oral, head, and neck cancers, xerostomia significantly impairs oral health-related quality of life (OHRQoL) by worsening difficulties in speaking, eating, and social interactions [39, 40].

Speech therapists are crucial in addressing xerostomia-related speech difficulties and are familiar with patients with advanced salivary gland dysfunction. They provide targeted interventions to improve articulation, manage swallowing difficulties, and enhance communication. By mitigating these functional limitations, speech therapy directly contributes to preserving the quality of life in affected patients, particularly those undergoing cancer treatments or managing chronic conditions like Sjögren's syndrome.

Preventive strategies further emphasize medication review, optimizing oral hygiene, and using saliva substitutes or sialogogues.

By collaborating closely with other specialists, xerostomia management transcends mere symptom relief to encompass the broader systemic and functional consequences, ultimately aiming to improve patients' overall quality of life.

6. Conclusion

Xerostomia, commonly known as dry mouth, can seriously undermine oral health by increasing the risk of dental caries, periodontal disease, oral infections, and functional impairments. A patient-centered clinical approach that targets symptom relief, oral health maintenance, and underlying causes is essential. Given the complexity of xerostomia-associated issues, a multidisciplinary strategy—integrating oral medicine specialists, rheumatologists, oncologists, and other healthcare professionals—is crucial. Early intervention and collaboration can minimize long-term impacts, improve oral function and comfort, and ultimately enhance overall quality of life.

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Hyposalivation and Xerostomia

Hülya Erten Can and Aslı Silkü

Abstract

Saliva is a vital bodily fluid that facilitates digestion, speech, and swallowing, while also protecting oral tissues through its antimicrobial and lubricating properties. Dry mouth, also known as xerostomia, is typically associated with reduced salivary flow or, in rarer cases, psychological factors. Key causes of decreased salivary flow include dehydration, certain medications, radiation therapy applied to the head and neck region, and specific systemic diseases such as Sjogren's syndrome. Individuals with salivary gland hypofunction are at a heightened risk for various oral pathologies, making symptomatic treatment and preventive approaches essential. This chapter provides a comprehensive overview of the assessment of dry mouth, alleviation of its symptoms, and preventive measures, based on the latest literature.

Keywords: artificial saliva, oral health, hyposalivation, saliva, xerostomia

1. Introduction

Despite stimulation of the salivary glands, a decrease in salivary flow rate is called hyposalivation, and the condition of dry mouth in people is called xerostomia (**Figure 1**).

Unstimulated saliva flow rate depends on the person, time, and conditions, but in the resting state 0.25–0.35 ml/min (mean 0.3 ml/min). The stimulation of saliva depends on the type of stimulus used differ. For example, in parotid secretion, 1.6 ml/min with paraffin; 1.7 ml/min with gum ml/min; and 0.8 ml/min secretion with 1% citric acid in submandibular saliva velocities were determined. Hyposalivation is defined as an abnormal amount of saliva based on the objective measurements, a term describing a state of decline. The reduced quality of life of the individual xerostomia is defined as a subjective feeling of oral dryness. For this reason, the diagnosis of hyposalivation can only be made by measuring the individual's salivary flow rate is diagnosed when it is found. Unstimulated salivary flow in case of hyposalivation for paraffin-stimulated saliva flow rate is ≤ 0.1 ml/min. For paraffin-stimulated saliva flow rate is ≤ 0.5 –0.7 ml/min.

Saliva plays an important role in maintaining the health of the oral cavity. The functions of saliva include removing dietary carbohydrates from the oral environment, neutralizing the pH of the oral cavity and dental plaque, remineralizing areas of demineralization in enamel, providing antimicrobial activity, contributing to digestion, making food swallowable, assisting speech and swallowing functions, and maintaining water balance. Therefore, xerostomia is an important health problem that negatively affects people's quality of life [1].



Figure 1. Mural depicts the treaty signed between Retief and Dingane in the Voortrekker monument in Pretoria, the person crouching to the right of the king is collecting the king's spit. I wonder why? What is the secret of saliva that has lasted through history.

2. Etiology

Causes of dry mouth include absence, underdevelopment or obstruction of the salivary glands, aging, smoking, mouth breathing, AIDS and salivary gland infections, head and neck radiotherapy applications, and some medications used. It can also be caused by systemic diseases such as uncontrolled diabetes, hypertension, autoimmune diseases such as rheumatoid arthritis, systemic lupus, sarcoidosis, Sjogren's syndrome, and psychological disorders that cause dysfunction in the salivary glands. Sometimes it is idiopathic, meaning that there is no known cause [2].

3. Factors affecting saliva secretion

The function of the salivary glands is under the control of the autonomic nervous system [3]. The secretory center in the medulla directs secretion [4]. Sympathetic and parasympathetic stimuli cause secretion, but the parasympathetic nerves have a greater influence. Secretion is caused by autonomic innervation or stimulation by substances that mimic it. The phase beginning with the thought or smell of food is called the cephalic phase [5]. Impulses from the cerebral cortex stimulate the secretory center, causing salivation. In what is known as the buccal phase, salivation begins when the senses of taste or touch in the mouth are stimulated [6].

The gastrointestinal phase occurs with the reflex from the stomach and upper digestive tract. Saliva secretion occurs when the wall of the esophagus is stretched by food entering the esophagus and these substances stimulate the mucosa in the

stomach. Saliva secretion can vary greatly in terms of both flow rate and chemical properties. At rest, in the absence of any stimulus, saliva is secreted only enough to keep the oral mucosa moist.

Salivary flow rate and content vary according to age, gender, sleep, diet, dehydration, emotional factors, infectious diseases, nervous system diseases, medications, and the type and application of stimuli. Chewing activity is the strongest stimulus in salivation. Smell is as strong a stimulus as taste.

Saliva secretion is the highest between the ages of 6 and 14. After the age of 20, secretion starts to decrease. In men, the secretion is higher [7].

4. Causes related to salivary glands

Absence, underdevelopment or obstruction of the salivary glands, infections, systemic diseases affecting the function of the salivary glands, and medications are among the causes of dry mouth [8].

Absence of major salivary glands is a very rare condition and was first described by Gruber in 1885 [9]. Salivary gland aplasia may occur in isolation or in association with other developmental anomalies, especially ectodermal anomalies.

4.1 Salivary gland stones (sialolith)

Calcified structural formations that occur as a result of obstruction of the salivary gland parenchyma or duct with calcium salts are called sialoliths. Sialoliths are the most common salivary gland diseases. They often develop in the submandibular gland or duct. Anamnesis, inspection, palpation, and measurement of the amount of salivary flow are important in the diagnosis of sialoliths. Patients usually present to the physician with recurrent pain and swelling during or after meals. In some cases, there may be pain in the gland area radiating to the head and neck. Sialoliths can be palpated clinically if they increase in size. When sialoliths become significantly larger, they can cause obstruction of the duct and reduce the flow of saliva. Sometimes these stones can cause acute inflammation of the salivary glands [10].

4.2 Salivary gland inflammation (sialadenitis)

It is a form of inflammation that progresses over time, usually as a result of decreased saliva production and the accumulation of thick saliva in the salivary ducts. The slowed salivation causes bacteria normally present in the mouth to spread through the ducts into the salivary gland. Chronic inflammation changes the structure and secretion structure of the salivary duct, causing the problem to become permanent over time. Stones that remain in the duct for a long time can also cause this problem to develop. Recurrent acute inflammations, weaknesses in the body's defense system, radiation treatments, smoking, and poor oral hygiene are among the causes of this disease [11]. The most prominent complaint is painful swelling that increases especially during meals, usually without signs of acute inflammation such as fever and redness. Sialadenitis is more common among older adults with salivary gland stones, but it can also occur in infants in the first few weeks of life. Without proper treatment, it can develop into a serious infection, especially in weakened or elderly people.

4.3 Viral infections

Systemic viral infections can sometimes lodge in the salivary glands, causing facial swelling, pain, and difficulty eating. The most common example is mumps. In adults and children with HIV and hepatitis C, there is an increase in salivary gland diseases from 4 to 8% [12].

4.4 Salivary gland tumors

Tumors arising from the salivary glands are 75–80% in the parotid gland, 20% in the submandibular salivary gland, and rarely in the sublingual or minor glands [13].

The majority of benign salivary gland tumors are mixed tumors or pleomorphic adenomas, which are more common in women between the ages of 30 and 60. In general, surgical treatment is performed for all benign tumors. During this surgery, the salivary gland where the tumor is located is removed as a whole. The important feature of the parotid salivary gland is that the facial nerve, which moves the facial muscles, passes through this gland on its way to the facial muscles after exiting from the lower part of the ear bone and branches into branches while in the gland. The part of the gland above the nerve and its branches is called the superficial lobe and the part below it is called the deep lobe. In benign parotid tumors, if the deep lobe is not involved by the tumor, only the superficial lobe is removed in surgery [14].

Although many different types of tumors can be seen in the salivary glands, the most common malignant tumors are mucoepidermoid carcinoma (45%) and adenoid cystic carcinoma (22%) [15]. Malignant tumors are classified as low, intermediate, and high grade according to the clinical behavior of the tumor, the rate of spread, and the degree of structural change. In cases of tumor spread to the lymph nodes in the neck or tumors with a high risk of metastasizing to the neck lymph nodes even if no obvious involvement is detected, the adjacent lymph nodes are also removed during surgery (neck dissection surgery). Radiotherapy is rarely the main treatment of choice for small tumors. For large tumors with a high risk of spreading, radiotherapy is given to the surgical site after surgery to prevent tumor recurrence in the same area.

Drug treatment (chemotherapy) may be preferred in patients who are not suitable for surgery due to other health problems and in patients with advanced regional spread and/or distant metastasis of the tumor [16].

Drug-induced xerostomia is one of the side effects observed in AIDS patients treated with prescriptions containing HIV protease inhibitors and HIV reverse transcriptase inhibitors. In the remaining cases of xerostomia, acute bacterial sialadenitis, Kaposi's sarcoma, or infiltration by non-Hodgkin lymphoma may be observed.

4.5 Cysts

Cysts are small sacs filled with fluid. They can form in the parotid glands of babies before birth due to problems with ear development. Other types of cysts can form in the major or minor salivary glands later in life. Cysts can also be caused by traumatic injuries, infections or salivary gland stones, or tumors [17].

5. Autoimmune diseases

5.1 Sarcoidosis

It is an autoimmune disease characterized by the formation of nodules called granulomas as a result of an overreaction of the immune system. Symptoms vary depending on the location and size of the granulomas. The severity of the symptoms also depends on the granulomas. In some cases, sarcoidosis may not show any symptoms. In other cases, the granulomas can turn into fibrosis, causing permanent damage to the lung [18].

Dry mouth and salivary gland enlargement may occur in 9% of patients with chronic sarcoidosis [19].

Heerfordt-Waldenström syndrome is a form of neurosarcoidosis. This syndrome is characterized by enlargement of the parotid or salivary glands, facial nerve palsy, and anterior uveitis. The anatomical pathology of the affected organs includes granulomas with peripheral lymphocyte deficiency. In approximately 6% of sarcoidosis cases, parotid gland enlargement is seen as a result of granulomatous inflammatory reaction.

5.2 Sjogren's syndrome

It is an autoimmune disease in which the body's immune system attacks different parts of the body, including the salivary glands, lacrimal glands, and sometimes the sweat and oil glands of the skin.

Sjogren syndrome can occur in two forms: primary and secondary. Primary Sjogren's syndrome occurs on its own and is not related to other diseases. Secondary Sjogren's syndrome occurs in combination with some types of inflammatory rheumatic diseases such as rheumatoid arthritis, lupus, and polymyositis.

Genetic factors, viral infections, and hormones are thought to play a role in Sjogren's syndrome, the exact cause of which is unknown. This disease can affect people of all ages and races and is more common in women (female/male ratio 9/1) and usually between the ages of 40–60 [20]. The definitive diagnosis of the disease is made by salivary gland biopsy. It may cause tooth decay and gum problems due to dryness in the mouth.

5.3 Chronic graft versus disease (GVHD)

It is a disease that occurs when the immune cells of a donor (graft) recognize the recipient's body (host) as foreign and mount an immune response against it. GVHD is divided into two main types, acute and chronic, each with different symptoms and treatment protocols [21]. Dry mouth is the most prominent complication. The squamous cell epithelium of the oral mucosa and the epithelium of the salivary gland in the initial stage of this disease are affected.

5.4 Systemic lupus erythematosus

It is an inflammatory connective tissue disease. It is a chronic, autoimmune disease that can affect different organs of the body, especially the skin, joints, blood, kidneys, and central nervous system. About 75% of patients with Systemic Lupus Erythematosus complain of pain and dry mouth [22].

5.5 Cirrhosis

It is an autoimmune, primary bile duct obstructing disease. Salivary gland calculi, dry mouth, and dysphagia may be seen in these patients.

5.6 Hypothyroidism

Hypothyroidism is a condition characterized by insufficient production of thyroid hormones, leading to various changes in body systems. One of its effects on oral health is the impairment of salivary gland function, resulting in hyposalivation.

In hypothyroidism, the metabolic rate decreases, which can adversely affect the energy requirements and secretory functions of the salivary glands. Additionally, prolonged hypothyroidism may lead to structural changes in the salivary glands, such as fibrosis and atrophy, further reducing saliva production. Hypothyroidism can also impair autonomic nervous system function, affecting the neural mechanisms that stimulate the salivary glands. Changes in fluid and electrolyte balance in hypothyroidism can further reduce the salivary glands' capacity to produce saliva.

5.7 Hyperthyroidism

Hyperthyroidism is a condition characterized by the excessive production of thyroid hormones (thyroxine and triiodothyronine) by the thyroid gland. This condition accelerates metabolic processes in the body and manifests with various physical and psychological symptoms.

Hyperthyroidism can lead to an imbalance in the autonomic nervous system, which can affect the stimulation of the salivary glands and result in hyposalivation. Additionally, the increased metabolic rate in hyperthyroidism can cause fluid imbalances in the body. This can reduce saliva production, leading to dry mouth.

One of the common symptoms of hyperthyroidism is sweating and fluid loss. Changes in the body's fluid balance can prevent the salivary glands from producing sufficient saliva. In some cases of hyperthyroidism (e.g., Graves' disease), the immune system becomes overly activated, leading to autoimmune reactions. This can damage the salivary glands and contribute to hyposalivation.

5.8 Eating disorders (anorexia nervosa, bulimia nervosa)

Eating disorders are psychological conditions characterized by abnormalities in an individual's eating behaviors, perception of body weight, or dietary habits. These disorders often lead to physical health problems, severe psychological issues, and impaired social functioning.

Eating disorders can lead to deficiencies in essential vitamins, minerals, and fluids in the body. Specifically, deficiencies in vitamin A, B-complex vitamins, zinc, and iron can negatively affect the function of salivary glands, causing hyposalivation.

Insufficient fluid intake and dehydration can further reduce saliva production. The frequent vomiting behavior often seen in bulimia nervosa can result in the loss of important electrolytes, such as potassium, leading to electrolyte imbalances that impair the function of salivary glands. Continuous vomiting exposes the oral cavity to stomach acid, causing inflammation and damage to the salivary glands, which can decrease saliva production.

Additionally, eating disorders are often accompanied by high levels of stress and anxiety, which can disrupt autonomic nervous system function and impact saliva secretion.

6. Medicines used

The most common cause of dry mouth among patients who visit the dentist is the use of medication for systemic diseases.

These medications, alone or in combination, can affect the salivary glands. Medication reduces salivary flow and changes the composition of saliva.

More than 500 drugs can cause this, including antianxiety drugs, anticholinergics, anticonvulsants, antidepressants, antiemetics, antihypertensives, antiparkinsonian, diuretics, bronchodilators, etc. [23].

Drugs are substances that typically exert powerful effects on the central nervous system and alter a person's state of consciousness. These substances can have pain-relieving, sedative, euphoric, or hallucinatory effects. However, one of the side effects of certain drugs can be hyposalivation. Drugs have strong effects on the central nervous system and can alter brain functions. These effects can indirectly impact the autonomic nervous system, which controls the stimulation of the salivary glands. Drugs can affect this system, leading to a reduction in saliva production.

Many drugs can cause fluid loss in the body. For example, stimulants or certain depressants can disrupt the body's fluid balance, leading to dehydration. Dehydration can result in the salivary glands not producing enough saliva.

6.1 Antihistamines

Antihistamines are drugs that block the effects of histamine and are commonly used to treat allergic reactions. However, one of the side effects of antihistamines is hyposalivation. This is primarily due to the anticholinergic properties of antihistamines. Many antihistamines not only block the effects of histamine but also affect the parasympathetic nervous system. This blocks cholinergic receptors that stimulate the salivary glands to increase saliva production. As a result, saliva production decreases, and xerostomia may occur.

6.2 Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs are commonly used to reduce inflammation, pain, and fever. However, one of the side effects of NSAIDs may be hyposalivation. NSAIDs inhibit the synthesis of chemical compounds called prostaglandins. Prostaglandins are substances that regulate many functions in the body, one of which is the stimulation of saliva production. Inhibition of prostaglandins disrupts the normal function of the salivary glands and may lead to hyposalivation. NSAIDs can also affect kidney function, which may lead to fluid imbalances. A lack of sufficient fluid in the body can reduce saliva production and cause xerostomia.

6.3 Antiemetics

Antiemetics are drugs used to treat nausea and vomiting. However, some antiemetics can have side effects, including hyposalivation. Many antiemetic drugs

have anticholinergic properties, which inhibit the parasympathetic nervous system. Cholinergic receptors help stimulate the salivary glands to increase saliva production. However, the anticholinergic effect blocks these receptors, reducing saliva production, which can lead to dry mouth.

6.4 Antipsychotics

Antipsychotics are drugs used to treat mental health disorders such as psychosis, schizophrenia, and bipolar disorder. However, one of the side effects of some antipsychotic drugs is hyposalivation. Many antipsychotic drugs have anticholinergic properties. Anticholinergic effects inhibit the parasympathetic nervous system, which leads to the blockade of cholinergic receptors that stimulate the salivary glands to increase saliva production. As a result, saliva production decreases.

Additionally, antipsychotics affect the brain's dopamine system. Dopamine is a neurotransmitter that influences many body functions, including salivation. Changes in dopamine levels can affect saliva production and lead to hyposalivation.

6.5 Chemotherapy

Various malignant tumors can be treated with chemotherapy or in combination with radiation. In a study of 127 cancer patients, dry mouth is the fourth most common symptom (in 78% of patients) [24]. Dry mouth has been shown to be associated with the use of chemotherapeutic drugs. In this study, dry mouth was associated with taste differences, chewing difficulties (dysphagia), and speech difficulties (dysphonia). The use of cancer drugs can thicken saliva, which can cause a feeling of dryness. About 65% of 50 patients with carcinoma and squamous epithelial cell tumors of the head and neck and squamous epithelial cell tumors of the head and neck, subjected to 12 weeks of selective overdose isolation and radiotherapy, experienced dry mouth [24].

Various drugs are used in chemotherapy. Some of these are chemotherapeutic drugs and hormones that directly affect the tumor and biological agents that strengthen the immune system. Some drugs are used to increase the effect of drugs that directly affect the tumor or to reduce or eliminate side effects.

Dryness, redness, and sores may occur in the mouth, throat and esophagus while receiving chemotherapy due to reasons such as inability to renew the cells in the mouth and esophagus, the body being more sensitive to germs, insufficient fluid intake, dry mouth, and poor oral care. Since this situation will both prevent your nutrition and create a favorable environment for you to catch germs, it is necessary to take the necessary precautions to prevent its occurrence.

6.6 Decongestants

Decongestants are medications used to relieve nasal congestion, commonly used for conditions such as the common cold, allergic rhinitis, or sinusitis. However, one of the side effects of decongestants can be hyposalivation. Particularly, decongestants classified as adrenergic agonists may have anticholinergic effects that suppress the parasympathetic nervous system. These effects block cholinergic receptors that stimulate the salivary glands, leading to a decrease in saliva production. Decongestants stimulate the sympathetic nervous system and constrict blood vessels, which reduces nasal congestion. However, this effect can systemically alter blood flow and affect the

fluid balance in other parts of the body. Fluid loss or insufficient fluid levels in the body can reduce saliva production.

6.7 Muscle relaxants

Muscle relaxants are medications used to relax muscles and prevent muscle spasms. These drugs are commonly prescribed to treat pain, muscle stiffness, or muscle spasms. However, one of the side effects of some muscle relaxants is hyposalivation. Specifically, drugs with cyclic anticholinergic properties have an inhibitory effect on the parasympathetic nervous system. This leads to the blockage of cholinergic receptors, preventing the stimulation of salivary glands.

Muscle relaxants also typically affect the central nervous system. These medications work by blocking certain nerve pathways in the brain and spinal cord to induce muscle relaxation. However, these effects can also impact autonomic nervous system functions, disrupting the normal functioning of salivary glands, which can result in hyposalivation.

6.8 Narcotic analgesics

Narcotic analgesics are powerful medications commonly used to relieve pain. These drugs act on the central nervous system to block the sensation of pain. However, some side effects of narcotic analgesics may include hyposalivation. Narcotic analgesics bind to opioid receptors in the brain, reducing sensitivity to pain. However, these medications can also affect the autonomic nervous system. The autonomic nervous system controls the neural mechanisms that stimulate the salivary glands. Narcotic analgesics can disrupt this system, leading to a decrease in saliva production. Additionally, narcotic analgesics can slow down the digestive system and reduce bowel movements. This can lead to changes in the body's fluid balance and negatively affect saliva production.

6.9 Sedative drugs

Sedatives are medications that suppress the central nervous system to produce calming, sedative, and sleep-inducing effects. These drugs are commonly used to treat anxiety, insomnia, and certain psychological disorders. However, one of the side effects of sedatives can be hyposalivation. Sedatives can slow brain functions and reduce control over the autonomic nervous system. The autonomic nervous system controls many involuntary functions in the body, one of which is saliva production. Sedatives can disrupt these functions, leading to dry mouth.

7. Radiotherapy

It causes various acute or chronic side effects that may affect healthy tissues during or after the application, reduce the patient's quality of life, and in some cases even lead to interruption or termination of the treatment. This situation occurs more frequently in the head and neck region, where structures such as mucosa, skin, subcutaneous connective tissue, muscle, salivary glands, teeth, and bone, which react differently to radiation, are not similar to each other.

Dry mouth is probably the most common permanent damage seen in patients undergoing radiotherapy to the head and neck region [19]. Reduction or cessation of salivary flow begins at 1-2 weeks of radiotherapy to the major salivary glands [20, 21]. A decrease in salivary secretion has been reported 24 hours after a single dose of 2.25 Gy.

In another study, dry mouth was present in all patients (100%) in the first week of radiotherapy. It is accepted that exposure of the parotid gland to radiation doses of 60 Gy or more causes permanent damage [23, 24]. The patients included in our study received 68-70 Gy radiotherapy. Six months after the end of treatment, nine (34.6%) of the patients reported that dry mouth was still present and 17 (65.4%) reported partial improvement in dry mouth [25].

Dry mouth (xerostomia) and difficulty swallowing (dysphagia) are common side effects in patients who have undergone radiotherapy for cancer in the head and neck region [26]. Depending on the volume of the salivary glands exposed to radiation, the total radiation dose, and the patient's characteristics, dry mouth may last for several months or years or may be permanent. In addition to decreased salivary flow, increases in salivary viscosity, sodium, chlorine, calcium, magnesium, and protein concentrations, decreases in pH, bicarbonate concentration, and IgA levels have been reported. Although salivary dysfunctions are not life-threatening, they have a negative impact on the patient's quality of life [27].

Intensity Modulated Radiation Therapy (IMRT) is an advanced and highly precise technique used in radiation oncology for the treatment of various cancers. It represents a significant advance in radiotherapy and enables more accurate and targeted delivery of radiation to tumor tissues while minimizing damage to surrounding healthy tissues. IMRT has revolutionized the way radiation therapy is delivered, providing improved therapeutic outcomes and reduced side effects for patients.

Technological evolution of treatment in head and neck radiotherapy, intensity-modulated radiotherapy, called IMRT, has become an effective method of salivary gland protection.

8. Plant extracts

The use of plant-based dietary supplements has become increasingly common today. Many different and numerous natural extracts are used uncontrollably by individuals to support their health or aid in dieting, often without medical supervision. The combined use of these extracts can lead to various complications, ranging from dry mouth to damage to vital organs or even fatal reactions. Some herbal preparations may exacerbate dry mouth complaints. Examples include extracts from the Lamiaceae family (such as sage), capsicum (red pepper), garlic, *Ginkgo biloba*, and *Hypericum perforatum*. Additionally, herbal preparations with strong diuretic properties, such as nettle (*Urtica dioica*) and dandelion (*Taraxacum officinale*), can also contribute to dry mouth. Caffeine, found in many medications and foods, is one of the most widely used molecules that can cause xerostomia.

9. Aging and salivary gland degeneration

9.1 Structural change

The relationship between aging and deterioration of salivary gland structure has been extensively investigated. Histologic analyses have shown that the acinar volume

of the submandibular glands decreases by approximately 30% with age, while that of the sublingual glands decreases by approximately 25%. Labial salivary glands and parotid glands decreased by approximately 12%. On the other hand, there was a gradual increase in lipid droplet infiltration in the salivary glands. There was also an increase in fibrotic tissue. In submandibular glands, age-related acinar degeneration was accompanied by ductal dilatation. In the submandibular glands, there was an 80% increase in the average proportion of intralobular ducts and a sharp decrease in extralobular ducts. Age-related degeneration of the parenchymal structures of the salivary gland may impair salivary gland function. In addition to histologic changes, aging also causes numerous changes in the body that can be attributed to the salivary gland. Dysfunctions such as a decrease in the number of receptors can severely reduce its function [6].

Reduced stimulation and blood flow to the salivary gland, impaired neuronal activity, and age-related conditions, as well as the use of medication in the elderly population, can also inhibit the function of the salivary glands.

9.2 Metabolic changes in the salivary gland

Metabolic processes of biological systems are influenced by genomic and transcriptomic processes. Aging is a complex process influenced by a combination of genetics, environment, nutrition, and lifestyles.

Metabolomics are powerful tools for analyzing and generating a wealth of data, providing information on aging-related changes in the body.

A lower salivary flow rate in Sjogren's syndrome patients correlates with higher choline and taurine concentrations [6].

In one study, investigating the diversity of the salivary metabolome using mass spectrometry, it was found that patients with Sjogren's syndrome were severely affected and 41 metabolites were reduced. These are mainly amino acids and carbohydrates.

9.3 Innervation of the salivary glands

The function of the salivary glands is under the control of the autonomic nervous system. The secretory center in the medulla directs secretion. Sympathetic and parasympathetic stimuli cause secretion, but the parasympathetic nerves have a greater influence.

The salivary glands are controlled by both the sympathetic and parasympathetic systems. More specifically, parasympathetic stimulation leads to the secretion of water and ions, while sympathetic stimulation leads to the secretion of proteins.

Sympathetic innervation of the salivary glands begins with preganglionic nerves that cluster from the thoracic ganglion and spread parallel to the superior cervical ganglion and then to the carotid artery. Parasympathetic innervation starts from the salivary nuclei in the brainstem, from where the facial nerve (CN VII) innervates the submandibular and sublingual salivary glands and the glossopharyngeal nerve (CN IX) innervates the parotid gland [28].

Secretion occurs through autonomic innervation or stimulation by substances that mimic it. The phase beginning with the thought or smell of food is called the cephalic phase. Impulses from the cerebral cortex stimulate the secretory center, causing salivation. In what is known as the buccal phase, salivation begins when the senses of taste or touch in the mouth are stimulated.

The gastrointestinal phase occurs with the reflex from the stomach and upper digestive tract. Saliva secretion occurs when the wall of the esophagus is stretched by food entering the esophagus and these substances stimulate the mucosa in the stomach. Saliva secretion can vary greatly in terms of both flow rate and chemical properties. At rest, that is, in the absence of any stimulus, saliva is secreted only enough to moisten the oral mucosa. Salivary flow rate and chemical structure vary according to age, gender, sleep, diet, dehydration, emotional factors, infectious diseases, nervous system diseases, medications used, and the type and application of stimuli. Chewing activity is the strongest stimulus for salivation. Smell is as strong a stimulus as taste.

Secretion increases in gum chewing, metal poisoning, pain and irritations, smoking, acute stomatitis. Intestinal parasites and nausea increase the amount of saliva. In children, excessive saliva at night to the point of wetting the pillow should suggest the presence of intestinal parasites. Enzymes and electrolytes in saliva may increase or decrease depending on the rate of saliva flow. Saliva secretion is highest between 6 and 14 years of age. After 20 years of age, secretion begins to decrease. Around the age of 60, the flow rate averages 0.025 ml/minute - 0.034 ml/minute. Men have more secretion [29].

10. Other causes

Vitamin D and B deficiencies, hemodialysis patients, high blood pressure, emotional changes such as fear, and excitement are also causes of dry mouth.

Tuberculosis is a rare infectious disease of the salivary glands. It is caused by infection of the lymph nodes around the parotid gland or by *Mycobacterium tuberculosis* reaching the parotid gland through the stenon duct. It may first give the impression of a tumor due to the mass it forms. Parotid secretion may contain necrotic material. Microbiological screening of saliva samples is one of the most appropriate methods for diagnosis.

Drugs are substances that typically exert powerful effects on the central nervous system and alter a person's state of consciousness. These substances can have pain-relieving, sedative, euphoric, or hallucinatory effects. However, one of the side effects of certain drugs can be hyposalivation. Drugs have strong effects on the central nervous system and can alter brain functions. These effects can indirectly impact the autonomic nervous system, which controls the stimulation of the salivary glands. Drugs can affect this system, leading to a reduction in saliva production. Many drugs can cause fluid loss in the body. For example, stimulants or certain depressants can disrupt the body's fluid balance, leading to dehydration. Dehydration can result in the salivary glands not producing enough saliva.

11. Symptoms of dry mouth

People with dry mouth may experience thirst, difficulty speaking, oropharyngeal pain, dysphagia, difficulty using dentures, mucus accumulation, plaque accumulation, changes in oral flora, mucosal changes, taste disturbances, bad breath, extensive dental caries, cracking of the tongue and lips, loss of tongue papillae, fungal infections, enlarged salivary glands, increased saliva density, and dark color [30].

A simple method involves using a tongue depressor or metal spatula, which is pressed firmly against the buccal mucosa for about 10 seconds. When the pressure on

the spatula is released, it quickly falls away from the cheek mucosa, which is considered that at least the minor salivary gland functions are normal. The opposite situation can be interpreted as xerostomia.

Additionally, in clinical settings, the presence of lipstick stains on the incisors of female patients can indicate dry mouth.

12. Prevalence of dry mouth

Nederfors et al. [31] found that the prevalence of dry mouth was 21.3% in males and 27.35% in females in a study conducted by subjective assessment in 3313 randomly selected children aged 20-80 years in Sweden.

Thomson et al. [32] determined that 10.6% of 972 individuals had dry mouth as a result of subjective evaluations.

Narhi et al. [33] reported that 41% of 341 individuals in Finland complained of dry mouth.

The prevalence of dry mouth (xerostomia) increases with age, with approximately 30% of people over 65 years of age.

In Sjogren's syndrome, patients also complain of dry mouth, and the prevalence of Sjogren's syndrome in older people ranges from 1 to 4%. Of the patients participating in the study, 44 (21%) had hyposalivation and 166 (79%) had normal salivary flow rate.

13. Dry mouth survey questions

1. Do you feel dry mouth at night or in the morning?
2. Do you feel dry mouth at different times of the day?
3. Do you keep water with you at bedtime?
4. Do you need fluids when chewing dry food?
5. Do you feel dry mouth when you eat?
6. Do you have difficulty eating any food?
7. How much saliva do you have in your mouth? Less, normal, or more?

With these questions, information can be obtained about the patient's subjective symptoms of dry mouth. In addition, dryness of the soft tissues, atrophic or fissural mucosa, cracking of the tongue, and loss of papillae, low salivary flow rate can be identified among the findings of intraoral examination.

14. Salivary flow rate measurement methods

Before saliva collection, all patients should be informed about the saliva collection protocol and warned not to do anything that may affect the saliva flow rate.

Anyone who does not have any health problems or factors affecting the unstimulated salivary flow rate in non-medicated individuals: body position, hydration, light, age, smoking, odor, gender.

The saliva collection procedure should be performed in the morning (9.00-10.00 am) for each patient, and patients should have eaten at least 2 hours before saliva collection, and should not engage in activities such as tooth brushing or chewing gum 30 minutes prior to saliva collection, and should only rinse their mouth with distilled water.

For a stimulated saliva sample; the patient is asked to sit leaning forward and collect saliva in his/her mouth and is asked to spit into a container. This is done once a minute for 5 minutes is repeated. The total amount of saliva divided by five and the unstimulated saliva flow rate calculated as ml/min. Another method for obtaining a stimulated saliva sample is to chew 1.5 g of paraffin or sugar-free chewing gum for a few seconds. The first saliva produced by the patient swallowed by the patient. Then, every 30 seconds for 5 minutes, the patient's cup stimulated saliva flow rate in ml/min from the amount of saliva produced by spitting calculated.

14.1 Saliva collection methods

14.1.1 Saliva drooling

Saliva is collected using stimulated and unstimulated methods. In the unstimulated saliva collection method, the patient should sit quietly in a silent room with their eyes open. The patient is instructed to drain the saliva accumulated in the mouth into a test tube using a funnel from the corner of the mouth.

In the stimulated saliva collection method, the patient chews paraffin for a specific duration and spits the resulting saliva into a test tube [34].

14.1.2 Saliva suction method

Saliva is aspirated from the mouth using a plastic dental syringe. The tip of the syringe is placed under the tongue, and the accumulated saliva is collected in a test tube. At the end of the procedure, the tip of the syringe is moved around the floor of the mouth to collect any remaining saliva.

14.1.3 Cotton roll weight measurement

Three cotton rolls (0.2 x 0.6 cm) are placed in the patient's mouth. One roll is positioned at the opening of the submandibular glands under the tongue, while the other two rolls are placed near the openings of the parotid glands.

At the end of the procedure, the cotton rolls are removed, and their weights are measured. However, cotton cannot absorb excessive saliva and only measures saliva retained in the hard and soft palate, not on the tongue's surface.

14.1.4 L-Salivo test

Another method is the L-Salivo Test, which measures moisture on the tongue surface. This test is applied to the tongue 2–3 hours after eating and detects moisture by absorbing a thin film of saliva [35].

Ogami et al. [5] measured salivary flow rates from mandibular labial salivary glands using paper strips based on the iodine-starch reaction.

15. Salivary gland function tests

The function and structure of the salivary glands can be assessed using scintigraphy with Technetium (Tc) radionuclides. The Tc99m radionuclide injected intravenously can be monitored by gamma cameras to track its distribution in the glands and saliva released into the mouth. Sialography, ultrasonography, MRI, and CT imaging techniques can be used to visualize a variety of conditions such as sialoliths, ductal blockages, tumors, and cysts that may contribute to dry mouth. However, these techniques are more useful for diagnosing salivary gland dysfunction rather than measuring salivary gland function.

Furthermore, salivary gland biopsies may be performed to diagnose systemic diseases like Sjogren's syndrome. The minor salivary glands located just beneath the mucosa of the lower lip are most commonly used for biopsy.

16. Treatment of dry mouth

For patients with dry mouth, identifying and addressing the underlying cause is essential. If the cause cannot be eliminated, such as in cases requiring mandatory medication, maintaining regular oral care to ensure optimal oral hygiene is critical. Reducing or completely eliminating cariogenic foods from the diet is necessary for these patients. The use of topical fluoride can help reduce caries activity. Daily use of low-dose fluoride mouthwash is recommended for caries prevention [36].

In the initial stage, symptomatic treatments should be applied to all patients who present with xerostomia, regardless of the underlying cause. Drinking small amounts of water frequently helps ease swallowing, moisturize tissues, and assist with oral hygiene. Many patients may already be practicing this solution, but they may also need encouragement.

Mouth rinses, gels, sprays, and artificial saliva preparations can be used to improve the quality of life and restore functions. Additionally, oral care products should be neutral or alkaline in pH, alcohol-free, and sugar-free. Overly flavored toothpaste and mouthwashes should be avoided as they can irritate dry oral mucosa, causing pain and burning.

One method to prevent dental caries is the application of 1.1% sodium fluoride and 0.4% stannous fluoride on a tray to be worn in the mouth. A topical 5% sodium fluoride treatment, applied by the dentist 3 or 4 times a year, can also be considered a preventive measure. Chewing gum, lozenges, and candies can stimulate saliva production and provide symptomatic relief; however, sugar-free and non-acidic products should be preferred. For dry lip mucosa, moisturizers rich in vitamin E can be beneficial for bleeding and painful lesions on the lips.

Alcohol and caffeine should be avoided as they can worsen symptoms. Particularly, during the winter months, complaints of dry mouth can increase, and improving the humidity in home and work environments can help alleviate these symptoms. As a result, recommendations aimed at increasing salivation (such as chewing sour lozenges or gum) should be prioritized over artificial saliva

preparations for individuals with xerostomia. This is because no liquid can match the oral benefits of the saliva naturally produced by the individual.

Treatment options for dry mouth include symptomatic treatments and systemic drug therapies. In symptomatic treatments, patients can sip small amounts of water frequently to keep the mouth moist. Instead of water, milk may be used due to its rich mineral content. Milk plays a significant role in moisturizing and lubricating the oral mucosa. It also buffers oral acids with its calcium phosphate content and aids remineralization, reducing the likelihood of tooth decay.

For patients experiencing pain and difficulty chewing due to reduced mucosal lubrication while wearing dentures, artificial saliva preparations can be used. Gels with moisturizing properties and viscosity similar to natural saliva are the effective symptomatic treatments.

Using artificial saliva preparations containing various enzymes, antioxidants, antimicrobials, and remineralizing agents can significantly enhance the quality of life for patients with dry mouth.

17. Our artificial saliva study

Development of A New Artificial Saliva Preparation For Use In Patients With Decreased Saliva (Artificial Saliva Project) [37].

The aim of our study is to use the artificial saliva, which we will create by utilizing the antioxidant properties of the extracts obtained from the plants growing in our Aegean region (Türkiye), in patients with dry mouth.

In addition to the advantages artificial saliva will provide for our patients, it is essential that it be inexpensive and readily available. Our primary objective is to improve the quality of life for cancer patients and helping with their oral hygiene, particularly by utilizing natural and local resources to prepare artificial saliva.

An intellectual property application was made to Dokuz Eylül University Technology Transfer Office (DETTO) for patent application procedures (5.12.2023). Upon this application, we were informed that it was decided to make a national patent application under Dokuz Eylül University (DEU) Rights ownership with the official letter from DEU Rectorate.

17.1 Content of artificial saliva preparations

There are many artificial saliva preparations with different ingredients in the literature [38]. Most of these preparations contain mucin or sodium carboxymethylcellulose. We prepared an artificial saliva preparation based on mucin and sodium carboxymethylcellulose. In these artificial saliva preparations, liquid extracts from endemic plants frequently found in the Aegean region were added to create 24 separate groups, including four control groups. Water-based extracts obtained from natural plants growing in the Aegean region (olive, artichoke, thyme, yellow cantonion, and *Equisetum arvense*) were added to the artificial saliva we developed. Samples were sterilized using UV light.

17.2 Viscosity measurements

The density of human saliva varies between 1003 and 1009 g/ml. It is hypotonic, and its viscosity is between 19 and 35 mPa.s (millipascalseconds) [5].

Viscosity was measured using the most commonly used Brookfield viscometer (Fungilab Expert L, Spain) with a TR11 spindle at a rotation speed of 10 rpm. All Brookfield viscometers operate on the well-known principle of rotational viscometry, where viscosity is determined by detecting the torque required to rotate a spindle submerged in the liquid at a specific speed. Findings showed that the viscosity values of both mucin- and sodium carboxymethylcellulose-containing groups, with low and high viscosity formulations, were equal to or higher than those of natural saliva.

17.3 Toxicity evaluation of artificial saliva

Since the prepared artificial saliva groups were to be used in the mouth, their cytotoxicity was evaluated first. In the evaluation, the survival incidence of gingival fibroblasts was analyzed by MTT test, one of the colorimetric tests. As a result, it was determined that the toxicities of all groups were within normal limits and did not show toxic effects.

The toxicity of the artificial saliva was evaluated using gingival fibroblast survival assays. Toxicity tests were conducted on 24 sterilized groups in artificial saliva preparations. It was determined that the preparations had no toxic properties.

17.4 The role of antioxidants

Antioxidants play a significant role in dentistry, particularly in the healing of periodontal issues, lesions such as aphthae on the oral mucosa, and damage to oral soft tissues. Various *in vitro* test systems are used to identify natural and synthetic compounds with antioxidant effects. One such system is the DPPH (1,1-Diphenyl-2-picrylhydrazyl) radical scavenging assay. The DPPH method was selected to investigate the antioxidant properties of the added plant extracts.

17.5 Antimicrobial properties

The antimicrobial properties of the artificial saliva preparation were evaluated using the agar diffusion method for activity against *Streptococcus mutans*, *Escherichia coli*, *Streptococcus sanguis*, and *Candida albicans*. It was determined that the preparation exhibited no antimicrobial activity. This finding is considered beneficial for maintaining the balance of the oral flora.

Intellectual property rights for this study have been secured, and a patent application has been submitted to the Turkish Patent Institute. Research continues to enhance the remineralization properties of the artificial saliva preparation.

We are continuing our research to provide remineralization properties to a new artificial saliva preparation containing antioxidants.

18. Alternative methods for treatment of hyposalivation

In addition to methods like local stimulants and lubricants, parasympathomimetic drugs, hormones, acupuncture, and gene therapy can also be used to treat hyposalivation. Salivary secretion is predominantly controlled by the parasympathetic system. The stimulation of receptors in the salivary glands can play a role in modulating salivary flow, either through changes in parasympathetic nerves or direct glandular alterations.

Research indicates that chewing sugar-free gum and using sugar-free and citric acid-containing lozenges enhance stimulation and improve taste disorders. Patients with dry mouth should avoid dry and spicy foods, as they can irritate the oral mucosa. Additionally, the use of ascorbic acid and citric acid increases salivary flow rates [39].

The therapeutic properties of chamomile extract (*Matricaria chamomilla*) have been investigated, particularly to study its effects on the protection and healing of oral mucosal lesions. Studies have shown that chamomile extract is effective in reducing symptoms of oral mucositis and discomfort in patients receiving radiotherapy or methotrexate treatment. Chamomile flowers are rich in components such as essential oils and flavonoids. These components exhibit sedative, antispasmodic, anti-inflammatory, and antimicrobial properties, contributing to the protection and healing of the oral mucosa.

Furthermore, saliva substitutes with flaxseed extract have also been investigated as alternative treatments to alleviate dry mouth. Flaxseed (*Linum usitatissimum*) contains biologically active components such as proteins, unsaturated fatty acids, insoluble fibers, and soluble fibers. These components offer therapeutic benefits by helping to moisturize and protect the oral mucosa. In this way, flaxseed extract can also play an important role in supporting oral health [40].

For patients with severe dry mouth, systemic cholinergic drugs such as pilocarpine are effective in both local and direct cellular stimulation. Pilocarpine is recommended by the FDA for treating dry mouth in patients with Sjögren's syndrome. However, pilocarpine may cause undesirable side effects and is contraindicated in patients with glaucoma or uncontrolled asthma.

Previous studies have demonstrated the benefits of acupuncture in increasing salivary flow rates after radiotherapy. It has also been shown to reduce symptoms of dry mouth.

Interferon is a protein with regulatory functions that recognize cell proliferation, differentiation, and surface antigens. The use of 150 IU of Interferon-alpha three times daily has been shown to enhance salivary production and alleviate symptoms of xerostomia and xerophthalmia in patients.

18.1 Transcutaneous electrical nerve stimulation (TENS)

TENS is defined as a method of delivering controlled, low-voltage electrical currents to the nervous system through electrodes placed on the skin.

TENS application has been shown to increase salivary flow rates in both healthy individuals and patients with xerostomia. According to Hargitai et al. [41], applying TENS to the skin over the parotid gland enhances salivary flow in healthy individuals. They also suggested that for TENS to be effective, a baseline level of salivary flow is required.

Studies have also demonstrated that TENS therapy increases salivary flow in patients with Sjögren's syndrome.

In 2003, Wong et al. [42] evaluated the effectiveness of TENS in 46 patients with radiation-induced xerostomia and residual salivary function. TENS was applied in an acupuncture-like mode to preselected acupuncture points based on Traditional Chinese Medicine principles. This treatment was conducted over a 12-week period, with 6 weeks of therapy followed by a 2-week interval. The study results showed that this treatment method improved total salivary production, and the effects lasted for at least 6 months after treatment completion.

18.2 Acupuncture

Acupuncture, one of the key branches of Traditional Chinese Medicine, is a treatment method performed by inserting needles into specific points on the body and has been practiced for many years. The mechanism of acupuncture involves its effects on the autonomic nervous system, leading to the release of neuropeptides and an increase in salivary secretion [43].

It has been noted that acupuncture elevates the levels of endogenous opioids such as enkephalins, serotonin, and endorphins in the central nervous system and plasma, promoting a sense of well-being and providing psychological and emotional relief [44].

In a study evaluating the effect of acupuncture on salivary flow rates, a treatment consisting of 12 sessions of acupuncture (20 minutes per session, twice a week for 6 weeks) was found to have a significant impact on increasing salivary production. This approach was identified as an effective treatment for alleviating radiation-induced dry mouth [45].

18.3 Acupressure

Acupressure is based on the principles of acupuncture. It is a non-invasive technique that involves applying pressure to acupuncture points using hands or devices to balance the body's energy flow. Stimulating acupuncture points through acupressure can alter the release of neuropeptides, thereby affecting brain chemistry.

Acupressure has been shown to help individuals regain a sense of control over their health, reduce stress, alleviate pain and medication side effects, improve sleep quality, and enhance overall well-being [46].

18.4 Other methods

For addressing dry mouth caused by antidepressant use, chewing 15 grams of cappuccino-flavored coffee for 5 minutes has been found to increase salivary flow, reduce dry mouth symptoms, improve speech ability, and provide relief for approximately 2 hours [47].

Additionally, performing oral care with solutions containing flaxseed extract, either with or without chlorhexidine, has been shown to reduce dry mouth symptoms and alleviate speech problems [37].

19. Conclusions

In patients presenting to the dentist with complaints of hyposalivation or xerostomia, it is essential to first identify the underlying cause. Once the cause is determined, oral hygiene should be improved, and a diet emphasizing protein and vegetables to enhance chewing function should be recommended [48]. Consumption of sugary and acidic foods and beverages should be controlled. Regular fluoride application to the teeth should also be advised. If there is no organic dysfunction in the salivary glands, patients should be encouraged to chew sugar-free gum to stimulate saliva production, use mouthwash preparations containing citric acid, and consider artificial saliva substitutes [9, 25, 38, 49, 50].

In conclusion, considering the pathologies that may arise from xerostomia, it is of great importance for dentists to investigate and evaluate xerostomia symptoms in their patients [9, 25, 38, 50, 51].

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Biomarkers in Saliva to Assess the State of Periodontal Disease

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Abstract

Periodontal disease is a multifactorial infectious and immunoinflammatory condition that affects the tissues surrounding the teeth, potentially leading to irreversible damage and tooth loss. Early detection and diagnosis are crucial to preventing its progression and associated systemic health risks. Traditional clinical diagnostic methods, such as probing depth and radiographic assessments, have limitations in detecting early-stage disease. Consequently, molecular biomarkers found in saliva have emerged as promising tools for the early diagnosis and monitoring of periodontal disease. These biomarkers include inflammatory mediators, enzymes, bone remodeling markers, and microbial metabolites, providing valuable insights into disease status and progression. Advances in omics technologies, including proteomics and genomics, have further enhanced the identification of potential biomarkers, paving the way for precision medicine in periodontology. This chapter reviews the role of specific salivary biomarkers in periodontal disease assessment, highlighting their diagnostic potential, clinical applications, and future research directions in the field.

Keywords: biomarkers, saliva, periodontal disease, diagnosis, omics

1. Introduction

Periodontal disease is a multifactorial infectious and immunoinflammatory condition that affects the periodontal tissue surrounding the teeth and is specifically caused by one or more microorganisms [1]. This condition leads to the onset of gingivitis, the signs of which are redness and bleeding gums. Gingivitis can progress to periodontitis, where the periodontal tissue gradually deteriorates, presenting pus secretion, dental mobility, or tooth loss (**Figure 1**) [1, 3, 4]. Gingivitis is considered a reversible stage since it is confined to the gingiva; however, periodontitis, characterized by involvement of the alveolar bone and periodontal tissue, is considered irreversible, precluding the restoration of the initial state of health [5]. As the disease progresses to more serious or severe forms, it negatively affects speech, nutrition (chewing), esthetics, self-confidence, and overall quality of life [6].

Periodontitis is a global disease that affects millions of people worldwide and requires regular and lifelong follow-up and management due to its chronic nature [6].

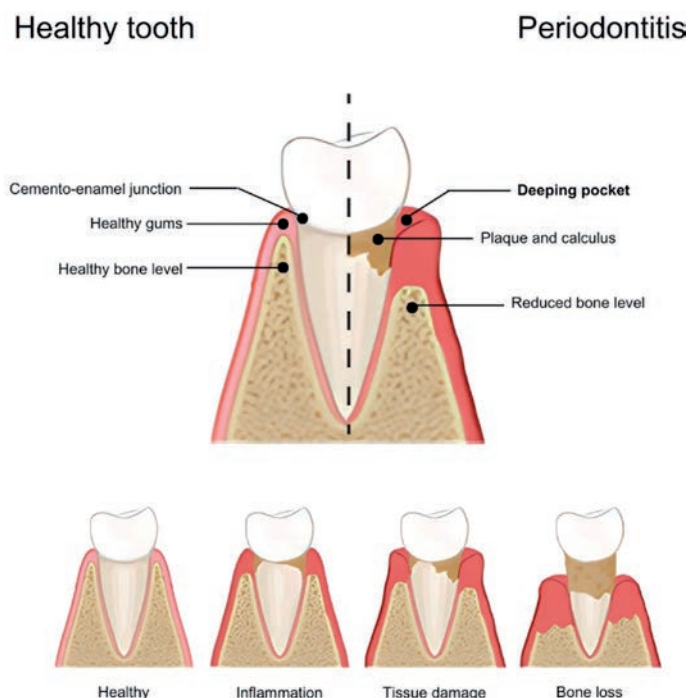


Figure 1.
Periodontal disease progression. Adapted from [2].

Epidemiological studies indicate that, in the USA, the morbidity of chronic periodontitis among adults in 2000 varied from 35 to 50%, with 10% of cases exhibiting severe disease and early tooth loss. According to the results of the 4th National Oral Health Epidemiological Survey of China, the percentage of subjects with healthy periodontal conditions was fewer than 10% among 35- to 45-year-old Chinese adults [7]. In 2021, around 1 billion people worldwide suffered from severe periodontitis, with an overall prevalence of 12.50%, and prognoses continue to rise [8]. The situation is exacerbated with age; as life expectancy rises, periodontitis is becoming a growing burden worldwide. In fact, between 1990 and 2010, the global burden of periodontal diseases increased by 57.3% [9].

The main etiologic agent for the onset and progression of periodontal disease is dental plaque biofilm, an organized collection of microorganisms residing in a complex intercellular matrix [10]. However, while bacterial activity is crucial to developing periodontitis, disease susceptibility and progression depend on a complex interaction with the host's immunoinflammatory response [11]. In fact, periodontitis is considered a microbiome-driven inflammatory disease affecting the gums [12].

The inflammatory component is prevalent in the etiopathogenesis of numerous pathologies, with growing epidemiological and experimental evidence associating periodontitis with an increased susceptibility to different health issues, including cardiometabolic, autoimmune, and cognitive neurodegenerative diseases, respiratory infections, and some cancers [12, 13]. The possible cause-effect connection between periodontal disease and some of these diseases is being studied [12, 13]. Therefore, early detection and treatment of periodontal disease could help reduce the risk of these comorbidities.

The treatment of periodontal disease aims to remove biofilm and bacterial deposits from the root surfaces of teeth to reduce inflammation and tissue loss. Regulation of the immune response to periodontal pathogens has been studied to mitigate inflammation, control bone damage, and promote bone regeneration. Drugs, stem cells, and other therapies targeting the immune microenvironment have shown promise in this regard [14].

2. Etiology of periodontal disease: The role of periodontal microbiota and inflammation

Periodontal disease develops when the host's immune system reacts disproportionately to periodontal pathogens (**Figure 2**).

The individual's immune and inflammatory response is influenced by external factors (such as dental plaque) and internal factors (such as the host's genetic makeup). It is intimately linked to the role of bacterial infection in the pathogenesis of periodontal disease. The immunophenotype is a crucial determinant of periodontitis severity, as individuals with a hyper-reactive genetic predisposition may overreact to even small amounts of bacterial biofilms harboring periodontal pathogens [1, 15].

Periodontitis is characterized by chronic inflammation of the tissues that support the teeth, accompanied by increased local concentrations of proinflammatory cytokines such as IL-1 α , IL-1 β , IL-6, and tumor necrosis factor-alpha (TNF- α), and regulatory cytokines such as IL-4, antagonist receptor of IL-1 (IL-1Ra), IL-10, and interferon-gamma inducible protein 10 (IP-10). Some cytokines recruit macrophages, neutrophils, and lymphocytes to the inflammation site [16]. In turn, neutrophils secrete several enzymes, including matrix metalloproteinases (MMPs) like MMP-8, MMP-9, calprotectin, and inflammatory mediators that combined cause the destruction of alveolar bone tissue and connective tissue [5, 17, 18].

Dental plaque biofilm, along with other environmental, lifestyle, and genetic risk factors, is the main etiologic agent responsible for the onset and progression of

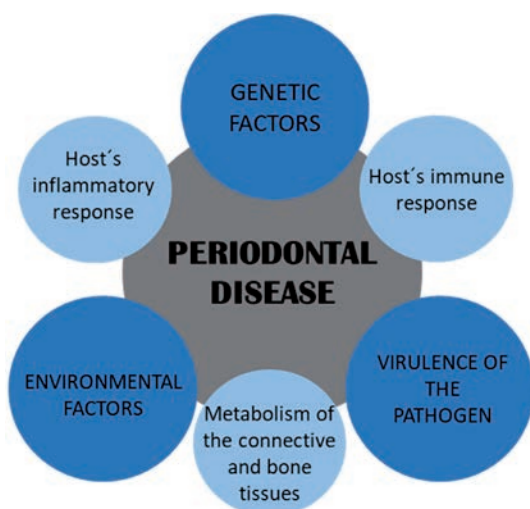


Figure 2.
Etiopathogenesis of periodontal disease.

periodontitis. In 1998, Socransky et al. categorized the periodontal bacteria according to their pathogenicity by assigning distinct color codes to clusters that mapped to various states of health and disease [19]. In this classification, the red complex pathogens (*Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola*) were mainly found in subjects with periodontitis, and the orange complex pathogens (*Fusobacterium*, *Prevotella*, and *Campylobacter* species) were often found in subjects with periodontitis (see **Figure 2** for the detailed bacterial composition of the red and orange complexes). The other complexes, such as the yellow complex (composed of different *Streptococcus* species) and green complex (composed of *Capnocytophaga* species), were strongly associated with periodontal health. Yellow, green, and purple complexes are the primary colonizers and are considered as a prerequisite for the appearance of the orange and red complexes (secondary colonizers) [10].

The keystone-pathogen hypothesis proposed by Hajishengallis et al. holds that certain low-abundance microbial pathogens can induce and aggravate a shift of the microbiome biofilm from a normally benign, symbiotic one to a dysbiotic one [20]. For example, *P. gingivalis*, one of the three bacterial components of Socransky's red complex, elicits an intense/destructive host immune response [19]. *P. gingivalis* attaches and forms biofilms on substrata of the commensal *Streptococcus gordonii* (a component of the yellow complex) [21]. It should be noted that the extent and rate of invasion of bacterial species can vary significantly in adjacent sites, such as in different teeth, or even in other sites of the same tooth [22, 23].

Pathogenic subgingival microbial species that accumulate and persist in biofilms interact with the vulnerable host, resulting in an increased inflammatory reaction, immune response imbalances, and the onset of periodontal disease [23, 24]. Periodontal disease is associated with increased levels of serum proteins and inflammatory mediators in the gingival crevicular fluid (GCF). GCF is an inflammatory exudate derived from the gingival plexus of blood vessels located within the gingival corium, immediately beneath the epithelium of the dentogingival junction. Evidence suggests that GCF from clinically normal tissue is an altered serum transudate, which only becomes an inflammatory exudate when the disease is clinically present [25].

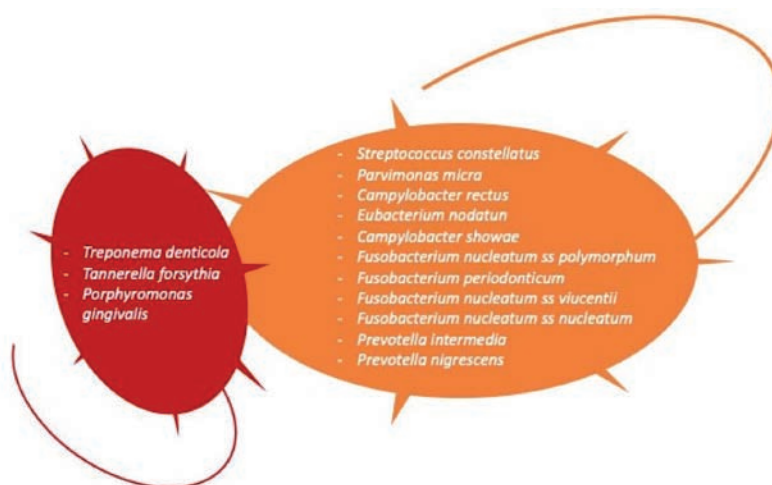


Figure 3.
Red and orange bacterial species according to Socransky [19].

Elevated concentrations of serum proteins and inflammatory mediators in the GCF will facilitate the proliferation of more pathogenic species, like those depicted in **Figure 3** (orange and red complexes), and their subsequent overgrowth [19].

It has been reported that clinically healthy gingival sites from subjects with periodontitis have a greater volume of GCF with higher levels of IL-1 β , IL-8, and higher proportions of orange and red complex species than clinically healthy sites from periodontally healthy subjects [26]. These findings suggest that these “clinically healthy” sites may be colonized by periodontal pathogens and exposed to a subclinical level of inflammation, thereby elevating the risk of periodontal disease onset and progression [26]. In this same sense, one study suggests that *Campylobacter rectus* and *Prevotella nigrescens* (two species of the orange complex) may be related to the recolonization of periodontal pathogens after professional cleaning, as their proportions increased in supragingival and subgingival plaque 4–7 days after professional cleaning [27].

3. Current diagnostic methods and clinical stages of periodontal disease

Early identification of periodontal disease is essential to initiate preventive treatment or use management strategies to halt further progression [17].

A specialized periodontal probe with a 0.5 mm diameter round tip is normally used for diagnostic evaluation to inspect and measure six areas per tooth in each gingival sites of the patient's dentition [28].

Along with the clinical history, conventional parameters for the diagnosis of periodontal problems typically include [1, 28–30]

- The probing depth (PD) of the gingival sulcus: distance between the gingival margin and the base of the periodontal pocket.
- The gingival index (GI): assesses the health of the gum according to its inflammation level.
- The clinical attachment level (CAL): the distance between the cemento-enamel junction and the base of the periodontal pocket.
- Gingival recession (GR): evaluates gum recession.
- The percentage of sites with bleeding on probing (BOP)
- Plaque index (PI): evaluates the plaque buildup along the gingival margin.
- Furcation involvement: evaluates pathological bone resorption in the area of the furcation.
- Dental mobility
- Radiodiagnostic assessment

According to the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases, the population can be divided into three groups [31, 32]:

1. Healthy subjects: no bleeding on probing (BOP <10%), probing depth (PD) of ≤ 3 mm, no radiographic bone loss, no clinical attachment loss (CAL), and no manifestations of other inflammatory lesions in the oral mucosa.
2. Subjects with gingivitis: have bleeding on probing BOP $\geq 10\%$, have PD ≤ 3 mm, no clinical attachment loss, or radiographic bone loss.
3. Subjects with periodontitis:
 - a. Mild to moderate (stage I/II): bleeding on probing BOP $\geq 10\%$, PD 5-7 mm, interdental CAL 2-4 mm.
 - b. Advanced (stage III/IV): bleeding on probing BOP $\geq 10\%$, PD > 7 mm, interdental CAL > 5 mm.

A new classification of periodontal diseases and conditions was introduced in 2018, showing the severity of involvement and tooth loss caused by periodontitis. It also considers the complexity of medical interventions required to eradicate or reduce infection and inflammation, in addition to other biological aspects, including the rate of progression, the influence of comorbidities and environmental factors, and the potential ramifications for the patient's overall health [33].

1. Initial periodontitis (stage I): Interdental CAL of 1-2 mm, radiographic coronal third bone loss (<15%), no tooth loss, maximum probing depth of ≤ 4 mm, with mainly horizontal bone loss.
2. Moderate periodontitis (stage II): Interdental CAL of 3–4 mm, radiographic coronal third bone loss (15–33%), no tooth loss, maximum probing depth of ≤ 5 mm, with mainly horizontal bone loss.
3. Severe periodontitis with potential for tooth loss (stage III): Interdental CAL of ≥ 5 mm, radiographic bone loss up to the middle third of the root and beyond, and tooth loss due to periodontitis of ≤ 4 teeth. In addition to stage II complexity: probing depth of ≥ 6 mm, vertical bone loss of ≥ 3 mm, and class II or III furcation involvement. It has a moderate ridge defect.
4. Advanced periodontitis with potential for tooth loss (stage IV): Interdental CAL of ≥ 5 mm, radiographic bone loss up to the middle third of the root and beyond, and tooth loss due to periodontitis of ≥ 5 teeth. In addition to stage III complexity: masticatory dysfunction, secondary occlusal trauma (degree of dental mobility ≥ 2), severe ridge defect, bite collapse with deviation, fan-shaped and with fewer than 20 teeth remaining (10 opposing pairs).

Patients in Stages I and II exhibit early to moderate stage periodontitis, without disease-related tooth loss, and are likely to respond predictably to conventional therapies based on bacterial load-reduction strategies. Conversely, for patients in Stage III and IV, one or more intrinsic or environmental risk factors may significantly impair the host's ability to respond defensively to bacterial infection and tissue damage [1, 18, 29].

4. Molecular biomarkers of periodontal disease: Comparison with traditional diagnostic methods

To date, the diagnosis of periodontal diseases is mainly based on periodontal probing to measure clinical parameters such as BOP, PPD, CAL, and bone loss. However, probing is considered an invasive test, frequently painful, and poorly accepted by patients. Moreover, these clinical indicators have limitations as they reflect past but not current disease activity, lack prognostic value, and do not reliably predict progression and response to treatment. Further, for these parameters to indicate the severity of the disease, there must be substantial damage, as they do not consistently detect early forms of the disease. In addition, complete clinical assessment of the oral cavity is often a lengthy and complex process for practitioners and researchers, which has prompted the development of abbreviated methods such as periodontal core coding and partial examination [1, 18, 34].

Due to the limitations of traditional clinical parameters, molecular parameters measured in oral fluids are becoming more prevalent as they can be used in the early diagnosis and prognosis of disease progression [1].

The growing interest in molecular biomarkers for periodontal disease stems from the benefits these biomarkers could offer over traditional diagnostic and monitoring methods. Among them, the following advantages are identified [2, 35–38]:

1. Improved diagnosis

Molecular biomarkers may account for earlier detection, as they can potentially identify periodontal disease in its early stages before clinical signs are detectable. In addition, they can complement clinical examinations, providing an objective assessment and information on the status and severity of the disease. Furthermore, many biomarkers can be detected in saliva or gingival crevicular fluid, allowing for non-invasive sampling and monitoring.

2. Disease activity monitoring

Molecular biomarkers can help define the stage of periodontal disease and monitor its progress. In addition, the combination of specific biomarkers allows obtaining different types of relevant information, such as characterizing the severity of the disease or allowing differentiation of whether the disease is in an active phase or in remission.

3. Guiding treatment and evaluation

Molecular biomarkers have the potential to assess periodontal therapies. Identifying specific molecular patterns can help tailor personalized treatments for each individual. In addition, they can be used to monitor the effectiveness of the interventions and adjust treatments accordingly.

4. Risk assessment

Molecular biomarkers may contribute to identifying patients at higher risk of periodontal disease; for example, genetic biomarkers can estimate individual

susceptibility to the disease. Certain biomarkers might predict the likelihood of disease progression. In this regard, validated predictive biomarkers a valuable tool for preventive policies.

Importantly, research on molecular biomarkers also contributes to understanding pathogenesis, providing insights into the molecular mechanisms of periodontal disease and, in their application, enabling the testing of new therapeutic approaches.

Biomarkers in periodontics can be categorized according to their diagnostic function into predictive, prognostic, and diagnostic markers [15]:

- Predictive markers identify risk factors and estimate the patient's overall risk prior to disease onset.
- Prognostic markers estimate the characteristics, stage, and extent of the disease already present.
- Diagnostic markers can identify disease onset, activity, and progression by biochemical or microbiological indicators.

Genetic markers are common in the predictive and prognostic marker groups.

An ideal biomarker for periodontal disease should fulfill several key characteristics to be clinically useful and reliable [34]:

- **Specificity:** The biomarker should be specific to periodontal disease, distinguishing it from other oral or systemic conditions.
- **Sensitivity:** It should be able to detect periodontal disease at early stages, before clinical signs are visible.
- **Predictive value:** The biomarker should accurately predict disease onset, progression, or treatment response.
- **Non-invasive sampling:** Ideally, it should be detectable in easily obtainable samples such as saliva or GFC.
- **Stability:** The biomarker should remain stable during sample collection, storage, and analysis.
- **Reproducibility:** Results should be consistent and reproducible across different laboratories and testing methods.
- **Rapid detection:** The biomarker should allow for a quick analysis, preferably chairside, to facilitate immediate clinical decision-making.
- **Cost-effectiveness:** The detection method should be affordable for routine clinical use.
- **Correlation with disease severity:** Levels of the biomarker should reflect the extent and severity of periodontal disease.
- **Responsiveness to treatment:** The biomarker should show changes in response to periodontal therapy, allowing for treatment monitoring.

- Biological plausibility: A clear biological rationale for the biomarker's relationship to periodontal disease pathogenesis should exist.
- Discriminatory power: The biomarker should be able to differentiate between health, gingivitis, and various stages of periodontitis.

Periodontal disease progression involves a complex interplay of microbial pathogens and host immune responses. Initially, pathogen-associated molecules trigger local inflammation. In response, host-activated cells release proinflammatory cytokines and proteases, leading to periodontal tissue damage and ultimately resulting in periodontitis. This multistage process releases various factors at different phases of the disease, presenting an opportunity to utilize these molecules as biomarkers. Consequently, a diverse array of biomarkers can be detected in oral fluids, including saliva and plaque biofilms, potentially representing valuable tools for diagnosing and monitoring periodontal disease progression. These biomarkers provide clinicians with crucial insights into the disease state, potentially enabling earlier intervention and more personalized treatment strategies.

5. Saliva as a source of molecular biomarkers of periodontal disease

Saliva has great potential as a source for identifying and analyzing potential periodontal disease biomarkers. Saliva is produced by the salivary glands, which are exocrine glands of the oral cavity. Saliva is composed of water, blood cells from oral micro-wounds, epithelial cell debris, organic molecules, growth factors, nucleic acids, DNA, mRNA, proteins, enzymes, electrolytes, immunoglobulins, hormones, and bacteria and their products that are of great use in the monitoring and early diagnosis of periodontal disease [1, 5, 39].

Saliva is helpful because it is a validated biological fluid for diagnostic procedures, and its collection is feasible for non-dental professionals. Saliva yields results in real time and provides information about the patient at the moment of collection: it can reveal the pathological and physiological state of the body [39, 40] and reflect both the specific oral microenvironment and the systemic environment associated with the person's health [1]. Saliva offers advantages over blood or GCF testing because saliva collection is easily accessible, noninvasive, and painless; does not require special equipment or a specially trained operator; and is more economical [1, 41]. In addition, saliva collection can be applied to a larger sample size and in different population types (children, adolescents, older adults, those with disabilities, or those with anxiety) [42]. On the other hand, the disadvantage of using saliva as a diagnostic tool is that it is subject to variability in its molecular composition due to multiple factors such as circadian rhythms [43], gender, age, dietary habits, collection methods, and the availability or lack of stimulation techniques [42].

According to their origin and function, several categories of molecular biomarkers of periodontal disease can be identified, many of them in saliva, as extensively reviewed [34, 35, 44–47]:

- a. *Host-derived biomarkers* are generated during the inflammatory response to bacterial load and are associated with the resulting soft tissue destruction. The diverse array of host-derived biomarkers includes various types of molecules, each offering unique insights into the disease process. Some key categories of these biomarkers include the following:

- Inflammatory mediators (cytokines, prostaglandin E2 (PGE2), C-reactive protein);
- Enzymes (MMPs, neutrophil elastase, cathepsins, aspartate aminotransferase, alkaline phosphatase (ALP), beta-glucuronidase);
- Bone remodeling markers (receptor activator of nuclear factor- κ B ligand (RANKL), osteoprotegerin (OPG), osteocalcin);
- Connective tissue breakdown products (pyridinoline cross-linked carboxyterminal telopeptide of type I collagen)

The accumulation of leukocytes in gingival epithelial tissues releases various enzymes and oxygen metabolites during the immune reaction, causing tissue damage. MMP-8 is one of the most representative enzymes involved in the breakdown of the extracellular matrix in periodontitis. MMP-8 promotes the degradation of collagen, determining periodontal attachment loss. Other enzymes released during the immune response or associated with cell death may have diagnostic potential. For example, ALP, which stimulates the calcification process and helps in bone remodeling, constitutes a marker for the progression of periodontal diseases. ALP shows higher salivary levels in periodontitis, and its elevated level in GCF seems to identify early attachment loss [48]. The lysosomal enzyme beta-glucuronidase shows higher levels in active disease sites and is also associated with clinical attachment loss. MMP-9 appears elevated in saliva and GCF in patients with recurrent attachment loss, and its levels decrease following periodontal therapy.

Activating macrophages in the lesion area is an important source of enzymes, cytokines, and inflammatory mediators. The severity of periodontitis is associated with increases in IL-1 β , TNF- α , prostaglandins (PG) such as PGE2, and MMPs, whereas their inhibition is related to the reduction of periodontal disease. Levels of PGE2 in saliva and GCF positively correlate with periodontal inflammation and serve as an indicator of impending bone tissue destruction. Studies have revealed significant differences in PGE2 concentrations between periodontitis patients and healthy individuals. Notably, PGE2 levels in periodontitis patients decrease significantly following periodontal treatment, reflecting the resolution of inflammation. Increased soft tissue destruction and increased IL-1 β and TNF- α can promote bone resorption by stimulating the release of osteoclastogenesis-associated factors. RANKL is increased while OPG is depressed in active bone resorption sites, resulting in an increased RANKL/OPG ratio. This ratio shift promotes osteoclastogenesis and subsequent alveolar bone loss. Once resorbed, the lost periodontal bone tissue cannot completely recover even though the inflammation is eliminated after treatment. Therefore, biomarkers associated with alveolar destruction must be detected as early as possible to prevent bone resorption and decrease irreversible destruction [15, 34].

b. Microbial biomarkers associated with the presence and activity of periodontal pathogens.

- Bacterial DNA or RNA
- Bacterial enzymes or metabolites (e.g., short chain fatty acids (SCFAs) and volatile sulfur compounds (VSCs))

Bacteria of the red and orange complex (see **Figure 2**) usually appear together in periodontal pockets and may cooperatively induce periodontal destruction. Some microbial test kits have been used to detect these bacteria and to assess their reduction or eradication associated with treatment. However, these bacteria account for only a small percentage of the strains found in the oral cavity. Moreover, the dysbiosis of free bacteria in saliva might be related to the onset, development, prognosis, and recurrence of periodontal diseases [22]. Next-generation sequencing analyses of 16S rRNA have enabled the identification of cluster ecotypes of plaque microbioma and their relationship with the prevalence and severity of periodontal disease. Such studies indicate that as the severity of disease increased, from healthy to gingivitis and periodontitis, the degree of dysbiosis in the salivary microbiome also increased [22]. In particular, the increased presence in the saliva of *Prevotella intermedia* and *Catonella morbi*, added to the decrease of *Porphyromonas pasteri*, *Prevotella nanceiensis* and *Haemophilus parainfluenzae*, may indicate generalized periodontitis in Stage I/II and could be used as a biomarker for an early and effective diagnosis [22].

Recently, evaluation of the bacterial metabolic activity by measuring the level of metabolites and the capacity of subgingival biofilm to produce them has been proposed as an approach for identifying risk determinants for the probability of periodontal disease progression. Studies have shown differences in the metabolic profiles of healthy individuals compared to those with periodontal disease. Oral dysbiosis can be reflected in the presence of SCFAs, including formic, acetic, propionic, butyric, and valeric acids produced by periodontal pathogens. Also, VSCs and amines produced by the periodontal microbiota may contribute to the inflammatory environment in periodontal pockets. In contrast, other metabolites, such as phenylacetate, may have a protective effect, which contributes to regulating the pH of the oral biofilm [49].

Comprehensive metagenomic studies and transcriptome and metabolome analyses are expected to contribute to the functional profiling of the oral microbiome and to obtain qualified information useful to stratify patients for precision treatments or preventive strategies. Targeted microbial analysis of periodontal disease pathogens can serve to identify effective antibiotic treatments and monitor patient responses.

c. *Genetic markers* to assess susceptibility to periodontal disease.

- Single nucleotide polymorphisms (SNPs) associated with periodontal disease susceptibility
- Epigenetic modifications

The presence of SNPs in genes involved in host immunity, inflammation, and tissue remodeling could be potentially linked with susceptibility to periodontitis. Several genome-wide association studies (GWAS) and candidate gene case-control studies have identified 65 genetic variants associated with periodontitis. Key genetic variations include the interleukin family (mainly IL-1 and IL-6), TNF- α , and Fc-gamma receptor genetic polymorphisms.

Recent studies have observed genetic pleiotropy between periodontitis and cardiovascular diseases (CVD), revealing that specific genetic variants are associated with both conditions. The highly pleiotropic genetic locus CDKN2B-AS1

(ANRIL) has been consistently linked to both coronary heart disease (CHD) and aggressive periodontitis. The ANRIL locus, in particular, is thought to play a crucial role in regulating inflammatory responses and cellular proliferation, processes relevant to both periodontal and cardiovascular pathology. Understanding these shared genetic factors may provide new insights into the underlying biological pathways connecting oral and cardiovascular health, potentially leading to novel therapeutic strategies for both conditions [50].

Epigenetic modifications play a crucial role in regulating gene expression and are intimately linked to the progression of periodontal disease. These modifications, including DNA methylation, histone modifications, and microRNA regulation, can significantly influence the host immune response and tissue homeostasis in the periodontium. Epigenetic modifications affect the level of gene expression and are closely linked to disease progression. Epigenetic analysis in periodontitis is in progress and offers potential applications in disease risk stratification and targeted therapy. For instance, genome-wide CpG methylation analysis has identified several genes affected by methylation in periodontitis patients [51]. For example, the IL-6 gene is hypomethylated in tissues of individuals with periodontal disease, suggesting an overexpression of this cytokine in inflamed tissues [52].

d. *Oxidative stress markers* in saliva could potentially serve as non-invasive markers for screening periodontitis.

- e.g., 8-hydroxy-deoxyguanosine, malondialdehyde

A decrease of total antioxidant capacity and a significant increase of malondialdehyde, nitric oxide, total oxidant status, and 8-hydroxy-deoxyguanosine levels or advanced glycation end products could represent sensitive biomarkers of periodontal disease [53].

5.1 Most promising salivary biomarkers of periodontal disease

Many studies have confirmed the ability of different bacterial and host-derived biomarkers in saliva to distinguish between oral health and various disease states, including dental caries, gingivitis, and periodontitis. A recent systematic review and meta-analysis identified five promising host-derived salivary biomarkers as candidates for the early diagnosis of periodontitis: MMP8, MIP-1 α , IL-1 β , IL-6, and hemoglobin [35]. *MMP8* is one of the strongest markers for tissue destruction and can reflect the grading and staging of periodontitis (sensitivity 65–87%, specificity 48–87%). *Macrophage inflammatory protein-1 alpha* (MIP-1 α), secreted by macrophages, is increased at the sites of periodontal inflammation and bone resorption, may identify subclinical inflammation in periodontally clinically healthy sites, and appears associated with periodontal bone remodeling, showing high sensitivity and specificity of 95 and 97%, respectively. *IL- β* , released by activated macrophages, lymphocytes, and fibroblasts, promotes the secretion of PGE2, releases MMPs, and determines bone and connective tissue destruction (sensitivity 54–88%, specificity 52–100%). *IL-6* is a pro-inflammatory cytokine secreted by macrophages in response to specific bacteria and by osteoblasts to stimulate osteoclastic activity. The levels of salivary IL-6 appear to be higher in patients affected by chronic periodontitis compared to healthy controls (sensitivity 52–80%, specificity 48–87%). Finally, detecting

Biomarker	Function and relevance	Sensitivity (%)	Specificity (%)
MMP8	Strong marker of tissue destruction, reflects the grading and staging of periodontitis.	65–87%	48–87%
MIP-1 α	Associated with periodontal inflammation and bone remodeling, identifies subclinical inflammation.	95%	97%
IL-1 β	Promotes PGE2 secretion, releases MMPs, and contributes to bone and connective tissue destruction.	54–88%	52–100%
IL-6	Secreted in response to bacteria and by osteoblasts to stimulate osteoclastic activity. Higher levels in chronic periodontitis.	52–80%	48–87%
Hemoglobin (Hb)	Indicator of microbleeding in gingival sulci, useful for preclinical diagnosis.	72%	75%
Other Biomarkers	CD9, CD81, suPAR, galectin-1, MMP-9, S100A8, LDH, AST, IL-1 β , IL-6, and TNF- α have diagnostic and prognostic potential in periodontal diseases.	—	—

Adapted from [54].

Table 1.
Sensitivity and specificity of promising salivary biomarkers of periodontal diseases.

hemoglobin (Hb) derived from microbleeding in gingival sulci may serve as an index for preclinical diagnosis (sensitivity 72%, specificity 75%) [35]. According to another recent systematic analysis, salivary CD9, CD81, suPAR, galectin-1, MMP-9, S100A8, LDH, AST, and pro-inflammatory cytokines (IL-1 β , IL-6, and TNF- α) have the diagnostic and prognostic potential to be used in periodontal diseases (**Table 1**) [54].

Biomarkers contribute to an accurate diagnosis and efficient treatments that improve periodontitis itself but also mitigate the development of other periodontitis-related diseases, and they can also serve to monitor the responsiveness to treatments [13]. In fact, after dental treatment, improvements at the level of biomarkers can be detected, including a decrease in proinflammatory cytokines, C-reactive protein, an improved lipid profile, a reduction of glycosylated hemoglobin (HbA1c), and even an improvement of the diversity in the oral microbiota, resulting in better health and the prevention of other diseases and comorbidities linked to periodontitis [13].

5.2 Validated biomarkers and clinical use

A few molecular biomarkers have already been translated as point-of-care (PoC) tests for periodontal diseases [34]. A PoC test is a rapid diagnostic kit designed to be used directly at the point of care, such as a dental clinic or doctor's office, without the need to send the sample to a laboratory. These kits provide results in just a few minutes and are typically easy to use, as they do not require specialized equipment or extensive personal training. PoC test kits are useful for making immediate clinical decisions and improving the efficiency of diagnosis and treatment.

To our knowledge, while several commercial tests based on various molecular biomarkers are available, the FDA (USA) has not yet approved a specific molecular test for periodontal disease diagnosis. In the European setting, three molecular tests for that purpose and based on detecting active MMP-8 have received approval. PerioSafe® and ImplantSafe® tests have received CE marking and have been validated for clinical use in multiple European countries. PerioSafe® is best suited for assessing

overall periodontal health and identifying patients at risk of periodontitis from saliva or a mouth rinse whereas ImplantSafe® is tailored for site-specific diagnosis, particularly around dental implants, to detect and monitor peri-implantitis or localized periodontal issues in GCF or peri-implant sulcular fluid [55]. Both tests constitute effective diagnostic tools for differentiating between healthy periodontium, gingivitis, and periodontitis, using cut-off points of 6.46 and 20 ng/mL of aMMP-8, respectively. In clinical practice, levels below 6.46 ng/mL indicate periodontal health, while levels between 20 and 60 ng/mL signal the need for more comprehensive treatment. Additionally, PerioSafe® facilitates epidemiological studies and has prognostic value, identifying patients at high risk of disease progression or those who may not respond to conventional treatments when levels exceed 60 ng/mL [34]. In addition, the test PerioMarker® analyzes the presence of the same enzyme (MMP-8) but uses a different technique and provides a positive/negative result. However, despite the promising value of these tests, they should be used in conjunction with traditional examinations and results interpreted alongside other clinical parameters.

Several commercially available saliva-based molecular biomarker tests exist for assessing periodontal disease risk, although they lack regulatory approval. Two genetic tests that evaluate a patient's susceptibility to periodontal diseases using saliva samples are the Periodontitis Susceptibility Trait (PST) and MyPerioID. Both tests analyze the presence of polymorphisms in the IL-1 gene, requiring the shipping of saliva samples to specialized laboratories for processing. Despite their commercial availability, these tests have faced some controversy and are not FDA-approved for clinical use [34].

Microbial tests have been developed to assess periodontal disease and pathogen abundance. The MyPerioPath test analyzes the presence of 13 bacteria associated with periodontal disease after DNA extraction and PCR amplification from saliva samples. The Omnigene Diagnostics utilizes specific DNA probes to identify eight key pathogens known to cause periodontal disease [34]. These molecular diagnostics tests are able to assess patient's oral microbiome and characterize the potential risk for periodontal disease, complementing traditional clinical examinations and radiographic assessments.

Up to date, most PoC kits for periodontal diseases rely on single molecular biomarkers measured in oral fluids. However, due to the complex causes of periodontal disease, it is unlikely that a single biomarker will provide an accurate diagnosis [37]. As a result, there has been increasing interest in the combination of biomarkers more than studying a single biomarker alone [37]. This multi-biomarker approach provides a more comprehensive view of the disease state, capturing various aspects of the inflammatory and tissue degradation processes characteristic of periodontal diseases. For example, combinations of salivary MMP-8, MMP-9, and tissue inhibitor of metalloproteinases-1 (TIMP-1) have shown a predictive accuracy of >90% for gingivitis and 100% for periodontitis [56]. Another study found that the dual combinations of IL-1 β , IL-6, and MMP-8 in saliva have an excellent ability to detect periodontitis and a good capacity to detect non-periodontitis [37] what makes them important early diagnostic biomarkers.

In this context, diagnostic tests integrating multiple metabolite analyses have been developed, combining PoC testing with lab-on-a-chip (LOC) technology. Microfluidic LOC systems enable the precise manipulation of small volumes of body fluids, consolidating complex laboratory procedures into a compact chip. An example of this is the Integrated Microfluidic Platform for Oral Diagnostics (IMPOD), which facilitates streamlines hands-free saliva analysis by integrating sample pretreatment with assay processes [57]. This is a portable and self-contained device capable of measuring levels

of MMP-8, TNF- α , IL-6, and C-reactive protein simultaneously. Devices applying similar principles are available to detect MMP-8, IL-1 β , and C-reactive protein for periodontitis diagnosis. A PCR chip for rapid amplification and detection of periodontal pathogens allows the quantification of different bacterial strains in clinical samples (a detailed review of PoC tests is found in [58]). These platforms rapidly quantify salivary biomarkers within 3–10 minutes, offering a practical and efficient solution for oral diagnosis [57]. However, although these LOC demonstrate their potential for chairside diagnostics, they still are not formally approved for clinical use.

Despite the intense research identifying host- and microbe-derived biochemical biomarkers for periodontal disease, significant heterogeneity in laboratory methods, data interpretation, and validation processes has hindered their translation into reliable clinical diagnostic tools. To facilitate standardization, a structured seven-point roadmap to enhance the comparability and reanalysis of research outcomes has been proposed. This approach aims to facilitate the translation of findings in periodontal research into clinical practice [38].

While biomarker combinations represent a paradigm shift toward precision periodontics, current clinical use remains supplementary to traditional methods due to unresolved standardization and cost-effectiveness concerns.

6. Future trends and perspectives

The integration of advanced biomarker technologies and personalized treatment protocols (including comprehensive physical examinations and classical strategies as mechanical removal of dental plaque and calculus) leads to precision periodontics, offering more accurate diagnoses, tailored interventions, and improved long-term prognoses for patients with periodontal diseases [59]. Emerging trends focus on enhancing precision, accessibility, and predictive capabilities through innovative technologies and interdisciplinary integration. Below are some key future perspectives involving salivary molecular biomarkers implementation:

6.1 Multi-omics biomarkers

Omics-based characterization of saliva constitutes one of the best molecular approaches to identifying robust biomarkers for periodontal disease progression, treatment response, and phenotypic stratification. Nowadays, high-throughput omics technologies enable comprehensive multi-analyte profiling across multiple biological layers. A vast amount of information can be obtained, including genetic information (genomics and epigenomics), molecular profiles (e.g. transcriptome and proteome analysis), and functional activity of biochemical pathways (metabolomics) which can refine the phenotype of individuals suffering periodontal disease including their genetic susceptibility. Furthermore, microbiome analysis can also be incorporated into these data sets and contribute to defining pathobiont ratios or dysbiotic community structures, aspects to model microbial interactions with the host. All these techniques are already in use from basic molecular studies to clinical research. Therefore, it is expected to reach a large body of evidence computed with complex bioinformatics tools—assisted with artificial intelligence (AI) and machine- and deep learning processes—able to integrate clinical, imaging, biological and large data sets to define periodontitis phenotypes. Current validation efforts should include lifestyle habits

(i.e., smoking-adjusted biomarkers thresholds), treatment-response biomarkers, and ethnicity-, gender- or age- specific biomarkers profiles. Altogether, this will allow to perform a patient stratification workflow for oral screening, personalized treatment and monitoring based on a comprehensive and validated panel of biomarkers [60].

6.2 Diagnostic devices

Current molecular diagnostics for periodontal disease consist in the determination of soluble biomarkers which can be determined with a PoC test. In this case, the sample is collected and directly applied at the PoC device obtaining rapid results with direct translation to clinical practice and treatment. More sophisticated technological advancements in the use of microfluidics assays and miniaturized biosensors can increase the performance of the diagnostic. They can integrate multiple laboratory functions and analyze determinations in a single, automatic and compact platform. Despite their potential for high-throughput screening, their clinical adoption faces two barriers to making it widely affordable: manufacturing scalability (which affects its affordability) and standardization [60].

However, this field is rapidly evolving, with a focus on developing biocompatible wearable sensors that are minimally invasive, able to provide continuous real-time data, and can be safely implanted for extended periods. At present, a miniaturized, battery-free, and wearable dental patch system that can be attached to the tooth surface has already been developed. This device can wirelessly monitor the oral microenvironment and deliver specific drugs on demand. Specifically, the prototype monitors the acidic microenvironment caused by bacterial metabolism and can deliver fluoride on-demand treatment. It uses near-field communication (NFC) for wireless energy harvesting and data transmission with smartphones [61], which supports monitoring and further treatment decisions.

This type of miniaturized portable devices achieve real-time monitoring of biomarkers of oral health and can also, on-demand, provide medication for personalized health care applications.

6.3 Economic and accessibility considerations

As commented, the development of robust molecular salivary biomarkers and their integration into clinical, at-home or wearable diagnostic devices could revolutionize periodontitis management by enabling early detection, reducing reliance on invasive procedures, and facilitating personalized treatment plans. However, this increased analytical power introduces challenges that can conflict with key diagnostic parameters such as affordability and user-friendliness. These considerations align with the evolving WHO diagnostic standards. Originally established in 2006 as the ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid/robust, Equipment-free, Deliverable) which have become widely accepted as the benchmark for an ideal test that can be used at the PoC. Recently, this has been updated to REASSURED diagnostic tests to include the additional criteria of R (Real-time connectivity) and E (ease of specimen collection) to improve future diagnosis [62]. For example, the use of wearable wireless biosensors would fulfill at least the two new criteria.

Strategic implementation of validated biomarkers through REASSURED-aligned platforms could optimize periodontal care pathways while maintaining cost-effectiveness –particularly crucial given the WHO’s emphasis on affordable diagnostics (typically <\$10 per test for basic assays).

However, the cost benefits of using PoC testing and LOC platforms to identify periodontal disease early are only realized if diagnosis is followed by timely treatment to prevent more expensive later stages. Simply diagnosing the disease does not always translate into cost-effectiveness for patients. In fact, the most significant financial burden for periodontal disease patients arises during the advanced stages of the disease, where treatment becomes more complex and costlier. In this context, a proactive approach—focused on early detection and management—will ultimately result in long-term savings for patients by minimizing disease progression and preserving oral health.

Moreover, an effective strategy to reduce the economic burden of periodontal disease on society should actively involve health care systems. The cost-benefit ratio associated with its prevention and management is often overlooked due to its *silent* nature. However, this pathological condition exerts a severe economic burden on both individuals and society. In 2019, the global treatment costs for periodontitis were estimated at US\$ 186 billion, with an additional US\$ 142 billion in productivity losses. The economic impact is equally substantial in Europe, where periodontal treatment costs for 32 countries were estimated at US\$ 2.52 billion in 2018. Moreover, the indirect costs in European countries, primarily associated with periodontitis-related edentulism, amounted to US\$ 156 billion. These figures underscore the urgent need for increased awareness, prevention strategies, and effective management of periodontitis to mitigate its economic burden (for a comprehensive review, see [63]. The health care systems could undertake the development of actions promoting oral health, the implementation of periodic checkups for prevention and early detection, and lifelong monitoring of periodontal health. All these actions may result in a more efficient handling of available resources with less impact on productivity and healthcare costs.

7. Conclusions

The use of saliva as a diagnostic fluid to determine periodontology biomarkers is a growing research area. These samples are non-invasive and easy to obtain, making this fluid attractive for clinical diagnostic use.

Incorporating periodontic biomarkers represents an important advance toward precision medicine in this field. Precision periodontics is based on identifying appropriate biomarkers for each patient's customized diagnosis and prognosis of periodontal disease. This would allow personalized treatment tailored to each patient's needs. In addition, the early identification of predictive biomarkers will enable healthcare professionals to tailor periodontal disease detection and preventive strategies for each patient.

Importantly, biomarkers alone are insufficient for diagnosing or treating periodontal disease and must be used in conjunction with clinical and radiographic assessments and the patient's medical and dental history.

Further studies are needed to identify and validate new biomarkers in saliva and to evaluate the efficacy and safety of their clinical use. Technologies and tools must also be developed that enable the accurate and rapid measurement of relevant biomarkers in saliva in addition to the use of predictive models that AI offers today.

Therefore, it is imperative to continue researching the viability of detecting biomarkers in saliva to facilitate personalized diagnoses and treatments for patients, as this approach within dentistry can advance precision medicine, resulting in greater effectiveness in clinical interventions.

All in all, the field of biomarkers in saliva to assess the state of periodontal disease is a rapidly advancing field that, no doubt, is going to improve diagnostics and

prevention strategies, enhance clinical outcomes and transform patient care, moving toward a more proactive, precise and patient-centered model of periodontal health management.

Conflict of interest

The authors declare no conflict of interest.

Acronyms and abbreviations

AI	artificial intelligence
ALP	alkaline phosphatase
ANRIL	antisense noncoding RNA in the INK4 locus
BOP	bleeding on probing
CAL	clinical attachment level
CHD	coronary heart disease
CVD	cardiovascular disease
GCF	gingival crevicular fluid
GI	gingival index
GR	gingival recession
GWA	genome-wide association
Hb	hemoglobin
HbA1c	glycosylated hemoglobin
IL	interleukin
IL-6	interleukin-6
IL-1	interleukin-1
IL-1Ra	antagonist receptor of IL-1
IP-10	interferon-gamma inducible protein 10
LOC	lab-on-a-chip
MIP-1	macrophage inflammatory protein-1alpha
MMP	matrix metalloproteinases
MS	mass spectrometry
OPG	osteoprotegerin
PCR	polymerase chain reaction
PD	probing depth
PG	prostaglandin
PGE2	prostaglandin-E2
PI	plate index
PoC test	point-of-care test
RANKL	receptor activator of nuclear factor-kB ligand
SCFAs	short-chain fatty acids
SNP	single-nucleotide polymorphism
TNF- α	tumor necrosis factor alpha
VSCs	volatile sulfur compounds
TIMP-1	tissue inhibitor of metalloproteinases-1

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Salivary Biomarkers for Screening and Monitoring of Oral Diseases

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Abstract

Salivary analysis tool for disease diagnostics has garnered growing scientific interest. The key advantages and the wide range of components consistently associated with oral diseases were discussed. A variety of salivary diagnostic technologies are available and have contributed to strategies for periodontal, dental, and mucosal alterations. Advanced nucleic acid (RT-Polymerase chain reaction (PCR), qPCR), salivary proteome, metabolomic, and lipidomic profiling (enzyme-linked immunosorbent assay (ELISA), mass spectrometry, ATR-FTIR) have identified biomarkers related to the diagnosis and progression of periodontal disease. Salivary studies with dental caries focus on host factors and cariogenic pathways like salivary antimicrobial proteins, flow, and bacteria clearance, emphasizing salivary markers in susceptible individuals. Human and microbial salivary proteomes have offered insights into the molecular pathogenesis of Molar Incisor Hypomineralization (MIH) (mass spectrometry-bioinformatic tools), identifying biomarker proteins found only in MIH subjects. Infrared profile (ATR-FTIR spectroscopy) coupled with machine algorithms can differentiate amelogenesis imperfecta and control saliva samples. Using immunological assay (ELISA) and an ultra-deep proteomics approach, we identified biomarkers that provide a good picture of the oral environment in the context of oral mucositis, providing a valuable window into treatment and/or disease progression monitoring. The combination of the biofluid saliva with analytical analysis has brought numerous perspectives for applications in diagnosing and managing oral diseases.

Keywords: saliva, salivary composition, oral disease, biomarkers, spectroscopy, artificial intelligence

1. Introduction

The role of saliva in protecting and maintaining the homeostasis of oral tissues is well established. However, its potential as a diagnostic and monitoring tool for oral and systemic conditions has garnered growing scientific interest [1–6]. The advancement of analytical methods, which now enable the precise identification of trace

substances, has significantly expanded research and possibilities using this biofluid [7–12]. This progress, driven by the refinement of analytical methods and data validation using platforms previously applied to plasma, urine, and other fluids, is a testament to the evolving nature of our field.

Saliva, a complex biofluid, is secreted by the major salivary glands (parotid, submandibular, and sublingual) and by numerous minor salivary glands distributed throughout the oral mucosa [13]. Once released into the oral cavity, it mixes with other components such as crevicular fluid, food debris, desquamated epithelial cells, microorganisms, and their products, increasing its biochemical complexity. Saliva contains around 99% water and 1% organic and inorganic components. The organic fraction includes amylase, glycoproteins, enzymes, mucins, lysozyme, histatins, cathelecidins, defensins, lipoproteins, statherin, lactoferrin, proline-rich glycoproteins, lactoperoxidases, agglutinins, immunoglobulins IgA, IgG, and IgM, ribonucleases, and matrix metalloproteases. The inorganic fraction includes sodium, calcium, phosphate, potassium, magnesium, and chloride ions [14, 15]. This intricate composition of saliva, with its wide range of components such as enzymes, hormones, antibodies, and antimicrobial peptides, underscores its multifaceted functions in lubrication, cleaning, digestion, calcium and phosphate homeostasis, microbial control, pH buffering, and demineralization and remineralization processes [13, 14, 16].

Salivary diagnosis is a field of research that aims to serve as an alternative or complement to conventional serological tests. The term “Salivaomics,” introduced in 2008 [17], along with recent advances in detection technologies, contributed to the identification of alterations in gene expression, transcriptional activity, protein and enzymatic profiles, and small molecule concentrations [18]. The main advantages of salivary diagnosis are its simplicity, non-invasiveness, and rapid collection. Sampling does not require specialized equipment or trained professionals and can be performed on individuals of all ages, providing a comfortable and reassuring experience [1].

There is no standardized method for saliva collection and preparation, which results in significant variations due to the influence of the sampling, transport, processing, and storage techniques employed [18, 19]. In addition, saliva is a dynamic fluid influenced by diet and various stimuli, with fluctuations in flow rate and secretion of ions and proteins [3]. To mitigate these limitations, the concomitant evaluation of additional salivary parameters is recommended to minimize potential confounding factors that may affect the assessment of a specific target. Measurement of unstimulated saliva flow rate (uSFR) stimulated salivary flow (sSFR), total protein concentration, pH values, and buffer capacity could be helpful as complementary metrics for better interpretation of salivary assay results.

A variety of methods and commercial kits are currently available for salivary evaluation. However, the choice should ideally be based on previously validated instruments and protocols [6, 12, 18]. If such data are unavailable, preliminary validation should be carried out. Salivary biomarkers with high analytical accuracy results have been detected using point-of-care (POC) devices or other standard methodologies (**Figure 1**).

Salivary changes were identified in the context of several systemic conditions, including oncological, skin, and kidney diseases [20]; breast cancer [4, 21]; diabetes mellitus [22, 23]; Alzheimer’s disease [24]; and Sjögren’s syndrome [25]. Importantly, the value of saliva in routine toxicological testing [26] and in identifying environmental chemical pollutants [27] has also been proven.

Given its constant exposure to all oral tissues, saliva captures molecular alterations occurring in the oral environment, making it a potentially valuable diagnostic fluid

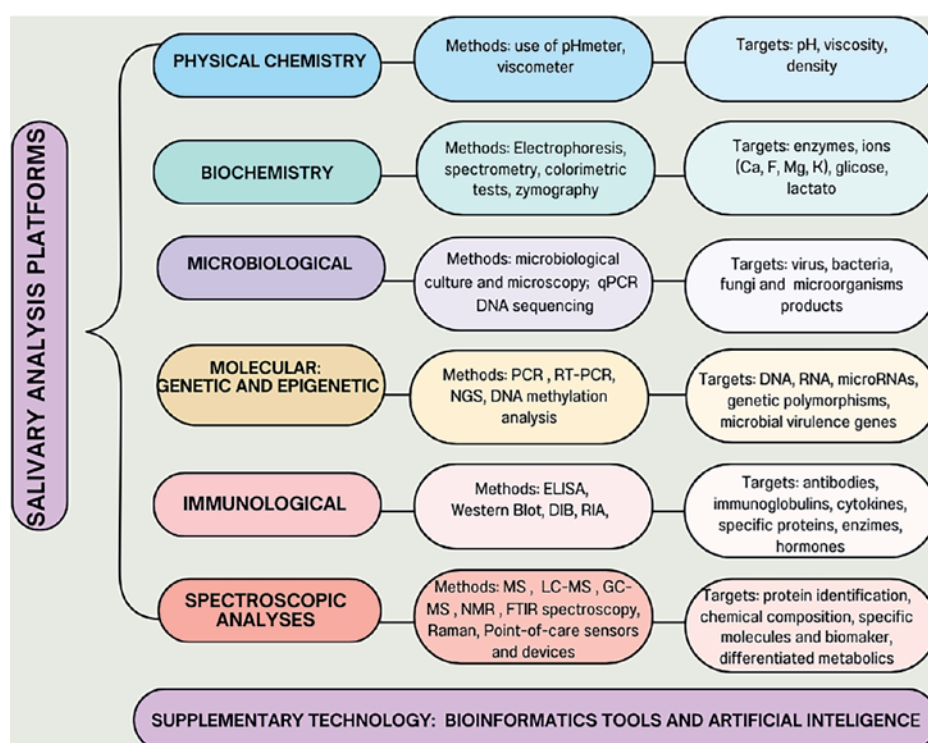


Figure 1.

Illustrative picture of salivary analysis platforms, methods, and targets. Polymerase chain reaction (PCR), quantitative PCR (qPCR), real-time PCR (RT-PCR), new generation sequencing (NGS), enzyme-linked immunosorbent assay (ELISA), dot immunobinding (DIB), radioimmunoassay (RIA), mass spectrometry (MS), liquid chromatography with mass spectrometry (LC-MS), gas chromatography with mass spectrometry (GC-MS), nuclear magnetic resonance (NMR), and Fourier transform infrared (FTIR) spectroscopy.

for screening and monitoring oral diseases. This tool has been suggested in studies of interest in dentistry, emphasizing specific biomarkers that can improve preventive and therapeutic dental care [7, 11, 28–30]. We focus on oral conditions currently targeted by salivary diagnostics, including periodontal disease and dental and mucosal alterations. These conditions represent a critical demand for professional practice and play a relevant role in the quality of life of individuals.

2. Salivary changes and oral diseases

2.1 Salivary biomarkers of periodontal disease: Advanced analytical platforms in medical and dental diagnostics

Periodontal disease, a chronic inflammatory condition, is associated with a dysbiotic biofilm that triggers host immune responses, leading to a sustained loss of periodontal ligament and alveolar bone [31]. The demand for early and reliable diagnostic approaches has underscored the potential of salivary biomarkers of periodontal diseases as a promising noninvasive strategy. Identifying specific nucleic acids, proteins, peptides, metabolites, inflammatory cytokines, and bacterial signatures in saliva that correlate with the onset and progression of periodontitis

can play a crucial role in implementing this diagnostic strategy in dental settings [7, 32]. Advanced analytical platforms, including real-time polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assays (ELISA), high-throughput omics technologies, and biophotonic devices such as attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) and Raman, can lead to more effective periodontal disease detection shortly. The oral fluid analysis in these cutting-edge techniques enables rapid, sensitive, and noninvasive diagnostics of periodontal diseases, establishing a novel framework for diagnostic tests, which may be adapted for chairside use to support timely interventions in medical and dental settings.

2.1.1 Advanced nucleic acid profiling in salivary diagnostics of periodontal diseases

Nucleic acid analyses in saliva, including bacterial genomic DNA from periodontitis-associated pathogens, emerged as critical assays for elucidating periodontal disease mechanisms. RT-PCR and next-generation sequencing (NGS) are advanced techniques that enable sensitive detection of subtle gene expression linked to specific bacteria and cytokine-driven signaling pathways associated with periodontitis. Quantifying specific mRNA transcripts allows the detection of host gene upregulation during active periodontal inflammation. These transcripts may include proinflammatory cytokines, including IL-1 β , IL-6, and TNF- α , as well as tissue-degrading enzymes like MMP-8 and RANKL. In parallel, bacterial DNA amplification can be used to characterize the oral microbiome precisely. Identifying genetic signatures from *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, two prominent periodontal bacteria, reveals which bacteria are driving the progression of periodontal disease. While less well-known, *Tannerella forsythia* and *Treponema denticola* are bacteria that can further contribute to this complex ecosystem. Altogether, bacterial nucleic acid profiling opens new avenues for early diagnosis and a more optimized comprehension of periodontal disease progression in medical and dental settings [33].

Bacterial signatures in saliva can be obtained employing molecular techniques targeting the genetic material of oral microorganisms. Quantitative PCR (qPCR) and 16S rRNA gene sequencing enable a highly sensitive measurement of the relative prevalence of specific pathogenic species. In periodontal disease, an increase in the levels of periodontal pathogens indicates a shift from a symbiotic microbial community to a dysbiotic environment. For instance, elevated counts of *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were consistently associated with the severity of periodontal destruction in saliva and gingival crevicular fluid [34]. This microbial imbalance not only acts as a diagnostic indicator but also provides prognostic value, as changes in the bacterial community structure often precede clinical manifestations of the disease. By integrating bacterial signature analysis into routine diagnostics, clinicians can more accurately predict disease progression and tailor treatment strategies to restore a balanced oral microbiome.

2.1.2 Proteins and peptides

The salivary proteome, a complex array of proteins and peptides, is a promising field in studying periodontal disease. It provides a comprehensive molecular snapshot of the level of periodontal tissue degradation. Molecular platforms, such as ELISA, Western blotting, and lateral flow immunoassays, are widely employed to

detect and/or quantify these proteins/peptides with high sensitivity and specificity. Several salivary components were identified as promising diagnostic biomarkers of periodontitis, including inflammatory cytokines and tissue-derived peptides. Among them, elevated levels of lactoferrin, vitamin D-binding protein, parotid secretory protein, elongation factor 2, complement C3, and members of the S100 protein family were associated with periodontal inflammation. Immunoglobulins such as IgG2 and IgA2 can also reflect the local immune response. Structural and enzymatic proteins, including α -amylase, cystatins (cystatin B and SN), and also the tissue-degrading enzymes matrix metalloproteinases (notably MMP-8 and MMP-9), are often found in higher concentrations in subjects with active periodontal disease. Other enzymatic markers include dipeptidyl peptidase IV and antimicrobial peptides such as LL-37, defensins, and salivary histones, and this enzyme also contributed to the proteomic fingerprint of periodontal status [35]. These salivary proteins and peptides collectively constitute a promising molecular panel for the early diagnosis, disease monitoring, and personalized management of periodontitis. However, their diagnostic and prognostic relevance must still be validated in large-scale and multicenter studies. Such studies are essential to assess the influence of systemic conditions (diabetes, obesity, and related comorbidities) on the expression of salivary and crevicular fluid biomarkers for periodontitis.

2.1.3 Metabolomic and lipidomic profiling of saliva for periodontal disease detection

Metabolomics and lipidomics, when applied, significantly contribute to a comprehensive molecular understanding of periodontal disease. High-resolution mass spectrometry allows simultaneous analysis of several metabolites and lipids related to the host–microbe interactions and disease progression. In metabolomic analysis, higher concentrations of cadaverine, 5-oxoproline, histidine, isoleucine, choline, and phenylalanine in saliva were related to periodontal disease severity. These metabolites reflect disruptions in local oral metabolism, oxidative stress, and amino acid pathways triggered by periodontal inflammation in the subgingival microenvironment [36]. The role of lipid metabolism in periodontitis is even less explored. Lipidomic analyses of gingival tissues have revealed significant differences between periodontitis patients and healthy individuals. Lysophosphatidylcholines (lysoPCs), diacylglycerols (DGs), and phosphatidylcholines (PEs) were upregulated classes of lipids in periodontitis, while triacylglycerols (TGs) were downregulated, suggesting similar regulation in the salivary milieu [37].

2.1.4 Infrared profile in saliva from subjects with periodontitis

ATR-FTIR spectroscopy, a technique that enables a rapid, reagent-free, and non-destructive platform to analyze the molecular composition of salivary components, works by measuring the absorption of infrared light by the sample. This generates a unique spectral fingerprint for each sample based on the biochemical changes associated with the respective composition. For instance, ATR-FTIR was used to detect variations in lipid, amino acid, carbohydrate, and nucleic acid content in saliva samples, allowing the differentiation of healthy controls from periodontitis patients and distinguishing chronic from more aggressive forms of the disease. It was shown that this sustainable tool effectively distinguished between different forms of periodontitis by revealing subtle shifts in these molecular components. It was necessary to improve the analysis of confounding factors such as smoking and systemic diseases

with inflammatory status [38–41]. The potential of ATR-FTIR arises from its analytical capabilities and feasibility for integration into point-of-care (POC) devices and artificial intelligence algorithms to detect bacteria [42]. Recent technological advances in infrared spectroscopic devices, including heated crystal systems that allow rapid sample drying of saliva, have positioned ATR-FTIR as a promising, sustainable tool for chairside use in dental settings. This innovation could enable clinicians to perform real-time evaluations of salivary composition, supporting immediate clinical decision-making to detect periodontitis. However, its clinical value still requires further validation to distinguish periodontal-specific inflammatory signatures from those associated with other oral or respiratory inflammatory conditions that may influence the salivary profile.

2.1.5 Practical considerations for clinicians

Salivary diagnostic technologies are reshaping oral care, offering noninvasive, rapid, and precise disease monitoring. These portable systems require minimal training, making them feasible for everyday dental and medical practice. Real-time PCR for quantifying microbial and host nucleic acids, immunoassays for detecting inflammatory proteins, and ATR-FTIR spectroscopy for rapid biochemical fingerprinting and omics-based approaches, including metabolomics and lipidomics, further enhance diagnostic precision. They are able to capture metabolic signatures linked to inflammation and tissue degradation. Detecting biomarkers, including *P. gingivalis* DNA, several cytokines, and metabolites, could support early diagnosis, guide therapeutic decisions, and enable personalized treatment strategies for dentists and physicians. Integration into point-of-care and self-collection frameworks may enhance usability in clinical and public health settings, which is critical for the early identification of periodontitis and its impact on managing systemic diseases, including insulin-resistant diabetes. Although promising, broader clinical validation in large, diverse populations is needed.

2.2 Salivary changes and dental alterations

Saliva, a key player in maintaining oral homeostasis, protects tooth enamel by regulating calcium homeostasis and promoting remineralization [1, 14, 16, 18]. When in contact with dental surfaces, in the healthy oral ecosystem, the saliva provides a dynamic interaction between the heterogeneity of the microbiome and metabolites. A dysbiotic state is believed to be a key factor in the onset of oral disease [43]. The emerging use of salivary analysis as an essential tool in studies involving enamel issues is a testament to its significance.

Dental caries represents one of the most prevalent oral diseases and results from imbalances in the demineralization-remineralization process, involving an intricate interplay of multiple factors (**Figure 2A**) [44–48]. Dental caries is a biofilm-mediated, sugar-driven, multifactorial, dynamic disease (46). Saliva can influence the direction and rate of the cariogenic pathway, contributing to the balance of oral microbiota [14, 16, 42, 47]. Saliva contains antimicrobial proteins (mucins, lactoferrin, lysozyme, statherin, histatins, and IgA) [49, 50] and promotes bacteria clearance through salivary flow and swallowing and inhibits the adhesion of cariogenic bacteria to tooth surfaces. Salivary markers such as alpha amylase activity [28, 29], acidic proline-rich protein-1, histatin-5, carbonic anhydrase 6, proteinase-3, lastoperoxidase, mucin-1 and statherin, mucins, glycoproteins (sCD14), interleukins (IL-2RA, 4,-13), urease,



Figure 2.
Clinical aspects of dental enamel. A- Molar enamel affected by caries. B- Molar enamel affected by HMI. C- Molars' enamel affected by amelogenesis imperfecta. D- A healthy molar enamel. (Author collection).

carbonic anhydrase VI, and urea [30, 51] have been proposed as potential tools to identify individuals susceptible to dental caries. These biomarkers offer promising insights for caries risk assessment and developing preventive and therapeutic strategies.

Other dental alterations have been attracting increasing attention, especially those involving tooth enamel, which offer opportunities for early intervention. The formation of enamel involves a complex biological process known as amelogenesis. It occurs in three well-defined stages: the secretory, transition, and maturation [52]. Disruptions in amelogenesis can lead to macroscopic quantitative or qualitative defects, which often present clinical and radiographic heterogeneity (www.phenodent.org) (**Figure 2B-C**). A deeper understanding of the pathogenesis of these enamel defects and the specific characteristics of the affected enamel alterations can contribute to developing more effective management strategies.

Molar Incisor Hypomineralization (MIH) is an intriguing dental condition (**Figure 2B**). This complex enamel defect affects one or more first permanent molars with or without the involvement of permanent incisors [53]. Demarcated opacities, resembling MIH, observed at the tips of permanent canine cusps, second permanent molars, and premolars have been included as possible concomitant diagnostic criteria of MIH by the European Academy of Pediatric Dentistry in 2022 [54]. The etiology of MIH is complex and multifactorial, suggesting the influence of systemic conditions and genetic factors that may act additively or synergistically [54, 55].

The clinical management of MIH is challenging due to rapid wear, progressing enamel loss, increased susceptibility to caries, loss of fillings, and severe hypersensitivity, all with unclear pathogenesis [53]. There is an urgent need for a deeper understanding of MIH at the cellular and molecular level.

Studies on the structural, mechanical, and chemical properties of MIH-affected enamel have demonstrated a reduction in both mineral quantity and quality (including decreased calcium and phosphorus content), reduced hardness and elastic modulus, and increased porosity, carbon/carbonate concentrations, and protein content (notably serum albumin, alpha-1 antitrypsin, and antithrombin III) compared to unaffected enamel [56].

Salivary physicochemical properties were investigated in MIH-affected individuals, revealing that lower salivary flow rates, moderately viscous saliva, and reduced pH were significantly more prevalent. These oral environment characteristics were more likely associated with the post-eruptive breakdown of the MIH teeth [57].

Salivary proteome studies indicated differential expression of proteins in MHI-affected children compared to healthy children, offering insights into the molecular pathogenesis of MIH [58–60]. Saliva of children with MIH presents a distinct protein composition compared to healthy children. Several proteins were found exclusively in healthy controls and associated with adaptive immunity and lysosomal function, suggesting that these proteins may play a role in maintaining oral health. In contrast,

identifying proteins found exclusively in the saliva of children with MIH supports their potential use as biomarkers for diagnosis or disease monitoring. Functional analysis indicated that these proteins are involved in inflammatory, immunological, and tissue degradation processes, reinforcing the hypothesis that MIH is a multifactorial condition with multiple contributing factors [58, 59]. The presence of proteins related to neutrophil activation and degranulation in the saliva of patients with MIH suggests that inflammation may play an essential role in the pathophysiology of MIH. The presence of proteins functionally linked to “cornification” and “keratinization” (synthesis of acute-phase proteins) reinforces these findings [59]. Salivary proteins were identified with high confidence by mass spectrometry. Gene ontology and biological pathways were conducted using bioinformatic tools, including the interactive platform “Enricher” [58, 59].

The microbial salivary analysis revealed a lower diversity in MIH-affected subjects, suggesting a dysbiotic environment in the MIH pathology. It was described as a downregulation of defense response to bacteria, immune system activity, regulation of peptidase activity, and the B-cell receptor signaling pathway. In contrast, leukocyte-mediated immunity, neutrophil activation and degranulation, and acute inflammatory response were upregulated in the MIH individuals [59].

Salivary proteomics changes in MIH children according to distinct degrees of severity, reinforcing the interplay between the MIH clinical characteristics and changes in the salivary proteome. The mass spectrometry and bioinformatic analysis identified proteins exclusively from MIH children, and 778 proteins overlapped across different severity levels. Gene ontology revealed distinct patterns of the biological pathways MIH in children, highlighting those involved with the severe condition (*i.e.*, calcium ion binding, microtubule binding, platelet-derived growth factor binding) [60].

Genetic factors encoding polymorphisms have been related to increased odds of developing MIH [55, 61]. These polymorphisms, variations in DNA sequence, can affect the expression or function of proteins involved in enamel development. Genomic DNAs were extracted from a saliva sample and genotyped using beadchip microarrays. It was also shown that genetic associations with MIH may differ across populations, possibly due to differences in the prevalence of MIH and frequencies of single-nucleotide polymorphisms (SNPs). In addition, the study highlighted the importance of replicating genetic association studies in different ethnic groups to confirm results and the need for GWAS (genome-wide association study) studies in larger groups to identify MIH predisposition genes [62].

Another condition that disrupts enamel development is Amelogenesis Imperfecta (AI) (**Figure 2C**), a heterogeneous group of rare genetic diseases that affect the quality and/or quantity of enamel in both primary and permanent teeth [63]. The clinical phenotype of AI can be classified into hypoplastic, hypomature, hypomineralized, and hypoplastic/hypomature with taurodontism, and the inheritance pattern is autosomal dominant, autosomal recessive, or X-linked [64]. The prevalence of AI varies in different populations, ranging from 1700 to 14,000 individuals, depending on the population studied [65]. More than 70 genes have been associated with “isolated” or “syndromic” forms of AI to date. These genes involve enamel matrix proteins, intracellular vesicle trafficking, ameloblast attachment, ion transport, mineralization, and matrix-protein degradation [66].

Progress in analysis techniques has broadened the understanding of AI, thus improving diagnosis and genetic counseling [52, 66]. New generation sequencing and bioinformatics analyses bring perspectives to associate genotype-phenotype and the

functional role of the affected protein. DNA samples have been obtained from saliva alone or in combination with blood to identify individual genetic variants [66–68].

Infrared profile compared salivary vibrational modes between AI patients and matched control individuals using ATR-FTIR spectroscopy coupled to machine algorithms [11]. It indicated that molecular changes in the saliva of AI individuals and matched controls have the potential to be detected by ATR-FTIR spectroscopy. Loading plot analysis found that AI can be associated with changes in DNA-related (vibrational mode at 1708 cm^{-1}), protein-related (vibrational mode at 1651 , 1572 , 1556 , 1507 , 1403 , and 1289 cm^{-1}), phosphate-related (vibrational mode at 1220 cm^{-1}), and carbohydrate-related (vibrational mode at 1012 and 980 cm^{-1}) regions. Changes in the loading plot of a protein region can be related to changes in salivary proteins [69], and changes in a phosphate region may be related to changes in salivary calcium and phosphorus [70]. According to the authors of this innovative study, ATR-FTIR spectroscopy of saliva can effectively differentiate between AI and control saliva samples, suggesting that this portable biophotonic has the potential to serve as a diagnostic platform for future studies on infrared biomarkers in large and multicentric cohorts with control and AI subjects [11].

The combination of saliva application on the ATR-FTIR platform has brought numerous perspectives for diagnosing and monitoring oral diseases, mainly clinical applications of interest to dentistry, as described in periodontitis.

2.3 Salivary changes and oral mucositis

Among the multiple functions of saliva, its essential protective and functional role in soft tissues of the mouth, oropharyngeal mucosa, tongue, oral epithelium, glands, and connective tissue represents a highlight in research in the health sciences. The measurement of molecules in saliva samples in the context of oral mucositis (OM) has received particular attention in the last 10 years. This symptomatic complication of cancer therapy can affect patients' function, quality of life, ability to tolerate treatment, and increase mortality. (71).

OM is an inflammatory condition of the oral and/or oropharyngeal mucosa secondary to antineoplastic therapy that typically manifests as atrophy, erythema, and ulceration [71]. Several inflammatory mediators, proteins, and inorganic components in situ coordinate the occurrence of OM. Targeted, extensively employed, and untar-geted techniques have expanded the knowledge about OM mechanisms and response to therapies and demonstrated the role of some molecules in predicting the development and severity of OM [72–74]. Furthermore, the potential of artificial intelligence (AI) in enhancing salivary analysis offers a promising future for OM research.

Among the most studied salivary targets are cytokines, particularly in conditions potentially causative of OM, such as chemoradiotherapy and hematopoietic stem-cell transplantation (HSCT). The increased levels of these cytokines in body fluids reflect activation of pathways related to inflammation and disease development, thereby providing a valuable window into treatments and/or disease progression monitoring. In this context, inflammatory cytokines have emerged as potentially essential biomarkers for identifying patients who would benefit from early preventive or therapeutic intervention for OM. Notably, patients with moderate-severe OM consistently exhibited higher levels of inflammatory cytokines in saliva and plasma at most time points, including at the pre-conditioning phase. However, it is worth noting that all patients reported a grade 0 on mucositis scales at these points, suggesting that patients at risk of moderate-severe conditions may have predisposing factors that lead

to the derangement in levels of inflammatory cytokines even before chemotherapy. This deviation is amplified as OM progresses and can persist for at least 6 months [75]. Among the cytokines associated with the development of OM, interleukin-1 β (IL-1 β), tumor necrosis factor (TNF), and IL-6 have been highlighted as essential factors and are usually found in high concentration as the grade of chemotherapy-induced OM [74–76]. IL-6 [75] and TNF [76] mainly predicted moderate-severe OM. Individuals with TNF- α concentration > 4.4 pg/mL were identified as at significant risk for OM with an odds ratio of 2.4 [76]. In contrast, reduced salivary levels of IL-10 were reported in patients receiving chemoradiation for head and neck malignancies [77]. However, results from some studies did not find associations between either salivary TNF- α or IL-10 with OM [72]. Overall, similar findings were obtained for radiation-induced OM, with patients with severe OM presenting elevated concentrations of interferon- γ (IFN- γ), TNF- α , IL-2, and IL-6 levels and reduced levels of IL-4, IL-10, and epidermal growth factor (EGF). IFN- γ , TNF- α , IL-2, and IL-6 were positively correlated with OM grade, while IL-10, IL-4, and EGF were negatively correlated. Notably, all targets effectively predicted the severity of radiation-induced OM [78].

Beyond cytokines, many other MO-related factors emerge in the salivary milieu. Chemokines, such as IL-8/CXCL8, were found to be elevated in the saliva of patients with chemotherapy-induced OM, and their levels were related to increased neutrophil recruitment and augmented concentration of nitrite and neutrophil extracellular traps (NETs) in saliva [73, 74]. Enzymes and growth factors also arise as key players in OM. Patients with OM showed significantly higher levels of metalloproteases MMP-2, MMP-9, and EGF than those without mucositis [72]. In contrast, the incidence of severe OM during chemoradiotherapy was negatively associated with the EGF, keratinocyte growth factor (KGF) [79], and transforming growth factor- β (TGF- β) [74] concentration in saliva. The detection of some proteins in saliva, such as albumin, might suggest oral mucosal damage/permeability, and it has been reported to increase within the first 2 weeks and 1 month after HSCT [80, 81].

Studies using an ultra-deep proteomics approach identified 5500 proteins in saliva [82]. Few studies used untargeted methods to evaluate saliva changes related to OM. Still, marked differences in the proteome of saliva were found in head and neck cancer patients undergoing radiotherapy who developed OM [83]. Unique and upregulated proteins mainly involved in biological processes related to B-cell activation and the adaptive immune response were predominant in the non-OM group, suggesting a more pronounced antibacterial immune response, while in OM patients, upregulation of peptides related to keratinocyte differentiation and epithelial repair was observed [84].

In addition to the compositional changes in proteins, changes in the inorganic components, such as Na, Cl, and K concentrations, have been reported to increase during HSCT [85]. On the other hand, the concentrations of the elements Al, As, Bi, Ca, Cd, Co, Cu, K, Li, Mn, Mg, Mo, Pb, S, Sb, Se, Sr, Ti, V, and Zn in saliva did not exhibit differences concerning the pre- and post-radiotherapy time points [86].

While salivary markers provide a good picture of the oral cavity scenario in the context of OM, few studies considered the influence of underlying disease and/or OM overlapping complications as secondary infections [74] and graft-versus-host-disease (GVHD) [81]. Temporal changes were also assessed in scarce studies, which is particularly relevant considering the significant fluctuation along OM development [72, 74, 75]. Importantly, salivary changes may occur independently of OM, as demonstrated by children [87, 88] and adults [80] undergoing chemotherapy, who

might experience changes in the levels of saliva IgA, IgG, and IgM with a tendency to decreased levels. Similarly, myeloperoxidase (MPO) levels showed a significant reduction in the saliva of adolescents and adults undergoing HSCT, with levels returned to typical values at the end of the treatment [73]. Moreover, several components of the salivary antioxidant system were decreased during HSCT, leading to decreased salivary antioxidative capacities [87]. EGF in saliva was also significantly reduced during chemoradiotherapy and radiotherapy [79, 89]. These variations may diminish the defense and repair mechanisms and explain the high risk of oral mucosal damage and secondary infections.

Remarkable changes in salivary flow were reported in parallel with variations in the saliva composition during OM. It may result from chemotherapy's effects on salivary gland secretion [81]. Hyposalivation also emerges as the primary consequence affecting all patients undergoing radiotherapy in the head and neck regions, as the salivary glands are directly exposed to the radiation beam [90]. In both situations, the impact of salivary flow on disturbances in the composition of whole saliva must be considered in the data analysis. There may be other underlying and confounding factors, such as bleeding from OM and periodontitis or cytokines released by tumors, which were not investigated in most studies. Hormonal changes, stress, smoking, dietary composition, dental and periodontal status, oral hygiene, age, and ethnic origin might also influence the results, impairing the generalization of data. Lastly, resting or stimulating saliva and the time of day of sample collection are also essential for comparing the results of different studies. Well, the pre-analytic procedures are relevant because of the high proteolytic activity of saliva, which could degrade some components before analysis [91].

The method widely used to assess the salivary biomarkers during OM is ELISA. ELISA is an immunological assay, and the technique relies on the interaction between the antigen (i.e., the target protein) and the primary antibody that recognizes the antigen of interest. The presence of the antigen is confirmed through the enzyme-linked antibody catalysis of the added substrate, and the products of the reaction are detected using a luminometer or a spectrophotometer. The quantification is based on a standard curve prepared from known concentrations of the target molecule [92]. Although several commercially available ELISA kits have been validated for use with saliva, particular physical characteristics of the sample might cause significant variations. Assay interferences (termed "matrix effects") can be defined as "the sum of effects of all components (both qualitative and quantitative) in a system, except the analyte to be measured" [93]. Therefore, pH, proteolytic enzymes, and viscosity are potential causes for matrix effects [94]. The viscosity is mainly due to different amounts of large glycoproteins, such as mucins. Handling highly viscous samples requires special attention to pipetting techniques to reduce pipetting errors, and tests of dilutions must be recommended to minimize matrix effects and data reliability [94].

3. Challenges and perspectives of salivary analysis

Factors related to sampling-such as whether resting or stimulated saliva, the methods to stimulate sampling, if restricting eating or rinsing the mouth before, duration of the collection, time of day), transport (ice or room temperature), processing (if centrifugation, dilution, or protease inhibitor(s) are used), and storage (temperature, for how long, and freeze-thaw cycles) can be critical for some analyses, compromising the results. In addition, saliva is a fluid deeply impacted by local conditions, such as

dental caries, disturbances of mucosal integrity, periodontal inflammation, systemic conditions, use of medications, and previous interventions such as surgery, radiation, and chemotherapy. Thus, despite the saliva and plasma concentrations correlating very well for some markers, data interpretation in light of potential modifiers in the oral cavity scenario is recommended. Other variables, including age, sex, pregnancy, smoking, xerostomia, hyposalivation, circadian cycle, anxiety, chronic stress, physical activity, and hydration status, might also influence particular endpoints. Moreover, a critical consideration must be made when using whole saliva to assess conditions that specifically affect the major salivary glands. Despite being the primary producers of saliva, once secreted into the oral cavity, the fluid's composition is modified by various local factors. Thus, direct collection from the major glands is preferable for investigations targeting gland-specific alterations. Therefore, salivary diagnosis based on multiple parameters and standardized procedures can bring more reliable results for a comprehensive interpretation and control of confounding factors, such as the variability between individuals and populations.

Recent advances have highlighted the potential of artificial intelligence (AI) and machine learning (ML) to enhance salivary spectroscopy. In ATR-FTIR applications, supervised algorithms such as Linear Discriminant Analysis (LDA), Support Vector Machines (SVM), Naive Bayes, Random Forest, Logistic Regression, Decision Tree, AdaBoost, k-Nearest Neighbors (k-NN), Gradient Boosting, and Neural Networks have enabled the classification of systemic and oral diseases, including systemic diseases (diabetes, Zika, chikungunya, and *Helicobacter pylori*) and oral diseases (oral cancer and amelogenesis imperfect) based on specific salivary infrared [8, 11, 22, 40, 41, 95, 96]. Pre-processing techniques such as rubberband correction, amide I normalization, and Savitzky-Golay filtering are applied to optimize spectral interpretation, reduce noise, and enhance signal extraction, improving model accuracy. This reagent-free and noninvasive biophotonic method offers sustainability, portability, and rapid analysis, an alternative for diagnostics and screening in low-resource settings.

Separately, a pioneering integration of ML with electrochemical biosensors was recently reported for COVID-19 detection in saliva, using bio-inspired peptides coupled with classification algorithms [97]. This innovative approach enables real-time, low-cost diagnosis and opens new avenues for electrochemical-based salivary screening in infectious, metabolic, and toxicological conditions. Similar to spectral methods, broader validation with multicenter cohorts and large validation datasets should be done to confirm diagnostic accuracy and reproducibility across clinical contexts.

These molecular tools represent a significant step toward precision dentistry. Improved patient outcomes through proactive disease management could potentially revolutionize the way we approach oral care. The challenge of the clinical applicability of salivary findings and how these technologies can reach populations in need remains.

4. Conclusions

Salivary analysis emerges as a robust diagnostic tool for screening and monitoring oral diseases. Recent findings on salivary changes during periodontal disease using platforms like RT-PCR, NGS, and ELISA showed promising biomarkers of

periodontitis, such as specific oral microbiome, inflammatory cytokines (IL-1B, IL-6, TNF-alpha, and lactoferrin), and tissue-remodeling enzymes (MMP-8, MMP-9, alpha amylase, cystatin B, and SN). Salivary antimicrobial proteins (such as mucins, lactoferrin, lysozyme, cathelicidin, statins, and IgA) and markers such as alpha amylase activity and CD14, IL-2RA have been proposed as potential tools to identify individuals susceptible to dental caries. Mass spectrometry and bioinformatic tools in MIH identified the salivary proteome with high confidence and indicated differential protein expression in affected individuals compared to healthy controls. Functional analysis showed that these proteins are involved in immune-inflammatory and tissue degradation processes, which are involved in the severity of MIH. The measurement of salivary molecules in the context of OM has received particular attention for identifying patients who would benefit from preventive or therapeutic intervention. Inflammatory cytokines (IL-1B, TNF, IL-6), chemokines (IL-8/CXCL8), enzymes, and growth factors (MMP-2, MMP-9, and EGF) have emerged as potential biomarkers in OM. The potential of a reagent-free, nondestructive platform, ATR-FTIR spectroscopy with artificial intelligence for diagnosing and monitoring oral disease, has brought numerous perspectives for application in dentistry. These platforms analyzed the molecular composition of salivary components and detected a biochemical fingerprinting of amelogenesis imperfecta and periodontitis, effectively differentiating saliva samples of affected individuals from controls. The salivary analysis, including the new analytical methods, represents progress in the dental setting and can potentially assist in diagnosing and managing oral diseases.

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Conflict of interest

The authors declare no conflict of interest.

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Evaluation of Salivary Biomarkers for Early Diagnosis of Oral Potentially Malignant Disorders

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Abstract

The following paper elaborates on a prospective study conducted at the Liaquat College of Medicine and Dentistry located in Karachi, Pakistan. The goals of the study were drafted to investigate the potential of salivary biomarkers analysis as an instrument of early detection of oral cancers. The samples were collected from patients who exhibited characteristics of untreated oral potentially malignant disorders (OPMDs) or oral cancer. With non-invasive screening and detection methods being one of the pivotal notions that rationalize the study, these samples were extracted with minimal to no invasion of the patients' tissues. A sample population of 60 people of varying ages was enrolled via establishing validated inclusion and exclusion criteria. Analyzing the salivary biomarkers MMP-9, Cyclin D1, and EGFR, multiple significant and relevant correlations were found. The study results revealed associations between various oral potentially malignant disorders, oral cancer, and each of the three biomarkers. Increased MMP-9 expression was found to accompany leukoplakia ($p < 0.05$). Cyclin D1 expression was in correlation with erythroplakia ($p < 0.01$). EGFR was expressed more in concurrence with other OPMDs ($p < 0.05$). It was also found that biomarker B (Cyclin D1) is positively correlated with cancer progression ($p < 0.01$) from premalignant or potentially malignant indicators present in the oral cavity upon clinical examination. All of these findings point toward the increasing value of salivary biomarker expression as a predictor for OPMDs and oral cancers.

Keywords: salivary biomarkers, early detection, malignant, disorders, OPMD, MMP2, OSCC

1. Introduction

Oral potentially malignant disorders (OPMDs) constitute a group of oral mucosal conditions with varying degrees of dysplasia, erythroplakia, or leukoplakia, which have the potential to transform into oral cancer if left untreated. Grouping from the World Health Organization differs between precancerous lesions and precancerous

conditions, as the two classes of OPMDs. A precancerous lesion is a region of tissue that differs histologically from other tissues of the same class in the same individual, and hence possesses the greater risk of exhibiting malignancy in the future, even though it is benign at the time of examination. The term precancerous condition describes a more generalized set of factors that predispose certain tissues to malignant transformation, despite the absence of localized changes in appearance or symptoms [1]. Another way to view this contribution of the WHO is as a compilation of the previously established terms precancerous lesion and precancerous condition under the umbrella term oral potentially malignant disorder.

Early detection of OPMDs is a crucial aspect of oral cancer prevention and management [2]. OPMDs possessing different appearances and symptoms also possess corresponding likeliness of malignant transformation [3]. High risk of malignant transformation has been attributed to oral leukoplakia, proliferative verrucous leukoplakia, oral erythroplakia, oral submucous fibrosis, dyskeratosis congenita, and oral graft versus host disease. Oral lichen planus and oral lupus erythematosus are examples of OPMDs that pose a lower risk of malignant transformation. Changes at the molecular level that can be held directly responsible for malignant transformation have yet to be pinpointed, but limited correlation with modifications in oncogenes and tumor suppressor molecules has been observed [3].

However, OPMDs do not indicate future malignancies with any surety, nor can they be used to predict the exact site where a malignant lesion will form in the future. Rather, these are only credible as a class among many classes of predisposing factors that increase the malignant potential of an organism or a region of its tissues [4]. The affected region is, however, more susceptible to cancerous development than other, unaffected, tissues. Similarly, patients diagnosed with OPMDs are more susceptible to cancerous development throughout the body than patients who do not exhibit such abnormalities.

Conventional methods for identifying OPMDs primarily rely on clinical examination and histopathological evaluation, which often occur at later stages of disease progression. Conventional oral examinations are deemed unreliable as screening tools for OPMDs, as the number of subjects possessing red or white patches in the oral cavity can range from 5% to 15% in most populations around the world, and only a small percentage of these observations are actually deserving of being regarded premalignant [5].

Staining of the oral soft tissues has been used as a minimally invasive screening technique. It is favored due to the harmless and quick nature of testing, even though the usefulness, i.e., sensitivity and specificity of tissue staining for OPMDs, is still very much subject to academic debate. The use of toluidine blue has been documented, where dysplastic tissue is shown to take up the dye more quickly and intensely than tissue of normal histology. Methylene blue is another dye used in oral mucosal screening, albeit being less than ideally specific, i.e., liable to producing false positives. Lugol's iodine is a third example of a staining agent that is able to not only detect the presence of premalignant tissue, but can also demarcate the margins of the region at risk. This allows the examining physician to foresee the site and size of potential malignancy.

One of the most significant reasons tissue stains have been favored as screening techniques in the case of mucosal and dermatological conditions is that they are easily accessible without surgical invasion. A similarly non-invasive and sensitive diagnostic tool is the assessment of biomarkers present in the saliva [6]. Biomarkers are molecular signatures that can indicate recent changes in regional tissues. These changes may

be responses to normal or pathological processes, pharmacological or biochemical agents, or changes in the organism's environment.

In the oral cavity, the prevalently present fluid, i.e., saliva, offers little resistance to examination. It is far less complex than other bodily fluids like blood, urine, inflammatory exudates, and seminal and vaginal secretions. Saliva is also easy to collect, store, and transport, making it a more convenient diagnostic sample than many others. Saliva is not only in contact with the oral mucosa for prolonged durations, but the locations of salivary glands and the paths of its secretion also ensure that it flows past submucosal layers of the buccal, labial, palatal, lingual, and sublingual tissues [7]. Thus, it may be stated that the saliva is the agent most extensively in circulation in the oral cavity that is accessible without any surgical invasion of the body.

The constant and copious circulation of saliva enables it to record or bear indications of histological changes around the oral cavity. Advances in molecular pathology have enabled the aforementioned biomarkers to soon become very telltale diagnostic indicators. It is only owing to the largely uncharted nature of this subject that the gaps between normal and pathological levels of certain salivary components have not already been identified [8]. However, screening for certain oral conditions can be conducted using salivary examinations with growing confidence, before moving on to more invasive and costlier techniques for confirmation. Pathologies limited to the oral cavity, ranging from dental caries to squamous cell carcinomas of the tongue, create detectable changes in the salivary molecular appearance [9, 10]. Discovery of cancer-related and premalignant dysplasia-related proteins in the saliva has come in the form of many different biomarkers. These substances vary in concentration and quantity from subject to subject, but evidence linking the degree of certain biomarker presence to pathological staging or prognosis is yet to be found.

Oral squamous cell carcinoma (OSCC) is one of the commonest tumors of the oral cavity and of the human body. More than 95% of oral neoplastic lesions are diagnosed as oral squamous cell carcinomas. In the efforts to investigate salivary biomarkers as diagnostic signs of oral cancers, OSCC patients were naturally the staple study population chosen by scholars. It was subsequently found that the biomarkers M2BP, MRP14, CD59, catalase, and profilin 1 have 90% sensitivity and 83% specificity for oral squamous cell carcinoma [11]. Another relevant molecule is the matrix metalloproteinase-2, which is present in the basement membrane and is degraded by collagenase type IV. The substance is normally associated with vascularity and inflammatory processes. Matrix metalloproteinase-2 is expressed to a greater degree in patients who are confirmed cases of oral squamous cell carcinoma. Many other biomarkers have been analyzed from salivary samples of patients with diagnosed malignant tissue present in their oral cavity.

Despite significant advances in cancer screening, diagnosis, and treatment over the past few decades, the view for OSCC remains dismal. Most OSCC patients are diagnosed at only an advanced stage with a poor 5-year survival rate [11]. Early detection of malignant lesions is the only hope if an attempt at the patient's survival is to be made [12]. The onset of about 90% of oral cancers happens when the patient is over 40 years of age [13]. Alarming, the average oral cancer patient is only diagnosed in their sixth decade of life. Usually, this happens owing to the lesion becoming aggressively symptomatic and impossible to ignore.

For oral squamous cell carcinoma, the earliest manifestations include a persistent lump, nodule, or indurated ulcer. Several studies have shown a higher prevalence of OSCC in males compared to females, with male-to-female ratios ranging from 2:1 to 4:1. This gender disparity has been attributed to lifestyle-related risk factors, such as

higher rates of tobacco and alcohol use among men [14]. OSCC can originate from various sites within the oral cavity, with the tongue being the most common location, followed by the floor of the mouth, buccal mucosa, gingiva, and palate [15]. The distribution of OSCC sites may vary based on geographic location, as certain risk factors, such as betel quid chewing in some Asian regions, can influence the site-specific prevalence [6]. It should also be noted that Central and South Asian populations are host to the highest frequency of oral cancers, followed closely by East Asia [14]. The common use of chewable tobacco products such as betel nut has repeatedly been named responsible for this high occurrence, as well as a lack of social and individual attention to oral hygiene.

Pakistan, located precisely in this region, is home to large numbers of this high-risk population. Salivary sampling and analysis to screen for oral cancers have little precedent in the country. Some literature can be found investigating the immunohistochemical assessment of MMP-2 as a diagnostic tool for OPMDs and OSCC. Numerous investigations of MMP2 expression in OPMDs and OSCC have been performed in Pakistan. In 2017, the Journal of Oral and Maxillofacial Pathology published an analysis of MMP-2 expression in 40 instances of oral squamous cell carcinoma and 20 cases of odontogenic mucosal diseases. Compared to instances with OPMD, the expression of MMP-2 was considerably greater in OSCC. The level of MMP-2 expression was likewise observed to correlate with OSCC differentiation favorably [16]. Another 2019 research analyzed MMP-2 expression in 30 instances of OSCC and 30 cases of OPMDs, and the results were published in the Journal of Cancer Research and Therapeutics. Compared to instances with OPMDs, the expression of MMP-2 was considerably greater in OSCC. MMP-2 expression was also found to be positively correlated with the OSCC clinical stage [17].

2. Methodology

The investigation was structured as a cross-sectional study targeting patients who exhibited signs of ongoing OPMDs but had never undergone any treatments for these. The sampling and analysis were done from March 2022 through March 2023, at the clinical setting attached to the Liaquat College of Medicine and Dentistry in Karachi, Pakistan. The open-source biostatistics software OpenEpi was used to calculate a suitable sample size for the study, and a population of 60 individual subjects was thus selected for the study.

As multiple clinicians were involved in the screening, evaluation, and diagnosis process, Inter-rater reliability was established via Kappa test to produce agreement upon nominal variables. This ensured that subjects were included based upon the same observations across the board, and rater subjectivity was minimized.

The following criteria were defined for inclusion of an individual into the sample population:

- Individuals aged 18 years or older
- Individuals presenting with erythroplakia, leukoplakia, or other potentially malignant oral disorders or lesions as identified during the clinical examination.

The inclusion of each subject was preceded by awareness counseling and signing of a consent form that detailed the procedures and purposes of the research. The

written and spoken education of patients was delivered and the consent was received using the local Urdu language as a medium besides English.

Diagnoses of leukoplakia, erythroplakia, and other OPMDs relied on definitions from the consensus report authored by Warnakulasuriya et al. [4], which is heavily reliant on the WHO's own definitions.

The following criteria determined the exclusion of potential participants from the study population:

- Individuals with a history of oral cancer.
- Individuals who have previously received treatment for oral potentially malignant disorders or oral cancers.
- Individuals afflicted by severe medical conditions or comorbidities that may interfere with the study procedures or confound the interpretation of study results.

Each patient who qualified through the inclusion and exclusion criteria was examined by dental professionals. Examination of the oral cavity was thoroughly conducted, and the past or current presence of any OPMDs was ruled out before extraction of any salivary samples. Salivary samples were obtained non-invasively using saliva collection strips. The participants were advised to avoid eating, drinking, brushing, smoking, or any such activity at least 2 hours prior to the sample collection to ensure uncontaminated saliva collection. The strip was used such that its absorbent end was identified and placed into the mouth under the tongue for approximately 5 minutes and then removed and subsequently checked for saturation. If the strip was not saturated with saliva, then the process was repeated again. The saturated strips were stored individually in a sealed container with patient's name, date, and time and were transported to a laboratory on the same premises and stored until testing, which was completed following only minimal delays.

Depending upon the specific biomarker being targeted, laboratory techniques such as enzyme-linked immunosorbent assays (ELISA) and molecular biology assays were used, among other similar analytical methods.

Hematoxylin and eosin (H&E) staining was applied to additional tissue samples taken from patients who showcased OMPDs. H&E staining was the chosen method of identifying levels of MMP-2 in OMPDs and OSCC to confirm diagnosis.

Tissue samples from the present lesions were surgically collected and also transported to the same laboratory to be biopsied.

The collected data were entered into SPSS version 27.0. The tool was used to apply descriptive statistical instruments to the data, including the Pearson correlation analysis. Values such as the median, mean, and mode were calculated to provide a central tendency measure and make the information presentable in the form of graphs.

3. Results

The sample population of 60 subjects belonged to a wide age range, and had a well-balanced gender ratio. Each patient had presented to the outpatient department with the clinical presentation of erythroplakia, leukoplakia, or other oral lesions, in

order of frequency. In total, 10 participants had progressed to oral cancer as well. This demographic information is tabulated as follows (**Table 1**).

Upon completion of immunohistochemical and biochemical testing of the samples, the results were compared to confirmed OPMD or OSCC presentation. Since the study aimed to evaluate the reliability of salivary biomarkers as indicators of OPMDs and neoplastic development, relevant variables under investigation were the sensitivity and specificity of increased levels of biomarkers, namely MMP9 (matrix metalloproteinase 9), cyclin D1, and EGFR (epidermal growth factor receptor). ROC curves for each biomarker were also plotted and recorded. ROC (receiver operating characteristic) curves are a statistical exercise that helps interpret the ratio of true positives to false positives. Values extracted by plotting ROC are stated in terms of area under the curve (AUC). An AUC value closer to 1.0 depicts a more reliable correlation between the variables (**Table 2**).

In terms of ROC, the biomarker that most reliably reports malignant potential is the MMP-9. Cyclin D1 is a close second, and EGFR is the least accurate of the three. However, even the 0.70 AUC value of EGFR reflects relatively high diagnostic accuracy and makes it worth investigating in clinical settings.

The sensitivity and specificity values of all three salivary biomarkers differ from each other only slightly. Cyclin D1 is the most sensitive out of the three, meaning that it serves as a relevant screening tool. EGFR is proven to be the most specific, making it the most reliable indicator if a clinician suspects and seeks to avoid false positives. MMP-9 offers balanced specificity and sensitivity.

MMP-9, cyclin D1, and EGFR were also studied to identify any existing correlation with OPMDs. **Table 3** describes the findings.

Raised levels of Cyclin D1 showed the strongest correlation with erythroplakia, as indicated by the low p -value of <0.01 . MMP-9 was found to be linked to leukoplakia,

Characteristic	Value
Total participants	60
Age range	18–65 years
Gender distribution	Male: 45%, female: 55%
Clinical presentation	Leukoplakia: 30%, erythroplakia: 15%, oral lesions: 55%
Disease progression to oral cancer	10 Participants (16% of the sample population)
Biomarker B (Cyclin D1) association with progression	$p < 0.01$

Table 1.
Demographic characteristics of patients.

Biomarker	Sensitivity	Specificity	ROC curve (AUC)
MMP-9	78%	72%	0.80
Cyclin D1	85%	68%	0.77
EGFR	62%	79%	0.70

Table 2.
Diagnostic accuracy of salivary biomarkers.

OPMD type	Associated biomarker	<i>p</i> -Value
Leukoplakia	MMP9	<0.05
Erythroplakia	Cyclin D1	<0.01
Other lesions	EGFR	<0.05
Correlation with age		
Biomarker levels		<0.05

Table 3.
Association of biomarkers with OPMD.

and EGFR was linked to other oral lesions with similar strengths of correlation (p -value <0.05). Biomarker levels were also found to be correlated with age, which means old age may be a significant predictor for OPMD onset.

Table 4 illustrates the likelihood of progression of OPMDs to oral cancer based on the study population. Around 16.7% of the participants received diagnoses for oral cancer following diagnoses of OPMDs. In the subjects who underwent malignant transformation, raised levels of biomarker B or cyclin D1 were notable and a correlation was detected (p -value <0.01) (**Table 5**).

Among the 30 cases of OPMDs present in the study population, MMP-2 expression was low in one-tenth yet moderate in more than half of the participants. High expression of MMP-2 was detected in 36.7% of the affected group (**Table 6**).

MMP-2 expression was high in all but one patient of oral squamous cell carcinoma, producing a 96.7% true positive rate. The singular patient, out of a group of

Clinical outcomes	Disease progression
Progressed to oral cancer	16.7% of the sample
Biomarker B association with progression	$p < 0.01$

Table 4.
Clinical outcomes and disease progression.

MMP2 expression	Number of cases (n)	Percentage (%)
Low expression <10%	3	10.0
Moderate expression 10–50%	16	53.3
Severe expression >50%	11	36.7

Table 5.
MMP2 expression in OPMDs.

MMP2 expression	Number of cases (n)	Percentage (%)
Low expression <10%	0	0.0
Moderate expression 10–50%	1	3.3
Severe expression >50%	29	96.7

Table 6.
MMP2 expression in OSCC.

30, exhibited moderate expression of MMP-2, and no patients of OSCC were found to have low expression of MMP-2.

4. Discussion

In our descriptive analysis, we have evaluated several relevant patients based on our inclusion criteria and have included all of the common OPMDs along with their histochemical relation to salivary biomarkers. The analysis of salivary biomarkers is one of the most non-invasive and accurate methods for screening, early diagnosis, and prognosis indicator. Several comparative studies have also tried to establish a relation between the occurrence of OPMDs and associated salivary biomarkers [18, 19]. However, some studies have solely focused on the recurrence of oral cancer and its immunochemical analysis [20].

In our present study, we selected 60 participants of varying age (between 18 and 65) with no gender bias, having some variety of OPMD. The salivary samples were collected by using non-invasive methods described as methods which do not affect the chemical composition of saliva. The saliva samples were carefully stored in a controlled environment and were later analyzed by using ELISA or molecular biology assays.

The study's aforementioned results demonstrate that the salivary biomarkers, particularly MMP9, Cyclin D1, and EGFR, show promise as potential tools for the early detection of OMPDs. These biomarkers exhibited varying degrees of sensitivity and specificity. Salivary matrix metalloproteinase (MMP) concentrations can be used as a non-invasive alternative for detecting and monitoring OSCC; in fact, as reported by Stott-Miller et al., salivary concentrations of MMP1 and MMP3 were 6–15 times higher in patients with OSCC than in healthy people. This pattern demonstrated an increase with advanced stages of the disease. The role of MMP2 and MMP9 was also more pronounced in tumor invasion with a significant difference observed in healthy people, patients with known OMPDs, and patients with oral malignancy. This also signifies the role of MMP1 and MMP9 as screening as well as prognostic indicators [21].

The role of microRNA (miRNA) in the diagnosis of oral cancer is yet to be fully established. MicroRNAs are small, noncoding segments of RNA containing 19–23 nucleotides. The abnormal appearance of microRNA appears to be linked with the development of certain body tumors; one particular kind, the microRNA 31, has been linked with the occurrence of OSCC [22]. Another key biomarker is the epidermal growth factor receptor (EGFR), which is a tyrosine kinase receptor. Tyrosine kinase is thought to play a role in maintaining various cellular activities, including the cell signaling pathways, overactivation of which is one of the primary factors in the development of OSCC. Several studies have concluded that overactivation of EGFR and other signaling receptors is strongly associated with several cancers, most notably the OSCC [23].

We also observed correlations between specific salivary biomarkers and associated OMPD. Initially, the patients with leukoplakia were tested. The mean age of leukoplakia onset is found to be after 40 years with a peak between 60 and 69 years with a strong male predominance of 6:1. A vast majority of cases of leukoplakia had tobacco and/or alcohol as etiological factors [18]. The saliva of patients with Leukoplakia demonstrated a significant increase in concentration of MMP9 compared to healthy patients. The level of MMP9 was also showed to be significantly increased when

compared to that of leukoplakia. Another interesting character observed was the variation in levels of MMP9 with the severity of the disease. It was noted that poorly differentiated OSCC had the highest levels of MMP9, moderately differentiated OSCC had slightly lower, and well differentiated OSCC had the lowest levels of MMP9. Similarly, the pattern of MMP9 variation in the case of epithelial dysplasia was consistent with that of OSCC, with poorly differentiated grade containing the highest level of MMP9 and well differentiated grade containing significantly lower levels of MMP9 [19].

Similarly, the incidence of Interleukins (IL), particularly IL6 and IL8, in the development of OPMDs and carcinogenesis was evaluated. The most tested was the IL-8, for which several comparative studies have highlighted its ability to distinguish between healthy and OSCC patients. However, further studies are required regarding its role in detecting OPMDs, which seems to be limited with only 60% of the studies providing such evidence. There is also debate about the stage of disease in which IL-8 is diagnostically significant. Some studies proposed that IL-8 is most active during initial stages, whereas some studies concluded that IL-8 is most active in advanced stages of the disease [20]. Further, the role of IL-6 was also evaluated, which also provided diagnostic clues as the levels of IL-6 were found to be elevated in patients of OSCC. Interestingly enough, the levels of IL-6 were found to be increased both in serum as well as in saliva, with saliva showing a more pronounced increase than serum. The salivary concentrations were more useful diagnostically. Dineshkumar et al. [24] reported a sensitivity of 99% and 82% for IL-6 salivary and serum levels, respectively [25].

Another biomarker, having the most importance as a disease progression predictor, was also evaluated in the salivary samples. Studies suggest that EGFR may not be associated with any particular OPMD, and is rather associated with epithelial changes and was thus found to be elevated in most other epithelial pathologies as well [26]. Further studies are required to be able to identify its precise role in the diagnosis of OPMDs. However, Comparative studies indicate that the malignant transformation potential of OPMDs increased significantly in patients with over-expression of EGFR. This further signifies the potential of EGFR more in disease prediction with little to no evidence suggesting its role in early diagnosis of OPMDs and OSCC [21].

A positive trend was also observed between salivary biomarkers and age. Studies suggest that certain mineral changes in the saliva composition may be a contributing factor in the high risk of OPMDs and OSCC in high age group individuals. Further, the salivary flow rate (SFR) was also significantly reduced in high age individuals as compared to young patients. The elderly population did demonstrate a decrease in Ca^{++} concentration in their saliva; however, any other molecular change was unremarkable between young and elderly aged patients [22]. Further studies are required to determine the correlation between age, salivary biomarkers, and their relation to OPMDs.

Our study also showed a potentially revolutionary finding in terms of Biomarker B (Cyclin D1) as an excellent tumor progression parameter. It was observed that Cyclin D1 was more expressed in patients in which OPMDs progressed to oral cancer, as compared to patients with only having OPMD. Further, other studies showed that Cyclin D1 was overexpressed in patients in which OPMD(s) had progressed to oral cancer or in patients having OSCC with advanced stages and subsequently poor prognosis. Cyclin D1 was increased in OSCC patients having poorly differentiated type, high metastatic potential, and increased proliferation rate of tumor cells.

Furthermore, an association was also found between the expression of Cyclin D1 and the low differentiation ability of tumor cells [23].

The results gained from our study can be utilized to isolate high-risk patients with particular risk factors. The non-invasive salivary biomarker analysis can be employed for screening purposes instead of invasive methods. Individuals with high-risk factors can then be further evaluated for appropriate treatment strategies and follow-ups. We believe that this method of biomarker analysis could lead to revolutionary changes in the approach to diagnose, treat, and predict various OPMDs and their progression to oral cancer.

We would like to hold in remembrance the restrictions of the study. Despite our best efforts, the small and limited sample size, along with unicentric study design, provides results which are more localized and point toward a specific population rather than a generalized overview. The short duration of the study is not enough to adequately monitor the progression of the disease in the long term. Furthermore, patient follow-ups at regular intervals to effectively monitor the progression of the lesions could not be accomplished. Further multi-scaled studies with long-term strategies along with regular patient follow-ups are required to authenticate the results of this study.

5. Conclusion

It is therefore concluded from the study that salivary biomarkers, particularly MMP9, Cyclin D1, and EGFR, are diagnostically significant in the early diagnosis of oral potentially malignant disorders. Similarly, Salivary Biomarkers can also be used to predict the malignant transformation of such lesions. One particular biomarker, Cyclin D1, holds excellent potential as a prognostic indicator. The limited parameters of the study mean that further research and studies are required to enhance the authenticity of the results.

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Conflict of interest

The study has no conflict of interest to declare by any author.

Ethical approval

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Salivary Biomarkers in the Diagnosis and Prognosis of Oral Disease

Ana Cláudia Garcia Rosa

Abstract

Salivary biomarkers have emerged as a noninvasive diagnostic tool for detecting and monitoring various diseases, including oral squamous cell carcinoma (OSCC), periodontal disease (PD), Sjögren's syndrome (SS), and oral potentially malignant disorders (OPMDs). Advances in salivaomics (including genomics, transcriptomics, proteomics, metabolomics, and microbiomics) have enabled the identification of disease-specific biomarkers that reflect pathophysiological changes. Key biomarkers include cytokines, enzymes, nucleic acids, metabolic markers, and oral microbiome alterations. In OSCC, IL-1 α , IL-1 β , IL-6, IL-8, TNF- α , VEGF, matrix metalloproteinases (MMP-1 and MMP-9), microRNAs, epigenetic markers (MGMT, DAPK1, and RASSF1A), metabolic markers (elevated lactic acid, LDH, and pipecolinic acid), and oral microbiome shifts (*F. nucleatum*, *P. gingivalis*) are linked to tumoral initiation and progression. In OPMDs, elevated TNF- α , IL-6, IL-8, MMP-9, and oxidative stress markers (ROS, NO) are potential indicators of inflammation and malignant transformation. In PD, IL-1 β , MMP-8, TNF- α , MMP-9, RANKL, and OPG correlate with disease severity and alveolar bone loss. In SS, diverse cytokine profiles reflect immune dysfunction and glandular inflammation. Despite technological advancements, challenges remain in optimizing point-of-care applications, standardizing biomarker protocols, and validating clinical applications. Future research should focus on integrating multiomics technologies and AI-driven salivaomics to enhance the accuracy, reliability, and clinical applicability of salivary biomarkers for diagnosing oral and systemic diseases.

Keywords: salivaomics, oral disease, periodontal disease, oral squamous cell carcinoma, oral potentially malignant disorders, Sjögren's syndrome, noninvasive diagnostics, artificial intelligence in salivaomics

1. Introduction

Saliva is a complex biological fluid composed of secretions from the major salivary glands (parotid, submandibular, and sublingual), minor salivary glands, and contributions from gingival crevicular fluid, plasma exudates, microbial components, and cellular debris [1, 2]. Saliva contains diverse molecules, including inorganic ions, nucleic acids (DNA and RNA), proteins (cytokines, immunoglobulins),

enzymes, and hormones, many of which originate from the bloodstream *via* transcellular and paracellular pathways [1–3]. The molecular composition of saliva reflects both systemic and local physiological changes, making it a suitable biofluid for diverse disease detection and monitoring [1, 2]. Salivary biomarkers are categorized into cytokines, proteins, enzymes, genetic markers, metabolites, and microbial components [3].

Based on its diagnostic potential, “salivaomics” was introduced in 2008 to analyze salivary molecules using omics technologies systematically [3]. The “omes” refer to the complete set of biomolecules present in saliva at a given time, including the proteins (proteome), RNA (transcriptome), DNA (genome), small metabolites (metabolome), and microbial communities (microbiome). In contrast, “omics” (genomics, transcriptomics, proteomics, metabolomics, and microbiomics) refers to high-throughput analytical methods that enable the identification, quantification, and functional study of these biomolecules. The integration of multi-omics approaches has significantly enhanced the understanding of disease mechanisms, metabolic pathways, and therapeutic responses, positioning saliva as an important biofluid for precision medicine [4–8].

Because of its painless, noninvasive collection, saliva is continuously explored as an emerging alternative to blood-based tests and biopsies in clinical and home-based settings. The salivary biomarker profile enables early disease detection, monitoring of therapeutic responses, and assessment of disease progression, making saliva a promising tool in medical and dental diagnostics. Moreover, salivaomics-based diagnostics align with personalized medicine principles, serving as a complementary tool for real-time monitoring and risk stratification across various conditions [1–3].

Considering its diagnostic promise, salivaomics has demonstrated significant potential in detecting and monitoring oral diseases, including oral squamous cell carcinoma (OSCC), periodontal disease (PD), Sjögren’s syndrome (SS), and oral potentially malignant disorders (OPMDs) [5–8].

Advancements in high-throughput analytical techniques have enhanced the detection and characterization of salivary molecular changes in oral diseases, supporting the integration of salivaomics into clinical practice [5–8].

Several studies have demonstrated the potential diagnostic applications of salivary biomarkers in multiple diseases. However, despite these advances, key challenges remain in fully integrating salivaomics into routine clinical practice [1, 9–12].

This chapter examines the current role of salivary biomarkers in diagnosing and monitoring OSCC, OPMDs, PD, SS, and other oral diseases, focusing on advances in multiomics, AI-based diagnostics, and precision dentistry.

2. Saliva as a diagnostic biofluid

Diagnosis using salivary fluid is an emerging field that integrates nanotechnology, molecular biology, and advanced analytics for the detection and potential clinical monitoring of various diseases [2, 3, 10]. As a biofluid with noninvasive, economical, and easily accessible collection, saliva offers several advantages over traditional blood-based diagnostic methods, making it highly suitable for point-of-care (POC) applications and large-scale screenings, though further standardization is needed for clinical implementation [3, 13].

Saliva contains several biomolecules, including nucleic acids, proteins, metabolites, and inorganic ions, which reflect physiological and pathological changes in the

organism, influenced by cellular activity and metabolic regulation [2, 10]. Therefore, the metabolic and molecular profile of saliva has the potential to provide early indicators of the progression of numerous diseases [1, 4]. In this context, salivaomics has identified specific biomarkers for OSCC, OPMD, PD, SS, and other oral diseases as well as systemic conditions, contributing to the early detection and continuous monitoring of these conditions [1–3, 10].

However, factors such as the timing of collection, sample processing, storage conditions, and pretreatment methods can influence the stability, integrity, and concentration of biomarkers in saliva. Therefore, standardized saliva collection, storage, and treatment protocols are essential to ensure biomarker stability, accuracy, reproducibility, and clinical applicability in salivary diagnostics [12].

Advances in artificial intelligence (AI) and machine learning (ML) have contributed to the analysis and identification of biomarker patterns in saliva-based diagnostics [8, 14]. Moreover, AI-assisted classification and predictive disease modeling can enhance the accuracy of biomarker-based diagnostics, improving sensitivity and specificity.

AI-powered platforms are being developed and tested for screening, monitoring, and risk assessment in oral diseases [14]. Despite its promise, the integration of AI into saliva-based diagnostics faces challenges, including data variability, external validation, and regulatory approval, which must be addressed before its full adoption into clinical practice [8, 14].

2.1 Salivaomics and biomarker identification

Salivaomics is a rapidly growing field that integrates multi-omics technologies (proteomics, genomics, transcriptomics, metabolomics, and microbiomics) to discover, analyze, and validate salivary biomarkers for disease detection, prognosis, and therapeutic monitoring [1, 3]. The ability to analyze multiple layers of biological information provides a more comprehensive understanding into disease pathophysiology, improving biomarker discovery and diagnostic accuracy [2].

Building on its multi-omics capabilities, salivaomics has advanced rapidly since its introduction in 2008 [3], with technological innovations expanding its potential clinical applications [13]. High-throughput technologies have identified over 3000 salivary proteins, significantly increasing from the limited number identified in the early 1980s [2, 10]. Additionally, methods such as next-generation sequencing (NGS), liquid chromatography-mass spectrometry (LC-MS), and polymerase chain reaction (PCR) have facilitated the detection of genetic, transcriptomic, proteomic, metabolomic, and microbiomic alterations [13]. These discoveries have improved our understanding of molecular mechanisms underlying disease processes, facilitating biomarker identification for oral pathologies whose early detection can support personalized treatment strategies [1, 9–12, 15, 16].

Each layer of salivary omics provides a distinct understanding of disease mechanisms. Genomic analysis identifies mutations, as well as epigenetic modifications, associated with diseases [1, 2]. Transcriptomics, which examines changes in gene expression, has identified salivary mRNA and microRNA signatures associated with diverse oral disease [2, 12].

Proteomics research focuses on identifying salivary proteins related to disease progression. Techniques such as mass spectrometry (MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) have significantly improved biomarker detection, enabling the high-accuracy identification of low-abundance proteins [10].

Metabolomics identifies disease-specific metabolic alterations by analyzing metabolites such as amino acids, lipids, and carbohydrates, which reflect biochemical and systemic changes in the organism [4–6]. Advanced analytical methods, including nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-mass spectrometry (LC-MS), have facilitated the detection of metabolic alterations associated with oral and systemic diseases [13].

Microbiomics investigates oral microbial imbalances (dysbiosis) associated with diseases such as PD, dental caries, and OSCC. Metagenomic sequencing and real-time PCR (RT-PCR) have identified microbial signatures that differentiate healthy from diseased states [2, 4, 8]. Whole genome sequencing (WGS) and 16S rRNA sequencing support the characterization of microbial DNA, aiding in oral microbiota analysis and disease predisposition studies [2, 8].

The lower concentration of many salivary biomarkers compared to blood requires highly sensitive detection methods [10]. Successful integration of salivaomics into clinical practice requires standardized saliva collection protocols, enhanced biomarker stability, and large-scale validation across diverse populations. Point-of-care (POC) biosensors, AI-driven bioinformatics, and diagnostic devices must be optimized to ensure high sensitivity and reproducibility [2, 8, 14, 17].

2.2 Technologies for salivary biomarker identification

2.2.1 Genomic and epigenetic techniques

Sequencing technologies, such as next-generation sequencing (NGS) and third-generation sequencing (TGS), enable deep genomic and transcriptomic profiling, facilitating the identification of disease-associated alterations. Whole genome sequencing (WGS) detects genetic mutations and structural variations, while RNA sequencing (RNA-Seq) enhances transcriptomic profiling by quantifying and identifying coding and noncoding RNAs [2]. Furthermore, 16S rRNA metagenomic sequencing, which characterizes bacterial taxonomic profiles, has been applied to analyze oral microbiota changes associated with PD and OSCC [2].

Epigenetic modifications, such as DNA methylation, are critical in the pathophysiology of OSCC and other diseases. Methylation-sensitive polymerase chain reaction (PCR) and sequencing technologies facilitate the detection of these epigenetic alterations, offering early diagnostic and prognostic biomarkers in salivary diagnostics [2].

2.2.2 Transcriptomic techniques

Microarray technology enables the high-throughput detection of differentially expressed mRNA and microRNA (miRNA) transcripts, facilitating large-scale biomarker identification. In OSCC, several mRNA biomarkers have been detected through microarray analysis. Specific miRNA dysregulations, implicated in tumor progression and epithelial-mesenchymal transition, have been identified [2].

2.2.3 Proteomic techniques

Proteomic analysis aims to identify and characterize salivary proteins to uncover disease-associated biomarkers. Techniques such as mass spectrometry (MS), two-dimensional gel electrophoresis (2D-GE), and tandem mass spectrometry (MS/MS) help identify proteins involved in disease processes. Methods such as MALDI-TOF

and LC-MS provide high-throughput and sensitive proteomic profiling, facilitating the detection of important biomarkers, including potential biomarkers associated with tumor progression and metastasis in OSCC [2, 12, 13].

2.2.4 Metabolomic techniques

Metabolomics identifies and quantifies small-molecule metabolites (lipids, vitamins, organic acids, carbohydrates, and amino acids) that reflect biochemical and systemic alterations in disease states. Salivary metabolomic profiling has revealed dysregulated polyamine metabolism in OSCC [5], elevated lactate levels associated with inflammation and microbial activity in PD [6], and altered amino acid pathways in SS, indicating immune and glandular dysfunction [7].

Analytical techniques such as nuclear magnetic resonance (NMR) spectroscopy and gas/liquid chromatography-mass spectrometry (GC-MS, LC-MS) enable the identification and quantification of these metabolic alterations, contributing to the early detection and monitoring of oral diseases [2, 5, 8, 10].

2.2.5 Liquid biopsy and molecular diagnostic techniques

Electric Field-Induced Release and Measurement (EFIRM) is a noninvasive liquid biopsy technique that detects circulating tumor DNA (ctDNA) and genetic mutations directly from saliva. This method was initially developed to identify EGFR mutations in patients with non-small cell lung cancer (NSCLC) [18].

Liquid biopsy techniques, including EFIRM, are being actively investigated for their potential to detect OSCC biomarkers with high sensitivity and specificity [2, 13]. Their noninvasive nature makes them promising tools for OSCC diagnosis and therapeutic monitoring.

2.2.6 Point of care and biosensing technologies

Point-of-care (POC) devices and wearable biosensors are portable diagnostic tools that enable early disease detection, risk stratification, and real-time biomarker monitoring. POC biosensors integrate microfluidic chips for fluid handling, electrochemical and optical sensors for biomarker detection, and nanotechnology for signal amplification and enhanced sensitivity [14, 17]. These biosensors enable rapid and noninvasive detection of salivary proteins, nucleic acids, and metabolites [2, 8].

AI-powered biosensors utilize machine-learning algorithms to detect biomarker patterns, enhancing diagnostic accuracy and disease monitoring. Their noninvasive and cost-effective nature makes them promising tools for early detection and screening of oral diseases [13, 17].

2.3 Salivary biomarkers in oral disease

Table 1 provides a summarized overview of key salivary biomarkers associated with various oral diseases.

2.3.1 Oral squamous cell carcinoma

Oral squamous cell carcinoma (OSCC) is the most prevalent malignant tumor of the oral cavity, comprising approximately 90% of all oral malignancies worldwide [9, 19].

Oral disease	Biomarker(s)	Category	Level of evidence	References
Oral Squamous Cell Carcinoma (OSCC)	IL-1 α , IL-1 β , IL-6, IL-8, TNF- α , VEGF, MMP-1, MMP-9, miRNAs (miR-21, miR-184), MGMT, DAPK1, RASSF1A	Cytokines, MMPs, miRNAs, Epigenetic markers	High	[2, 8, 9, 19]
Oral Potentially Malignant Disorders (OPMDs)	IL-6, IL-8, TNF- α , MMP-9, oxidative stress markers (ROS, NO)	Cytokines, Oxidative Stress	Moderate	[2, 8, 12, 15]
Periodontal Disease (PD)	IL-1 β , IL-6, TNF- α , MMP-8, MMP-9	Cytokines, Bone Metabolism	High	[2, 11, 16]
Sjögren's Syndrome (SS)	IL-1, IL-6, IL-10, IL-17, TNF- α , β 2-microglobulin, calprotectin, oxidative stress markers (lactate, alanine, malate), T-cell proliferation markers (arginine, isoleucine)	Cytokines, Proteins, Metabolites	Moderate	[2, 7, 20]
Peri-Implantitis	IL-1 β , IL-6, TNF- α , MMP-8	Cytokines, MMPs	Low	[21]
Dental Caries	Lactoperoxidase, Proline-rich proteins, Histatin-5, IgA	Salivary Proteins	Low	[22, 23]
Medication-Related Osteonecrosis of the Jaw (MRONJ)	NTX, Bone-specific alkaline phosphatase (B-AP), Hypotaurine	Bone Metabolism, Oxidative Stress	Low	[24, 25]
Temporomandibular Joint Osteoarthritis (TMJ OA)	AI-assisted salivary biomarker panels (Multi-Omics)	Multi-Omics	Low	[14]
Pemphigus Vulgaris (PV)	Desmoglein 1 & 3 Autoantibodies	Autoantibodies	Low	[10, 26]
Oral Candidiasis (OC)	Tyrosine, Choline, Phosphoenolpyruvate	Metabolites	Low	[27]

Table 1.
Summary of key salivary biomarkers for oral diseases.

The 5-year survival rate for OSCC varies significantly depending on factors such as the stage at diagnosis, tumor location, and treatment accessibility [9, 12, 15]. Major risk factors for OSCC include tobacco and alcohol consumption and betel nut chewing. While human papillomavirus (HPV), particularly HPV-16, is a well-established risk factor for oropharyngeal squamous cell carcinoma (OPSCC), its direct association with OSCC is less pronounced [9, 12, 19]. Some cases of OSCC arise from oral potential malignant disorders (OPMD) such as leukoplakia, erythroplakia, and lichen planus. Although OPMD can potentially progress into OSCC, not all cases undergo malignant transformation. The risk of progression depends on factors such as lesion subtype, size, presence of dysplasia, and patient habits (e.g., smoking and alcohol use) [9].

Salivary biomarkers have emerged as promising alternatives to biopsy-based diagnostic methods for OSCC. Proteomic analyses have identified biomarkers, including interleukins [(IL-1 α , IL-1 β , IL-6, IL-8), and tumor necrosis factor-alpha (TNF- α)], matrix metalloproteinases (MMP-1 and MMP-9), and proteins such as cytokeratin fragment 21-1 (Cyfra 21-1), mucin-1 (MUC1), and vascular endothelial growth factor (VEGF) [8, 12, 19]. Transcriptomic analysis has identified

upregulated mRNA markers, including H3 histone family member 3A (H3F3A), mitogen-activated protein kinase kinase 3 (MAP2K3), and beta-2-microglobulin (B2M) [8]. MicroRNA profiling has identified upregulated microRNAs (miR-21, miR-184) and downregulated miR-145, which may contribute to OSCC progression [8]. DNA methylation markers such as O6-methylguanine-DNA methyltransferase (MGMT), death-associated protein kinase 1 (DAPK1), and Ras association domain-containing protein 1A (RASSF1A) are linked to OSCC through aberrant hypermethylation [8, 19].

Metabolomic studies have revealed increased levels of lactic acid, betaine, and pipercolinic acid and decreased levels of phenylalanine and valine in OSCC patients [8]. Microbiome dysbiosis has been implicated in OSCC development, with increased colonization of *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, particularly in smokers. *P. gingivalis* enhances tumor progression by increasing TNF- α and metalloproteinase production while inhibiting p53-mediated apoptosis. *F. nucleatum* secretes lipopolysaccharides and pro-inflammatory cytokines, facilitating cancer development and metastasis [8].

While numerous salivary biomarkers have been identified as potential diagnostic and prognostic tools for OSCC, many remain experimental. Large-scale clinical validation studies, standardization of collection methods, and robust biomarker panels are required before widespread implementation in routine clinical practice [28].

2.3.2 Oral potentially malignant disorders

Oral potentially malignant disorders (OPMDs) are a heterogeneous group of conditions characterized by an increased risk of malignant transformation to OSCC. These disorders exhibit distinct clinical, histopathological, and molecular features, influencing their likelihood of progressing to malignancy. The most well-known OPMDs include oral leukoplakia (OL), erythroplakia, oral lichen planus (OLP), and oral submucous fibrosis (OSF) [12].

Within this group of disorders, oral leukoplakia (OL) is the most prevalent, presenting as a white patch or plaque on the oral mucosa with no identifiable cause. While OL's annual malignant transformation rate is generally less than 1%, lesions exhibiting epithelial dysplasia are at a higher risk [19]. Erythroplakia, characterized by red patches, has a significant malignant potential [12]. OLP, a chronic inflammatory disorder associated with immune dysregulation, has a malignant transformation rate of approximately 1.14%, particularly in atrophic and erosive forms [2]. SF, predominantly resulting from areca nut chewing, leads to progressive fibrosis and carries a 1% risk of malignancy [12].

Salivary biomarkers have emerged as promising tools to assess the progression and malignant potential of OPMD. Elevated salivary levels of IL-8 have been associated with OSCC and may indicate an increased risk of malignant transformation in OPMDs [8, 12, 19]. Salivary biomarkers, including TNF- α , IL-1 β , IL-6, IL-8, lactate dehydrogenase (LDH), and MMP-9, have been widely studied, with diverse reports demonstrating high sensitivity and specificity for diagnosing OSCC and OPMDs [12, 15]. Elevated LDH levels were found in OSCC and high-risk OPMD, supporting its potential role in identifying OPMD patients with a higher risk of malignant transformation [15]. Oxidative stress markers have been linked to the pathogenesis and progression of OPMDs [11, 15, 19]. Increased levels of reactive oxygen species (ROS) and nitric oxide (NO) have been observed in patients with OPMDs, particularly in conditions such as OLP [11].

Metabolic profiling in OL has identified alterations in key metabolic pathways. Increased levels of lactic acid, sphinganine-1-phosphate, pseudouridine, and D-glycerate-2-phosphate, along with decreased levels of valine and phenylalanine, have been observed. These metabolic changes may correlate with dysplastic progression [5].

Molecular diagnostics have revealed that miRNA profiling indicates dysregulation of specific miRNAs in both OPMD and OSCC. Among these, miR-320a is upregulated in both OPMD and OSCC, whereas miR-136 exhibits stage-dependent variation (downregulated in OPMD but upregulated in OSCC), suggesting its involvement in disease progression [8].

Advancements in artificial intelligence permitted the development of models that analyze multi-omics salivary biomarker data, enhancing the differentiation between dysplastic and non-dysplastic OPMDs. Machine learning algorithms have been employed to validate biomarkers for disease progression, improving diagnostic precision. While IL-8 has been identified as a potential predictor of malignant transformation in previous research, further validation in AI-driven studies is required [14].

2.3.3 Periodontal disease

Periodontal disease (PD) is a chronic inflammatory condition affecting a significant portion of the global population [16]. The disease is characterized by the progressive destruction of periodontal tissues, including the gingiva, periodontal ligament, and alveolar bone. PD often remains asymptomatic in its early stages, leading to delayed diagnosis and treatment. Major periodontal pathogens include *Pgingivalis*, *Aggregatibacter actinomycetemcomitans*, and *F.nucleatum*, contributing to dysbiosis, immune evasion, and tissue destruction [16]. Smoking exacerbates PD severity, resulting in increased pocket depth, heightened inflammation, and elevated rates of tooth loss [16].

Proinflammatory cytokines, including IL-1 β , IL-6, and TNF- α , are strongly associated with chronic periodontal inflammation [11, 16]. Matrix metalloproteinases (MMP-8 and MMP-9) contribute to connective tissue breakdown and disease progression [11, 16]. Bone metabolism markers such as receptor activators of nuclear factor kappa-B ligand (RANKL) and alkaline phosphatase (ALP) regulate osteoclastic activity and alveolar bone resorption [11]. Enzymatic indicators of periodontal tissue damage include aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [11].

Additional salivary biomarkers, such as fatty acids, dipeptides, immunoglobulins, and nitric oxide, have been linked to periodontal inflammation and alveolar bone loss [1]. Smoking significantly affects oxidative stress-related biomarkers and tissue degradation. Elevated levels of malondialdehyde (MDA), salivary cortisol, and albumin in smokers indicate increased inflammation and extracellular matrix remodeling, while reduced levels of superoxide dismutase (SOD) and LDH suggest compromised antioxidant defense and altered tissue regulation [11, 16]. Several factors influence the salivary metabolome in PD, including diet, oral microbiota composition, smoking, and systemic conditions. Salivary metabolic biomarkers associated with PD include fatty acids, dipeptides, and microbial metabolites [6].

AI-assisted salivary biomarker platforms have been applied to periodontal disease diagnosis, utilizing machine learning-based analyses of 16S rRNA sequencing data and metagenome biomarkers. These models enable dimensionality reduction, classification, and predictive modeling, achieving high accuracy in distinguishing periodontal disease from health [14].

2.3.4 Sjögren's syndrome

Sjögren's Syndrome (SS) is a chronic autoimmune disease primarily affecting the salivary and lacrimal glands, leading to symptoms such as xerostomia (dry mouth) and keratoconjunctivitis sicca (dry eyes), often accompanied by systemic complications. The global prevalence of primary SS is approximately 0.06%, with significant geographic variability. In the United States, an estimated four million individuals are affected, making it one of the most prevalent autoimmune diseases. SS is characterized by lymphocytic infiltration and progressive destruction of exocrine glands, especially the salivary and lacrimal glands [2, 11, 15, 29].

Autoantibodies, particularly anti-Ro/SSA and anti-La/SSB, have been proposed as early detection markers of the disease; however, their presence in saliva is less common [10]. Elevated salivary levels of proinflammatory cytokines, including IL-1, IL-6, IL-10, IL-17, and TNF- α , reflect the inflammatory profile associated with SS [2, 3, 7, 20]. Proteomic studies have identified altered expression of proteins such as β 2-microglobulin, calprotectin, carbonic anhydrase VI, NGAL, and Siglec-5 in SS patients [20]. Metabolomic profiling has revealed increased salivary levels of succinic acid, isovaleric acid, and alanine, indicating metabolic disturbances linked to the disease [30]. A recent experimental study identified increased levels of lactate, alanine, malate, arginine, leucine, valine, and isoleucine in the saliva of SS patients compared with healthy individuals [7].

AI advancements are transforming SS diagnosis by refining biomarker discovery, disease classification, and risk prediction. Recent AI-based models have been studying predictive biomarkers correlating with disease severity and progression [14].

2.3.5 Dental caries

Dental caries results from demineralization of enamel and dentin due to acidogenic and aciduric bacteria, mainly *Streptococcus mutans*, *Streptococcus sobrinus*, and *Lactobacillus acidophilus* [2, 22]. Elevated levels of salivary proteins, including lactoperoxidase (LPO), proline-rich proteins (PRP1/2), histatin 5 (Hst5), and mucins, have been detected in individuals with active caries, probably reflecting protective mechanisms or microbial interactions [22, 23]. However, findings on IgA and total protein levels remain inconsistent [23]. AI-based salivary diagnostics have been applied to analyze the composition of the salivary microbiome in high-risk individuals, utilizing 16S rRNA sequencing and microbiome-derived biomarkers to predict susceptibility to dental caries [14].

2.3.6 Peri-implantitis

Peri-implantitis is an inflammatory condition triggered by the accumulation of bacterial biofilm on the implant surface. The inflammation leads to peri-implant tissue destruction, bone resorption, and loss of osseointegration. Several salivary biomarkers have been associated with peri-implant disease progression, including the pro-inflammatory cytokines IL-1 β , IL-6, IL-10, TNF- α , and MMP-8 [21].

2.3.7 Medication-related osteonecrosis of the jaw

Medication-related osteonecrosis of the jaw (MRONJ) is a complication associated with antiresorptive (e.g., bisphosphonates, denosumab) and antiangiogenic therapies, characterized by impaired bone healing and necrosis. Salivary biomarkers

linked to MRONJ include salivary concentrations of N-terminal telopeptide (NTX) and bone-specific alkaline phosphatase (B-AP) have been detected, reflecting alterations in bone metabolism associated with MRONJ [24]. Increased salivary levels of hypotaurine are potential early detection markers for MRONJ, suggesting oxidative stress involvement in its pathogenesis [25].

2.3.8 Temporomandibular joint osteoarthritis

Temporomandibular joint osteoarthritis (TMJ OA) is a degenerative joint disease characterized by progressive cartilage degradation, subchondral bone remodeling, and synovial inflammation. The disease leads to pain, joint stiffness, and impaired jaw function. AI-assisted saliva liquid biopsy platforms enable differentiation between TMJ OA and other conditions or disease stages through salivary biomarker analysis [14]. These models enhance biomarker discovery, dimensionality reduction, and disease classification, potentially improving early diagnosis [14].

2.3.9 Pemphigus vulgaris

Pemphigus vulgaris (PV) is an autoimmune blistering disorder that leads to epithelial detachment and blister formation in the skin and mucous membranes. PV frequently affects the oral mucosa, which is often one of the first sites of involvement. The disease is caused by autoantibodies targeting desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3). Traditionally, these autoantibodies are detected in serum samples, but studies have demonstrated that saliva-based diagnostics can also be used for this purpose [10, 26]. Detecting Dsg1 and Dsg3 autoantibodies in saliva provides a noninvasive alternative to serum-based testing, facilitating the diagnosis and monitoring of PV [10, 26].

2.3.10 Fungal and viral infections

Oral candidiasis (OC), a common fungal infection caused by *Candida* species, may be diagnosed using salivary biomarkers. A recent pilot study has identified elevated salivary metabolites in OC patients, including tyrosine, choline, and phosphoenolpyruvate [27]. Additionally, several viral infections, such as HIV, human papillomavirus (HPV), and hepatitis C, can potentially be detected through salivary diagnostics, as viral markers and specific antibodies are present in saliva, making noninvasive screening a viable alternative to blood-based tests [3, 31].

3. Clinical implications and future perspectives

Molecular analyses of salivary components have emerged as an innovative and noninvasive method for detecting and monitoring oral and systemic diseases, offering an alternative to traditional blood analysis and tissue biopsy [1, 3, 7, 11, 17]. The diverse biomolecules in saliva, including cytokines, proteolytic enzymes, genetic markers, microbial components, and metabolites, provide biochemical parameters that allow the identification and monitoring of conditions such as dental caries, PD, OSCC, OPMD, and SS.

Advances in analytical techniques, sequencing technologies, and bioinformatics tools have improved the sensitivity and specificity of devices and equipment used in

salivary diagnostics [2, 14, 17]. However, despite these technological improvements, challenges remain regarding the standardization of saliva collection and storage methods and the variability of salivary biomarkers, delaying clinical validation and widespread adoption [2, 19, 17].

Salivomics offers a noninvasive, cost-effective, and patient-friendly approach, making it suitable for large-scale screening [2]. Unlike blood-based diagnostics, saliva collection is simple and does not require specialized personnel [3].

Among the most relevant salivary biomarkers, inflammatory cytokines, such as interleukins (IL-1 α , IL-1 β , IL-6, IL-8) and tumor necrosis factor-alpha (TNF- α), are associated with inflammation and disease progression, particularly in OSCC, OPMD, and PD [8, 11, 12, 15, 16, 19]. Matrix metalloproteinases (MMP-1, MMP-8, and MMP-9) mediate connective tissue breakdown in OSCC, malignant transformation in OPMD, and extracellular matrix remodeling in PD [8, 11, 12, 15, 16, 19]. Epigenetic modifications, including hypermethylation of MGMT, DAPK1, and RASSF1A, are linked to OSCC development [19].

High-throughput analytical technologies, including mass spectrometry (LC-MS, MALDI-TOF-MS), two-dimensional gel electrophoresis (2D-GE), and next-generation sequencing (NGS), have significantly enhanced the identification and quantification of salivary biomarkers, improving diagnostic accuracy [2]. The development of point-of-care (POC) biosensors and liquid biopsy technologies has further advanced salivomics [17]. Rapid diagnostic devices, such as the rapid strip test (RST) for MMP-1, have shown high sensitivity for early OSCC detection, while ELISA and magnetic bead-based assays (MBBA) have enhanced biomarker quantification and clinical reliability [2, 19]. Collectively, these advancements have expanded the clinical applicability of saliva-based diagnostics, improving precision and accessibility in both medicine and dentistry.

Artificial intelligence (AI) has significantly contributed to biomarker discovery, enhancing the differentiation between dysplastic and non-dysplastic OPMDs, refining OSCC metabolomic classification, and validating salivary microbiome signatures for early disease detection. Machine learning-based metagenome analyses have further improved periodontal disease classification and risk assessment using 16S rRNA sequencing data [14]. Despite these advancements, several limitations remain, including the lack of standardized collection methods, lower biomarker concentrations in saliva compared to blood, and the need for large-scale clinical validation to ensure diagnostic accuracy before clinical implementation [14].

Despite these advancements, salivomics research continues to face challenges, particularly the lower concentrations of salivary biomarkers compared to blood, necessitating highly sensitive detection technologies [9, 17]. Establishing standardized collection protocols, optimizing storage conditions, and advancing analytical techniques are necessary to ensure reliable biomarker quantification [2, 17]. Another major limitation is the reliance on single biomarkers, which often lack sufficient diagnostic accuracy, increasing the risk of false positives and negatives [17]. Multi-biomarker panels, rather than single biomarker assessments, may further improve sensitivity and specificity, reducing diagnostic variability. Additionally, many POC technologies remain in prototype development and fail to meet the clinical standards required for regulatory approval [17]. Validating biomarker panels and ensuring regulatory compliance are necessary to support clinical adoption.

The variability in biomarker detection remains a challenge in salivomics. Several factors influence biomarker stability and reproducibility, including sample collection methods, storage conditions, circadian rhythms, diet, hydration status,

and individual variability [2, 9, 17]. A possible solution is adopting standardized saliva collection devices and protocols that minimize contamination and maintain biomarker stability. Recent advancements have improved the development of commercial saliva collection kits with built-in stabilization buffers, which can help reduce variability and extend biomarker integrity for clinical use [32, 33]. Integrating automated sample processing and AI-driven normalization algorithms can also adjust for biological and environmental factors, such as hydration status, sample collection variability, and individual metabolic differences, thereby improving biomarker reproducibility and diagnostic accuracy. These strategies will help bridge the gap between research and clinical application, ultimately making salivaomics a reliable tool in routine diagnostics.

The future of salivaomics is promising, with the integration of AI, multi-omics platforms, and POC devices expected to accelerate the transition from research to clinical practice. Addressing challenges will enable saliva-based diagnostics to become reliable tools for personalized medicine.

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Conflict of interest

None.

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Section 3

Therapeutic Strategies and Emerging Technologies

Management and Treatment of Sialorrhea

Livia Barroso Ribeiro

Abstract

Sialorrhea, or excessive salivation, results from impaired saliva transfer from the oral cavity to the oropharynx, manifesting as visible drooling or internal accumulation, causing significant discomfort and impacting patients' social function, oral health, and quality of life. Its prevalence is notably high in neurological conditions, affecting approximately 70% of patients with Parkinson's disease, 50% with amyotrophic lateral sclerosis (ALS), and between 10 and 80% with cerebral palsy. Clinically, sialorrhea can lead to serious complications, including aspiration pneumonia. The pathophysiology of sialorrhea frequently involves dysfunction in oral clearance rather than true hypersecretion and is linked to a complex neuroanatomical network that regulates salivary secretion. Sialorrhea management requires a comprehensive, individualized, and often multidisciplinary approach. The main strategies include: anticholinergic agents, botulinum toxin (BoNT) therapy, surgical options, and speech-language therapy. Optimal sialorrhea management is facilitated by a coordinated multidisciplinary team, which may include speech-language pathologists, neurologists, otolaryngologists, dentists, physical therapists, and psychologists. This collaboration ensures comprehensive assessment, individualized treatment planning, and holistic care, addressing both the physical and psychosocial dimensions of the condition.

Keywords: sialorrhea, dysphagia, salivary glands, sialorrhea therapeutics, sialorrhea medication, saliva, botulinum toxin

1. Introduction

Sialorrhea, or excessive drooling, arises from impaired saliva transfer from the oral cavity to the oropharynx [1, 2]. Although it is often characterized by visible leakage, it may also present as internal pooling, causing significant discomfort [3, 4]. The clinical significance of sialorrhea extends well beyond physical symptoms and profoundly affects patients' social functioning, oral health, and overall quality of life [2]. Excessive sialorrhea can lead to aspiration, potentially resulting in life-threatening complications such as pneumonia, particularly in elderly patients or those with sensorimotor impairments [5, 6].

The epidemiological profile of sialorrhea varies according to its underlying etiology, with particularly high prevalence rates in neurological conditions. Approximately 70% of patients with Parkinson's disease, 50% with amyotrophic lateral sclerosis (ALS), and between 10% and 80% with cerebral palsy experience clinically significant sialorrhea [5]. These statistics underscore the importance of recognizing and addressing this symptom in neurological care settings.

Sialorrhea is an absolute or relative increase in saliva, usually noticed due to leakage from the oral cavity. This leakage typically manifests during nocturnal rest and is later observed during the day, leading the patient to hand towels or handkerchiefs [5]. In severe cases, particularly among elderly patients or those with compromised sensorimotor function, sialorrhea can lead to aspiration, resulting in potentially life-threatening complications including aspiration pneumonia [6, 7].

The reflex arc regulates salivary secretion and produces various physiological actions involving multiple neural pathways. The afferent branch contains chemoreceptors in the taste buds and mechanoreceptors in the periodontal ligament region. Afferent innervation from cranial nerves V, VII, IX, and X leads to impulses in the salivary nucleus in the medulla oblongata. The efferent impulses are primarily parasympathetic; they originate from the chorda tympani nerve (a branch of cranial nerve VII) and travel to the submandibular, sublingual, and other minor glands via the lingual nerve (a branch of cranial nerve V). Efferent fibers to the parotid gland are supplied by the lesser petrosal nerve (a branch of cranial nerve IX), which travels through the fibers of the auriculotemporal nerve (cranial nerve V) and reaches the gland [8].

This complex neuroanatomical framework provides multiple potential intervention points for sialorrhea management. Therapeutic approaches may target afferent stimuli, central processing, efferent pathways, or the salivary glands. The selection of appropriate interventions requires careful consideration of the underlying etiology, symptom severity, patient characteristics, and functional status.

Sialorrhea represents a significant clinical challenge with extensive implications beyond mere discomfort. It affects multiple dimensions of patient well-being, ranging from physical health to psychosocial functioning. Understanding its pathophysiology, which involves complex neural pathways and salivary gland function, is essential for effective management. This condition requires a comprehensive, individualized approach that addresses both the underlying causes and the manifold consequences.

2. Salivary gland anatomy and physiology: The basis for understanding sialorrhea

2.1 Structural organization of salivary glands

The parotid glands are composed of serous alveoli. The secretions produced by the alveolar cells of the salivary glands are conducted to the mouth through a highly branched ductal complex that converges into a common duct. Within the gland, the most undersized ducts, lobular ducts, are classified according to their histological characteristics. The most distal striated ducts are preceded by intercalated ducts. Myoepithelial cells are identified around the acini and intercalated ducts. The ducts converge and join to form increasingly larger ducts, with their walls progressively modifying and thickening toward the main excretory duct [9].

Parotid glands consist almost exclusively of secretory acinar units with serous cells (serous acini). They produce aqueous secretions that are rich in enzymes and antibodies. The serous acini are elongated and branched, making the parotid gland a compound acinar gland. Serous cells have a pyramidal shape [9].

The vascular supply to the salivary glands is derived predominantly from the external carotid arterial system and its glandular branches, providing robust perfusion to support metabolic and secretory demands. Venous drainage occurs via tributaries that connect to the external jugular venous system, completing the circulatory network that is essential for glandular function.

2.2 Salivary production and composition

The total salivary output demonstrates a remarkable volume, with an average daily production between 1 and 1.5 liters. The salivary flow from unstimulated glands comprises 20% of the parotid glands, 65% of the submandibular glands, 7–8% of the sublingual glands, and less than 10% from numerous minor glands. When stimulated, the parotid glands contribute more than 50% of the total salivary secretion [9].

2.3 Physiological functions and regulation

Salivary glands play a critical role in maintaining oral homeostasis. Among these is the provision of continuous moisture to the oral cavity, which protects mucosal surfaces from abrasive damage, while simultaneously facilitating the clearance of microorganisms and food particles during swallowing [10].

Salivary secretion operates under sophisticated neurological control, mediated by the superior salivary center within the central nervous system, although there are specific triggers. The three triggers or stimuli that initiate secretion are chewing (mechanical), taste (acidic substances are more stimulating and sweet substances less stimulating), and olfactory stimuli. Other factors that influence secretion include psychological factors, pain, types of medications, and systemic diseases that affect the glands [11, 12].

The autonomic innervation fibers of the salivary glands involve dual sympathetic and parasympathetic components, with each system producing distinctive secretory responses. Different neurotransmitters and hormones stimulate different receptors and salivary glands to elicit different responses. When sympathetic innervation predominates, secretion contains more proteins from the acinar cells, whereas parasympathetic stimulation produces more aqueous secretions [1]. This dual regulatory mechanism allows adaptive responses to varying physiological demands.

2.4 Pathophysiological considerations in sialorrhea

The pathophysiology of sialorrhea involves two key mechanisms: hypersalivation (excessive production) and dysfunctional oral clearance, in which normal saliva volume cannot be adequately managed [4]. In many clinical contexts, particularly neurological disorders, the predominant mechanism involves impaired oral processing, rather than hypersecretion. Dysphagia, especially that involving impairment of oral clearance, is a frequent cause of retention of sialorrhea [13].

When salivary production exceeds an individual's capacity for effective oral transport and swallowing, several consequential manifestations may develop, including intraoral stasis, extraoral leakage, and aspiration of salivary fluid in more severe

presentations [12]. These phenomena contribute significantly to the clinical and functional impact of the condition.

Neurological diseases are the most common cause of sialorrhea, occurring in approximately 50% of ALS patients, 70% of Parkinson's disease patients, and between 10% and 80% of cerebral palsy patients [2, 5]. The high prevalence in neurological populations underscores the importance of effective management strategies tailored to specific patient populations.

3. Common causes of sialorrhea

Sialorrhea stems from diverse etiologies with varying presentations and management implications. Understanding these distinct causative mechanisms is essential to develop targeted therapeutic approaches.

3.1 Neurological disorders

Diseases such as Parkinson's disease, cerebral palsy, amyotrophic lateral sclerosis (ALS), and stroke can affect swallowing control [2, 5, 13, 14].

3.2 Medication-induced sialorrhea

Several medication classes can induce or exacerbate sialorrhea through various mechanisms, such as antipsychotics and benzodiazepines, which can increase salivary production [15].

3.3 Infectious and inflammatory conditions

Acute and chronic infections involving the oral cavity, oropharynx, or dentition may precipitate temporary hypersalivation via local inflammatory mechanisms. Infections in the mouth, throat, or teeth can cause temporary hypersalivation [16].

3.4 Gastroesophageal reflux disease

Gastroesophageal reflux can stimulate increased salivary production through a vagally mediated reflex mechanism. This salivary response represents a physiological protective adaptation intended to neutralize refluxed acid and protect the esophageal mucosa. In patients with chronic reflux, this compensatory mechanism may contribute to clinically significant sialorrhea [16].

3.5 Hormonal and pregnancy-related factors

Physiological alterations in hormonal status, particularly during pregnancy, can induce transient hypersalivation (ptyalism gravidarum) [4].

3.6 Dental and oral structural abnormalities

Malocclusion, macroglossia, and other structural abnormalities affecting the oral containment of saliva can contribute to sialorrhea through mechanical mechanisms [4].

Sialorrhea stems from diverse etiologies, with neurological conditions representing the most prevalent cause. Although Parkinson's disease, cerebral palsy, and ALS account for most cases, other factors, including medication side effects, orofacial conditions, and systemic disorders, can also contribute to this challenging symptom. The recognition of these varied causes is essential for implementing targeted therapeutic strategies and achieving optimal symptom control.

4. Clinical implications of sialorrhea

Sialorrhea's impact extends beyond hypersalivation to encompass a spectrum of physical, functional, and psychosocial implications that significantly impact patient well-being and quality of life.

4.1 Physical implications

4.1.1 Perioral and Chin dermatological complications: Chronic salivary exposure to perioral skin often leads to dermatological complications, including irritant dermatitis, maceration, and secondary infections

- i. Irritant contact dermatitis is characterized by erythema, pruritus, and discomfort [3, 4];
- ii. Progressive skin maceration leads to compromised epithelial integrity;
- iii. Development of fissures and cracks that may serve as potential portals for secondary infections;
- iv. Opportunistic fungal and bacterial infections included candidiasis and impetigo;
- v. Chronic discomfort and localized pain may significantly affect the quality of life.

4.1.2 Enhanced risk of oral and periodontal pathology

The altered oral environment associated with excessive salivation creates conditions conducive to pathological microbial proliferation [1, 10]:

- i. Dysregulation of the normal oral microbiome with overgrowth of opportunistic organisms;
- ii. Increased susceptibility to dental caries, particularly affecting anterior dentition;
- iii. Development or exacerbation of gingivitis and periodontitis;
- iv. Persistent halitosis resistant to conventional oral hygiene measures;
- v. Compromise of overall oral health status necessitating more intensive dental interventions.

4.1.3 Aspiration risk and pulmonary complications

Perhaps the most clinically notable complication of sialorrhea, particularly in patients with concomitant dysphagia or diminished consciousness, is salivary aspiration. Difficulty in saliva control significantly increases the risk of aspiration, potentially causing [6, 12]:

- i. Recurrent aspiration pneumonia requiring antibiotic therapy and potential hospitalization;
- ii. Progressive impairment of respiratory function due to chronic microaspiration;
- iii. Increased healthcare utilization, including emergency department visits and hospitalizations;
- iv. Development of serious respiratory complications including bronchiectasis and pulmonary fibrosis;
- v. Long-term deterioration of pulmonary function contributes to increased morbidity and mortality.

4.1.4 Compromised oral hygiene maintenance

The practical challenges associated with managing excessive salivary flow significantly impact the effectiveness of oral hygiene practices [4, 10]. Excessive saliva makes maintaining adequate oral hygiene difficult, leading to the following:

- i. Difficulty in maintaining successful removal of dental biofilm due to continuous fluid dilution;
- ii. Reduced efficacy of brushing and interdental cleaning techniques;
- iii. Increased frequency of professional dental interventions is required to maintain oral health;
- iv. Challenges in preserving optimal gingival health and preventing periodontal disease;
- v. An elevated risk of various oral pathologies requires aggressive preventive strategies.

4.2 Psychosocial implications

4.2.1 Social embarrassment and stigmatization

Individuals with visible sialorrhea frequently experience considerable psychosocial distress related to social perception and interaction [2, 8]:

- i. Difficulties participating in communal dining experiences due to visible drooling;
- ii. Embarrassing situations during social gatherings lead to anticipatory anxiety;
- iii. Discomfort in formal and professional contexts affects career progression and opportunities;
- iv. Challenges in situations where appearance and adherence to social norms are emphasized.

4.2.2 Interference with interpersonal communication

This condition substantially impacts verbal and nonverbal communication in multiple domains [2]:

- i. Impaired ability to maintain sustained conversations without interruption to manage secretions;
- ii. Diminished confidence in public speaking and group communication contexts;
- iii. Negative effects on intimate and romantic relationships;
- iv. Altered dynamics in established friendships and family interactions;
- v. Compensatory modifications in communication patterns and social behaviors.

4.2.3 Progressive social isolation

Fear of embarrassment and social judgment frequently precipitate a pattern of increasing social withdrawal [2, 8]:

- i. Active avoidance of social engagement and the public setting;
- ii. Preference for isolation and activities conducted privately at home;
- iii. Gradual contraction of social networks and interpersonal connections, then reduction of social circles;
- iv. Abandonment of previously valued community and group activities;
- v. Progressive limitations of meaningful personal interactions and relationships, restricting personal contacts.

4.2.4 Psychological and emotional consequences

The cumulative impact of these social challenges commonly manifests as notable psychological sequelae [2]:

- i. Development of social anxiety and condition-specific phobic responses;
- ii. Increased vulnerability to depression and other mood disorders;
- iii. Erosion of self-confidence and negative alterations in self-perception;
- iv. Chronic psychological distress affects overall emotional well-being;
- v. Persistent frustration is related to limited symptom control and unpredictability.

4.2.5 Impact on family systems and caregivers

The implications of sialorrhea extend beyond the affected individual to impact family systems and caregivers [2]:

- i. Increased requirements for supportive care and assistance with symptom management;
- ii. Additional financial burden related to treatment, hygiene products, and supportive care;
- iii. Emotional strain on family relationships due to altered social participation patterns;
- iv. Caregiver fatigue and risk of burnout in cases requiring intensive management;
- v. Necessity for adapting to family routines and activities to accommodate conditions.

Sialorrhea profoundly affects patients social functioning and psychological well-being, often leading to embarrassment, social withdrawal, and a diminished quality of life. This creates barriers to normal social interaction, potentially resulting in isolation and emotional distress that extends to family members and caregivers. Addressing these psychosocial dimensions is as important as managing physical symptoms when developing a comprehensive treatment plan.

5. Therapeutic approach for sialorrhea

The management of sialorrhea requires a comprehensive approach considering the etiology, severity, patient characteristics, and potential side effects of interventions. The treatment options range from conservative behavioral techniques to pharmacological interventions, minimally invasive procedures, and surgical approaches in refractory cases [17].

5.1 Pharmacological management

5.1.1 Anticholinergic medications

Anticholinergic agents are the first-line pharmacological interventions for sialorrhea. Various approaches are available for the treatment of sialorrhea [18]. One option

is oral therapy involving anticholinergic agents, such as glycopyrrolate, benztropine, scopolamine, and tropicamide. Anticholinergic agents decrease acetylcholine regulation and ultimately decrease saliva secretion through the parasympathetic autonomic nervous system [19, 20].

Several mechanisms have been proposed to explain the relationship between medication use and decreased salivary secretion; however, anticholinergic action seems to be the most important [21]. Five subtypes of muscarinic receptors for acetylcholine mediate cholinergic signals to autonomic organs. Blocking cholinergic receptors, especially the M3 subtype, can reduce salivary secretions [19, 20]. However, available anticholinergics are not selective for M3 receptors and can produce undesirable adverse effects, such as mental confusion, hallucinations, intestinal constipation, urinary retention, and drowsiness [22].

Commonly prescribed anticholinergic agents include the following:

1. Glycopyrrolate

- a. Mechanism of action: Selective muscarinic M3 receptor antagonists;
- b. Advantages: Limited blood–brain barrier penetration, reduced central nervous system effects;
- c. Efficacy: 30–40% reduction in salivary production in clinical studies.

2. Scopolamine

- a. Formulation: Transdermal patches (1.5 mg released over 3 days);
- b. Advantages: Convenient administration and consistent drug delivery;
- c. Limitations: Higher risk of central side effects due to blood–brain barrier penetration.

3. Atropine

- a. Forms of administration: Oral, sublingual, or ophthalmic drops were administered sublingually;
- b. Disadvantage: Significant cerebral penetration with an increased risk of central nervous system effects.

5.1.2 Side effects of anticholinergic therapy

Anticholinergic medications can be inefficient over the long term, resulting in undesirable side effects. The most common side effects were constipation, urinary retention, fatigue, and drowsiness [4, 22]. Studies have demonstrated that the response rates to these medications vary from 70 to 90%, but approximately 30–35% of patients discontinue treatment due to adverse effects [4, 22]. Anticholinergic drugs are contraindicated in cardiac diseases, glaucoma, pyloric stenosis, prostatic hypertrophy, and hepatic or renal insufficiency.

Anticholinergics are effective, but they present considerable adverse effects that may limit their prolonged use.

1. Peripheral effects

- a. Xerostomia (paradoxically exacerbating swallowing difficulties);
- b. Blurred vision;
- c. Urinary retention;
- d. Intestinal constipation;
- e. Tachycardia;
- f. Mydriasis (pupillary dilation).

2. Central effects (more pronounced with agents that cross the blood–brain barrier)

- a. Mental confusion;
- b. Cognitive and memory alterations;
- c. Hallucinations;
- d. Agitation;
- e. Drowsiness.

5.1.3 Contraindications to anticholinergic therapy

Anticholinergic medications are contraindicated in several conditions.

- 1. Closed-angle glaucoma
- 2. Significant prostatic hypertrophy
- 3. Myasthenia gravis
- 4. Gastrointestinal or urinary obstruction
- 5. Severe heart failure
- 6. Dementia.

5.1.4 Alternative pharmacological approaches

Apart from traditional anticholinergics, other medication options include.

1. Tricyclic antidepressants (such as amitriptyline)
2. Anticholinergic effects as a secondary mechanism of action
3. It may be a suitable alternative when depression is a comorbid condition
4. Clonidine
 - a. Alpha-2-adrenergic agonists that reduce salivation as a secondary effect;
 - b. Limited clinical utility due to potential hypotensive effects.

5.1.5 Treatment efficacy and implementation

Pharmacological management of sialorrhea demonstrates efficacy in 70–90% of cases, although discontinuation rates of 30–35% occur because of adverse effects. Treatment protocols are typically initiated with low doses and are gradually titrated based on the clinical response and tolerability.

5.2 Surgical options

Surgical approaches offer a more definitive management for refractory sialorrhea but introduce higher invasiveness and potential complications [4, 23]. The surgical options include denervation, duct transposition or ligation, and salivary gland excision. Substantial complications include salivary fistulas, cyst formation, taste alterations, hearing impairment, and irreversible changes in saliva production [23, 24].

Radiation therapy is now infrequently employed and is generally reserved for elderly patients who are neither surgical candidates nor able to tolerate pharmacological interventions [4].

5.2.1 Salivary duct procedure

- i. Salivary duct ligation involves occlusion of major salivary gland ducts, redirecting saliva posteriorly to facilitate swallowing [23, 24];

Salivary gland resection represents a definitive procedure involving the removal of the submandibular or parotid glands, substantially reducing saliva production [24].

5.2.2 Duct redirection techniques

- i. Technique that changes the path of the salivary ducts, directing saliva to the posterior part of the mouth, facilitating swallowing;
- ii. It reduces saliva accumulation in the anterior region of the mouth, preventing involuntary leakage [23, 24].

5.2.3 Neurectomy (nerve sectioning)

- i. Surgical interruption of the nerves innervating the salivary glands;
- ii. It is primarily indicated for severe neurological sialorrhea that is refractory to conservative management [4, 23].

5.2.4 Advantages of surgical intervention

- i. Provides long-term or permanent symptom control [24];
- ii. Indicated for severe cases unresponsive to less invasive approaches [23, 24];
- iii. May significantly enhance the quality of life of appropriate candidates [24].

5.2.5 Disadvantages and risks

- i. Risk of excessive dry mouth (xerostomia) [4, 24];
- ii. Possible infections or surgical complications [24];
- iii. It can cause changes in swallowing and taste [4, 24];
- iv. Need for speech therapy after surgery [12].

5.3 Long-term implications of salivary gland excision

Although surgical removal of salivary glands successfully controls sialorrhea, it has considerable long-term physiological consequences that warrant careful consideration [4, 24]. Among the surgical approaches, removal of salivary glands (primarily submandibular and, in some cases, parotid glands) has been used to permanently reduce saliva production [23]. However, this radical intervention has significant long-term implications that should be carefully considered before making therapeutic decisions [4, 24].

5.3.1 Permanent physiological alterations

Bilateral salivary gland removal frequently results in severe and irreversible xerostomia, or dry mouth, with multiple consequences [1]. The consequences include the following:

- i. Chronic oral discomfort, including burning sensations and mucosal irritation [10];
- ii. Necessity for frequent fluid intake [10];
- iii. Difficulty maintaining prolonged speech or extended periods [10];
- iv. Altered taste perception [10];
- v. Impaired food moistening during mastication [10, 25].

5.3.2 Impact on digestive function

Saliva plays a fundamental role in the initial digestive process [1]. This drastic reduction compromises the following.

- i. Salivary amylase activity affects the initial carbohydrate digestion [10, 25];
- ii. Proper formation of food bolus [12, 25];
- iii. Oral buffering capacity to neutralize acids [1, 10];
- iv. Food lubrication, potentially complicating esophageal passage [10, 25].

5.3.3 Long-term oral health complications

5.3.3.1 Increased susceptibility to oral infections

The antimicrobial properties of saliva are critical for oral health [1]. Their marked reduction leads to the following:

- i. An elevated incidence of dental caries despite adequate oral hygiene [1, 10];
- ii. Increased risk of recurrent oral candidiasis [10];
- iii. Greater susceptibility to periodontal disease, such as gingival and infectious diseases [1, 10];
- iv. Persistent halitosis resistant to conventional management [10].

5.3.3.2 Dental health deterioration

Salivary gland removal is associated with progressive dental compromise.

- i. Accelerated enamel demineralization [1, 10];
- ii. Progressive dental erosion [10];
- iii. Dentinal hypersensitivity [10];
- iv. Increased need for restorative dental interventions [10];
- v. Premature loss of dental elements [10].

5.3.4 Orofacial functional changes

5.3.4.1 Speech impairment

Permanent reduction in saliva can alter aspects of communication [10, 12].

- i. Difficulty in articulating sounds, especially during extended speech [10, 12];
- ii. Need for frequent interruptions for hydration while speaking [10];
- iii. Reduced intelligibility during prolonged communication [12];
- iv. Increased fatigue during verbal expression [12].

5.3.4.2 Swallowing dysfunction

Paradoxically, surgery intended to address swallowing-related conditions may create new dysphagia patterns [12]:

1. Dysphagia in dry food.
2. Need for modifications in food consistency.
3. Increased risk of choking in solid foods.
4. Extended meal duration.

Surgical removal of salivary glands for sialorrhea control represents a definitive and irreversible intervention that, although effective in controlling the target symptoms, has long-term implications that significantly affect the patient's quality of life [4, 24].

Decision-making should involve multidisciplinary assessment and thorough discussion with patients and caregivers, carefully weighing the benefits of long-term complications. This approach should be considered only after exhausting less invasive alternatives and fully understanding the permanent adaptations required [2, 7, 13].

5.4 Botulinum toxin therapy

Botulinum toxin (BoNT), a neurotoxin derived from *Clostridium botulinum*, inhibits acetylcholine release at neuromuscular and autonomic junctions [20, 26]. Its efficacy is well established in the management of spasticity and dystonia [27, 28]. Beyond muscle relaxation, BoNT blocks presynaptic acetylcholine release in the parasympathetic ganglia [20, 28]. It blocks saliva secretion when injected into salivary glands [20, 28]. The primary neurological application of BoNT involves focal dystonia, particularly cervical dystonia [20, 28].

Depending on the target tissue, BoNT can block neuromuscular cholinergic innervation or autonomic cholinergic innervation of exocrine glands and smooth muscles [20, 26]. Structurally, BoNT consists of one protein formed by light and heavy chains [20, 26]. The heavy chain has two functional domains: a C-terminal domain that binds to peripheral nerve terminal receptors and another. This N-terminal domain translocates the light chain to the cytoplasm of nerve cell cytoplasm [20, 26]. The light chains are zinc metalloproteinases that block the release of the neurotransmitter acetylcholine at cholinergic synapses through cleavage of the SNARE protein (soluble N-ethylmaleimide-sensitive factor (NSF) attachment receptor), leading to blockage of parasympathetic neurotransmission [20, 26]. Botulinum toxin acts by blocking acetylcholine release at the presynaptic terminal through deactivation of fusion proteins, preventing acetylcholine from being released into the synaptic cleft and thus preventing depolarization of the postsynaptic terminal; consequently, muscle contraction is blocked [20, 26].

The main secretomotor fibers of the salivary glands are parasympathetic and susceptible to BoNT inhibition [22]. Importantly, BoNT does not affect the synthesis or storage of acetylcholine, resulting in a temporary effect [20, 26, 29]. Chemical denervation lasts several months, and neurotransmission and muscle activity recovery require new nerve endings and functional connections at motor plates [20, 26].

Several recent studies have demonstrated the efficacy of using botulinum toxin to relieve salivation symptoms [21, 28, 30–36].

5.5 Speech therapy management

Speech therapy for sialorrhea (hypersalivation or excess saliva) focuses on improving oral control and swallowing function [12, 24], particularly in patients with cerebral palsy, neurological diseases (e.g., Parkinson's disease and ALS), and oral motor difficulties [12].

5.5.1 Speech therapy treatment for sialorrhea

1. Enhance oral awareness and voluntary saliva control [12, 37].
2. Strengthen the orofacial musculature (lips, tongue, and cheeks) [12, 37].
3. Improved coordination of breathing, sucking, and swallowing [12].
4. Teach strategies to reduce involuntary saliva output [12, 37].

5.5.2 Therapeutic techniques

1. Myofunctional Exercises
 - a. Strengthening of the mouth and tongue muscles [12, 37];
 - b. Lip-sealing training prevents saliva output.
2. Sensory and Proprioceptive Stimulation
 - a. Tactile and vibratory stimuli increase oral perception [12, 37];
 - b. Techniques to improve oral sensitivity and response to saliva [12, 37].
3. Swallowing and Posture Training
 - a. Techniques for improving voluntary swallowing [12];
 - b. Adjustment of body and head postures to facilitate swallowing [12].
4. Respiratory Pattern Training
 - a. Exercises to strengthen respiratory patterns and avoid mouth breathing [12].
5. Auxiliary Resources
 - a. Straws, special drinking fountains, and strategies such as thermal and gustatory stimulation assist in oral closure and effective swallowing [12].

The management of sialorrhea encompasses a spectrum of therapeutic approaches ranging from pharmacological interventions to surgical procedures, botulinum toxin

administration, and rehabilitative techniques. Treatment selection requires careful consideration of the underlying etiology, symptom severity, patient characteristics, and potential adverse effects. Although anticholinergic medications offer beneficial symptom control for many patients, their utility may be limited by substantial side effects. Botulinum toxin injections are a minimally invasive alternative with favorable efficacy and safety profiles [38]. While offering more definitive management, surgical interventions carry the risk of permanent physiological alterations that warrant thorough evaluation. Rehabilitative approaches using speech therapy provide non-invasive options that may complement medical and surgical management. The optimal therapeutic strategy typically involves individualized, multidisciplinary assessment and frequently incorporates multiple treatment modalities to achieve satisfactory symptom control and improve quality of life.

6. Multidisciplinary team approach

The valuable management of sialorrhea requires a coordinated multidisciplinary approach that addresses the complex biological, functional, and psychosocial dimensions of the condition [39]. The composition of the team may vary based on the health-care setting and available resources but typically includes the following specialists:

6.1 Speech-language pathologist

A speech-language pathologist conducted a comprehensive assessment of oral motor function, swallowing mechanics, and speech production. Their interventions included the following:

- i. Detailed evaluation of swallowing dynamics and the development of a targeted therapy protocol;
- ii. Rehabilitation of compromised oral functions;
- iii. Implementation of techniques to enhance oral muscle control and coordination;
- iv. Guidance on optimal posture during feeding to minimize aspiration risk;
- v. Training in compensatory techniques to manage saliva effectively [12, 37].

6.2 Neurologist

The role of the neurologist encompasses the following:

- i. Comprehensive assessment of the underlying neurological conditions contributing to sialorrhea;
- ii. Prescription of appropriate pharmacological interventions;
- iii. Evaluation and implementation of botulinum toxin therapy;

- iv. Monitoring of neurological disease progression and treatment responses;
- v. Management of associated neurological symptoms that may impact sialorrhea [10].

6.3 Otolaryngologist

The otolaryngologist provides the following expertise.

- i. Evaluation of facial and cervical structures affecting saliva production and control;
- ii. Performance of surgical intervention when indicated;
- iii. Administration of botulinum toxin injections using anatomical landmarks or imaging guidance;
- iv. Assessment of salivary gland structure and function;
- v. Management of complications related to therapeutic interventions [23].

6.4 Dentist

The contributions of the dental specialist are as follows.

- i. Maintenance of optimal oral health to minimize complications;
- ii. Treatment of dental conditions that may exacerbate salivary management difficulties;
- iii. Provision of specific oral hygiene recommendations adapted for patients with sialorrhea;
- iv. Assessment of dental occlusion and its impact on oral competence;
- v. Implementation of preventive strategies to minimize oral complications [10].

6.5 Physical therapist

The physical therapist addressed the following questions:

- i. Optimization of body posture to facilitate saliva management;
- ii. Implementation of respiratory exercises to enhance physiological control;
- iii. Strengthening of relevant muscle groups to improve functional capacity;
- iv. Development of techniques for effective management of respiratory secretions;
- v. Prevention of respiratory complications, particularly aspiration pneumonia [12].

6.6 Psychologist

The psychological component of care includes.

- i. Provision of emotional support to address the psychosocial impact of the condition;
- ii. Interventions targeting self-esteem and body image concerns;
- iii. Development of strategies for coping with social challenges;
- iv. Guidance for families and caregivers;
- v. Management of anxiety, depression, and other psychological sequelae [2].

6.7 Integrated multidisciplinary approach

The optimal management of sialorrhea depends on effective coordination among various healthcare specialties to ensure comprehensive care addressing both physical and psychosocial aspects of the condition [2, 4, 12]. This collaborative framework facilitates holistic patient assessment and enhances the effectiveness of the interventions through coordinated implementation and monitoring [12].

Each professional has unique expertise that, when coordinated, addresses the complex physical, functional, and psychosocial dimensions of this condition. This collaborative model ensures comprehensive assessment, individualized treatment planning, and holistic care that optimizes the outcomes and quality of life for affected individuals.

7. Final considerations on sialorrhea and future directions

Sialorrhea represents a relevant challenge that impacts multiple aspects of the individual's life, demanding a broad and integrated approach [2, 4, 8]. Effective care requires attention to several fundamental aspects.

7.1 Individualized treatment planning

Each patient presents unique characteristics regarding their condition, including [2, 4, 8]:

- i. Variations in symptom severity and presentation pattern;
- ii. Diversity of underlying etiological factors;
- iii. Differential impacts on quality of life and functional capacity;
- iv. Individual variations in treatment response;
- v. Specific care requirements based on comorbidities and functional status.

Treatment	Mechanism	Efficacy	Duration of Effect	Side Effects	Contraindications	Level of Evidence
Anticholinergic						
Glycopyrrolate	Muscarinic	70–90%	Continuous	Xerostomi	Glaucoma, prostatic hypertrophy, myasthenia gravis, cardiac disease	Low
Scopolamine	Muscarinic	60–80%	3	Similar	Same as	Low
Atropine	Non-selective	60–85%	Continuous	High incidence of central side effects	Same	Low
Botulinum						
BoNT	Inhibits acetylcholine release at nerve terminals	80–90%	3–6	Temporary	Neuromuscular	Moderate
Surgical						
Salivary	Redirects or blocks salivary flow	>90% reduction in	Permanent	Excessive dry mouth, potential gland swelling	Poor	High
Salivary	Removes	>95% reduction in target gland secretion	Permanent	Xerostomia	Poor	Very
Surgical						
Behavioral	Improves oral motor control and swallowing	Variable, 40–70%	Requires	None	None	None
Fonte: <i>Elaboração própria.</i>						

Table 1.
 Comparative analysis of sialorrhea treatment approaches.

7.2 Importance of family and social support

The engagement of family members and broader support networks constitute a critical component of effective management [2, 12].

- i. Enhancement of treatment adherence through daily reinforcement;
- ii. Facilitation of consistent symptom management strategies;
- iii. Provision of essential emotional support;
- iv. Implementation of necessary environmental and lifestyle adaptations;
- v. Ensuring continuity of care across different settings and contexts.

7.3 Future perspectives

The evolving landscape of sialorrhea management offers several promising directions [2, 4, 8].

- i. Development of novel therapeutic approaches with enhanced efficacy and safety profiles;
- ii. Advancement in pharmacological options with improved target specificity;
- iii. Refinement of rehabilitative techniques to enhance functional outcomes;
- iv. Expanded understanding of underlying neurophysiological mechanisms;
- v. Enhancement of patient-centered intervention strategies and outcome measures.

Sialorrhea management requires a holistic approach that recognizes both its multifactorial etiology and wide-ranging impact on patient well-being. Successful intervention depends on individualized treatment plans that incorporate the expertise of multiple disciplines, engage family support systems, and adapt to patients changing needs over time. As therapeutic options continue to evolve, the integration of emerging evidence with patient-centered care principles will further enhance our ability to effectively address this challenging condition (**Table 1**).

8. Conclusion

Sialorrhea is a complex condition with multifactorial etiology and comprehensive impact on patient well-being. Effective management requires individualized treatment plans, strong family and social support, and integration of diverse therapeutic modalities. Continued research is crucial for developing more targeted interventions and deepening understanding of underlying neurophysiological mechanisms, aiming to improve patient outcomes and quality of life.

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Perspective Chapter: New Technologies in Salivary Gland Surgery

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Abstract

New technologies are increasingly widespread in medical practice. Particularly, the 3D view is considered among the most useful innovations in surgery. It allows the operator to reconstruct the patient's anatomy in his own mind, going beyond his personal imagination. In the last few years, a new facility has been experienced; it is the exoscopy. Exoscopy is a magnified vision system, similar to microscopy, but which allows a tridimensional view of the surgical anatomy. In this chapter, after a brief discussion of surgical anatomy, pathological anatomy, and the traditional surgical approach to salivary glands, we have reported our experience with the exoscope. In our opinion, this innovative tool is highly useful for reducing many complications consequent to injuries to some critical structures that are often encountered during the procedure and which are difficult to see with the naked eye.

Keywords: salivary glands, parotid gland, submandibular gland, pleomorphic adenoma, Warthin tumor, exoscopy

1. Introduction

1.1 Surgical anatomy of the parotid gland

The parotid glands are a pair of totally serous salivary glands located inside a space of the face named “parotid lodge.” It is sited below the auditory canal, behind the posterior edge of the mandibular ramus until the angle of the mandible, in front of the mastoid process of the temporal bone. The posterior margin of the parotid gland is leaned against the anterior margin of the sternocleidomastoid muscle while the anterior margin extends along the lateral face of the masseter muscle. The parotid tail overlaps the posterior belly of the digastric muscle.

The parotid gland is held by a capsule that comes from the investing layer of the deep cervical fascia. The parotid gland is separated from the submandibular gland by a thickening of the deep cervical fascia, which corresponds to the stylomandibular ligament.

The parotid gland presents an upper end and a lower end. The upper end of the parotid gland surrounds the external auditory meatus, and from it comes four

structures: two nerves, one artery, and one vein. Specifically from the upper end of the parotid gland come out the Superficial Temporal Artery (STA), the Superficial Temporal Vein (STV), the AuriculoTemporal Nerve (ATN), and the temporal branch of the Facial Nerve (FN) (**Figure 1**). The lower end of the parotid gland overlaps the posterior belly of the digastric muscle, and from it comes out the cervical branch of FN, the RetroMandibular Vein (RMV) or its two branches (anterior and posterior); finally, the External Carotid Artery (ECA) enters into the parotid gland through its lower end.

The parotid gland presents an anterior border and a posterior border. The anterior border of the gland overlaps the masseter muscle, and from it, the Parotid Duct (PD), also named the Stensen duct, the Transverse Facial Artery (TFA), and the remaining three branches of the FN come out: the zygomatic branch, the buccal branch, and the mandibular branch. The posterior border of the parotid gland overlaps the anterior margin of the sternocleidomastoid muscle. The posterior border of the parotid gland is crossed by the main trunk of the FN, which enters into the gland and in whose context it divides initially into two main branches (cervicofacial and temporofacial) and after into its five final branches: the temporal branch and zygomatic branch from the temporofacial (superior branch) and the buccal, marginalis mandibulae, and cervical branches from the cervicofacial (inferior branch).

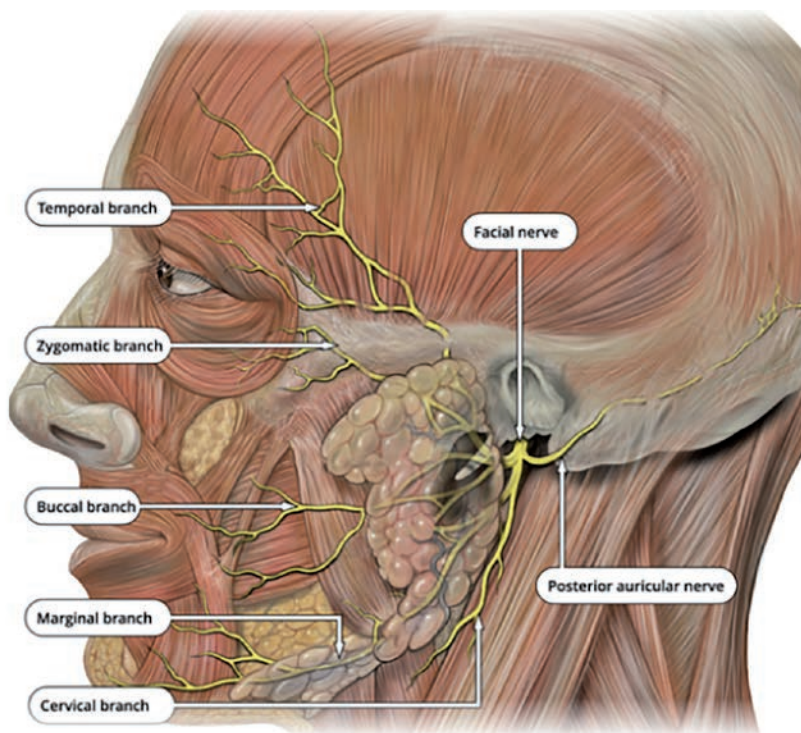


Figure 1. FN and its five branches emerging from the parotid gland. The main trunk and the two main branches (cervicofacial and temporofacial) are evident inside the context of the parotid gland, and, finally, the five branches emerge from the upper end (temporal branch and zygomatic branch), from the anterior margin (buccal branch and marginalis mandibulae branch), and from the lower end (cervical branch). The posterior auricular nerve is also evident; that is another motor branch of the FN for the posterior auricular muscle and the occipital muscle.

Within the context of the parotid parenchyma, the ECA gives rise to its two terminal branches: the Internal Maxillary Artery (IMA) and the STA. IMA passes through the deep lobe of the parotid gland between the neck of the mandibular condyle, and it continues into the pterygomaxillary fossa. STA continues upward until it emerges from the upper end of the gland. From STA origins, the TFA heads forward, emerging from the anterior border of the gland.

The STV follows the opposite direction to the homonymous artery. It enters the gland through the upper end and after receiving the Middle Temporal Vein (MTV) and the TFA. Within the deep lobe of the parotid gland, the STV joins the maxillary vein (coming from the pterygoid plexus), and it becomes RMV or Posterior Facial Vein (PFV). The last emerges from the lower end of the gland, and it divides into its anterior and posterior branches. The anterior branch joins the Facial Vein (FV), becoming the Common Facial Vein (CFV), while the posterior one joins the External Jugular Vein (EJV). The CFV heads posteriorly, draining into the Internal Jugular Vein (IJV).

The course of FN divides the parotid gland into two lobes: the superficial and the deep lobes. The superficial lobe presents a lateral face that is covered by the parotid-masseteric fascia (derived from the investing layer of the deep cervical fascia), while above the fascia there is the SMAS (Superficial MusculoAponevrotic System) and the skin.

The deep lobe presents an anteromedial surface and a posteromedial surface. The first hugs the posterior border of the ramus until the angle of the mandible, the masseter muscle, and the medial pterygoid muscle (commonly defined as the three Ms). The posteromedial surface takes continuity with the mastoid process and the structures attached to it (sternomastoid muscle and the posterior belly of the digastric muscle), with the styloid process and the structures attached to it (stylopharyngeus, styloglossus, and stylohyoid muscles, stylohyoid and stylomandibular ligaments). The space included among these structures takes the name of “parotid bed,” and it is occupied by the carotid sheath and the structures contained within it: ICA, IJV, and the last four CNs (Glossopharyngeal, Vagus, Accessory, and Hypoglossal nerves). The main trunk of the FN enters the parotid gland just from the posteromedial surface.

The FN is also the most superficial structure inside the parotid gland. In fact, all other structures are inside the deep lobe, and they are the ECA, the RMV, and ATN. So, inside the deep lobe, the ICA divides into its two terminal branches, which are the Internal Maxillary Artery (IMA) and the Superficial Temporal Artery (STA) at the neck of the condyle level. Here the Transverse Facial Artery (TFA) originates from the STA, and it courses anteriorly just above the Parotid Duct (PD). Inside the deep lobe, the Superficial Temporal Vein (STV) courses, and it becomes the RetroMandibular Vein (RMV) after joining with the Middle Temporal Vein (MTV).

The vascularization of the parotid gland is guaranteed by the small vessels starting from the ECA, the STA, the IMA, and the TFA. The sensitive innervation comes from the Great Auricular Nerve (GAN) for the capsule and from the ATN for the parenchyma. The parasympathetic innervation originates from the Inferior Salivatory Nucleus, and it runs through a branch of the Glossopharyngeal nerve (IX CN), which is the Lesser Petrosal Nerve, which continues with the ATN at the Otic Ganglion.

Finally, the Parotid Duct (PD) is there. It comes out from the anterior border of the parotid gland, where the middle third joins the upper third; it runs forward below the TFA and above the buccal branch of FN; it crosses the lateral face of the masseter muscle until its anterior border, where the PD turns medially and pierces the buccinator muscle, opening onto the oral mucosa at the superior second molar level.

Although the traditional anatomy considers the parotid glands composed of two lobes based on the course of FN, in 2016, the ESGS (European Salivary Gland Society) proposed a new interpretation of the parotid gland anatomy for better fitting to surgical needs [1].

The surgical anatomy of the parotid gland, according to ESGS, extends the division of the gland into five levels, as reported in **Figure 2**.

- Level I (lateral-superior)
- Level II (lateral-inferior)
- Level III (medial-inferior)
- Level IV (medial-superior)
- Level V (accessory)

As the images in **Figure 2** show, the ESGS 2016 classification involves the identification of two planes, sagittal and axial, developed by an imaginary line that joins the main trunk of FN and the Stensen duct. The levels I and II are on the lateral side of the gland, corresponding to the superficial lobe of the traditional anatomy. The I and II levels are, respectively, higher and lower than the imaginary line. The level III and IV are on the internal side of the gland, corresponding to the deep lobe of the traditional anatomy. The levels III and IV are, respectively, lower and higher than the imaginary line. The level IV is an agglomeration of gland parenchyma that lies along the course of the PD.

1.2 Surgical anatomy of the submandibular gland

The Submandibular Gland (SG) is one of the three major salivary glands (submandibular, parotid, and sublingual). It produces mixed saliva but mostly serous, unlike the sublingual gland, which produces mostly mucous saliva. It lies in the digastric triangle, also named the submandibular triangle, just because the

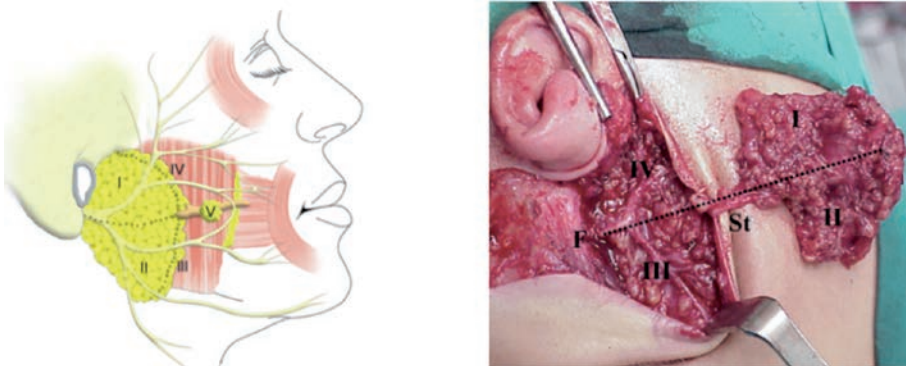


Figure 2.
ESGS 2016 Surgical Anatomy of Parotid gland. The five levels are well evident in relation to the planes formed by the imaginary line that joins the main trunk of FN with the PD; the accessory level along the course of the PD is also evident [1].

submandibular gland is the main component of this space. The digastric triangle is limited superiorly by the inferior border of the mandible (from the angle to the symphysis), inferiorly by the anterior and the posterior belly of the digastric muscle (joined by the “intermediate tendon”), medially by the mylohyoid muscle, and laterally by the platysma muscle (covered by the subcutaneous fat and the skin).

The SG lies leaning against the medial face of the mandibular body within a space excavated under the mylohyoid line named “submandibular fossa.” The medial surface of the mandibular body covers the SG almost totally; just its lower pole overflows below the inferior border of the mandible, and it is crossed by the Anterior Facial Vein. The posterior pole of the SG makes contact with the stylohyoid muscle.

The SG is wrapped by a capsule, which is an extension of the parotid-masseteric fascia. The SG is separated from the PG by the Stylomandibular Ligament (a thickening of the same fascia).

The SG presents an almond shape, and it is composed of two parts: a superficial part and a deep part, respectively below and above the mylohyoid muscle (**Figure 3**).

The superficial part of the SG makes contact with the Facial Artery (FA). The vessel originates from the ECA, and it runs forward and upwards, passing medially to the posterior belly of the digastric muscle and the stylohyoid muscle. Once the FA enters the digastric triangle, it makes a first loop passing between the mandibular fossa of the mandible and the lateral face of the SG; after that, the FA makes a second loop passing below the inferior border of the mandible, and then it continues on the face. The course of the FA is divided into a cervical tract and a facial tract; the crossing point between the first and the second tract is just the inferior border of the mandible. The superficial part of the SG makes contact with the Anterior Facial Vein (AFV), whose course follows the opposite course of the FA until the inferior border of the mandible,

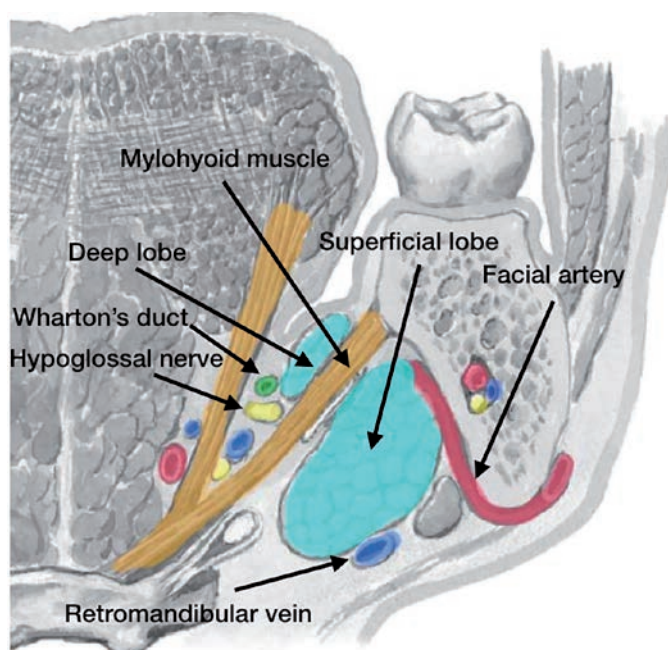


Figure 3.
SG position in the digastric triangle of the neck. The superficial part and the deep part are separated by the mylohyoid muscle.

where the AFV continues its course passing above the superficial part of the gland. Then, the AFV joins the anterior ramus of the RMV, forming the Common Facial Vein (CMV). The superficial part of the SG also makes contact with the cervical branch of FN. Also, the marginalis mandibulae branch of FN, which runs along the inferior border of the mandibular body, makes contact with the superficial part of the SG.

The superficial part of the SG makes contact with the Hypoglossal Nerve (HN). This nerve, from the carotid triangle, crosses laterally the ECA and medially the posterior belly of the digastric muscle and the stylohyoid muscle, forming the superior side of the “Farabeuf’s triangle” (EJV, Thyrolingualofacial Venous Trunk, HN). Then, HN enters the digastric triangle through the “Bèclard triangle” (hyoglossus muscle, posterior belly of digastric muscle, and major horn of hyoid bone). Once HN has entered the digastric triangle, it runs along the hyoglossus muscle together with the Comitant Vein of the Hypoglossal Nerve (or Ranine Vein).

HN forms the “Pirogov’s triangle” (intermediate tendon, mylohyoid muscle, HN) through which the Lingual Artery passes.

The superficial part of SG also makes contact with the mylohyoid nerve (a branch of the mandibular nerve that innervates both the mylohyoid muscle and the anterior belly of the digastric muscle).

The deep part of the SG lies between the mylohyoid muscle and the hyoglossus muscle. From the anterior and superior face of the deep part, the Submandibular Duct (SD) originates. It runs forward and medially with an Italian S-shaped course, and it opens onto the oral pelvis. Because of the antigravity course and because of the partially mucous saliva, the SG is more predisposed to the formation of salivary stones. The deep part of the SG makes contact with the Lingual Nerve which runs together with the SD between the mylohyoid muscle (laterally) and the hyoglossus muscle (medially).

The SG is vascularized by the glandular branches of the FA while its parasympathetic innervation starts from the Superior Salivary Nucleus, and it gets to the SG through the chorda tympani nerve (a branch of the FN) with the interposition of the submandibular ganglion. This innervation is the same both for SG and for the sublingual gland.

2. Pathological anatomy of salivary glands

2.1 Classification of salivary gland tumors according to “WHO Blue Book 2017”

Tumors of salivary glands, including both malignant and benign tumors, are 2–3% of head and neck tumors. The parotid gland is the most interesting site (80%), and most (over 60%) are benign tumors [2]. The incidence of salivary gland tumors is statistically greater in males (0.7/100,000) than in females (0.5/100,000), generally manifested between the 4th and the 7th decades of life; most neoformations are benign tumors (85–90%). Malignant tumors are most frequent among people of advanced age, and they involve more frequently the minor salivary glands [3].

The last classification of salivary gland tumors was proposed in 2017 by the WHO (World Health Organization), and it has been added to the “Blue Book for Tumors and Tumor-Like Lesions of the Neck and Lymph Nodes” [4].

The classification expects the following types of benign tumors:

- Pleomorphic adenoma
- Oncocytic cystic adenoma (Warthin tumor)

- Monomorphic Adenoma (oncocytoma)
- Papillary cystadenoma
- Canalicular adenoma
- Cystadenoma
- Mucinous adenoma (Mucosa-type adenoma of the minor salivary glands)
- Benign mesenchymal tumors (fibromas, lipomas)
- Fibromatous tumor
- Oncocytic cell tumor
- Pleomorphic tumors of the minor salivary glands.

Pleomorphic adenoma (PA) is also named “Benign Mixed Tumor” because it is typically composed of a mixed cellular component [5]. Pleomorphic adenoma is the most common salivary gland tumor, accounting for 60–70% of all major and minor salivary gland tumors, even if it is much more common in PG. It is also the most common benign minor salivary gland tumor, accounting for 70% of all tumors.

The term “pleomorphic” refers to the variety of cells and structures that make up the tumor, which may have a combination of epithelial, myoepithelial, and mesenchymal components (fibrosis, cartilage, bone, etc.), and its histological features were first described by Nasse in 1892. The PA is not equipped with a real capsule but with a pseudocapsule, which is simply a perilesional fibrous thickening [6].

It is more frequent among women (2:1) [7], and, generally, it presents as a palpable nodule in the parotid region of the skin. Atypical manifestations could be tinnitus, hypoacusia, or ear pain [8]; sometimes PA can grow, achieving a huge size [9]. Although it is possible, that FN paralysis can also be a presentation of PA, it is more frequent for malignant tumors, and, in this case, it represents an unfavorable sign [10]. The PA presentation of the submandibular gland, minor salivary glands, and sublingual gland (rare) is typically a local swelling [11].

The risk of malignant progression is low, but it exists; in these cases, the neoplasm takes the name of Carcinoma ex Adenoma Pleomorphic (CxPA). The risk is higher in patients more than 50 y.o., in smokers, and for neoformation larger than 2 cm [12].

Generally, the main reason for treatment for PA is esthetics, but the risk of malignant progression (albeit low) and the progressive growth (albeit slow) make surgery indicated.

Warthin Tumor (WT) is the second tumor of salivary glands for frequency. It is also named Oncocytic Cystic Adenoma because it is histologically characterized by a dense lymphoid stroma and a double layer of oncocytic epithelium with a papillary and cystic architectural pattern [13].

WT was first described by Hildebrand in 1895, and it was initially named cystadenolymphoma. That term was then abandoned because it was improper. The risk of malignant transformation is rare, and it is estimated at 0.3% [13].

Unlike the PA, WT is more frequent among men than women, and it involves above all advanced age, the 6th decade for women, and the 7th for men [13].

Three types of WT are known: epithelial predominance, mixed, and lymphoid predominance. Some authors suggest the subtype lymphoid predominance constitutes the early stage, while the mixed one followed by the epithelial predominance type represents more advanced development stages of the same tumor. Typically, TW is constituted by varying proportions of papillary-cystic structures lined by oncocyctic epithelial cells and a lymphoid stroma with germinal centers [13].

WT is the only benign neoplasm of SGs associated with tobacco smoking. Another known risk factor is the EBV infection, although it is closely associated with multiple/bilateral Warthin tumors. The malignant transformation of WT can include both epithelial and lymphoid components, but the most common malignant transformations of WT are mucoepidermoid carcinoma, squamous cell carcinoma, undifferentiated carcinoma, oncocyctic adenocarcinoma, and adenocarcinoma. Unlike PA, the disproportionate growth of WT is rare, and, generally, the size of WT is not over 4–6 cm in diameter; but, more frequently than PA, WT are multiple and/or bilateral tumors [13].

There is no mutual agreement for the treatment of WT. Most authors believe that the best treatment consists of ECD (ExtraCapsularDissection), while many authors consider Superficial Parotidectomy (for neoplasm in level I or II) or Total Parotidectomy with Nerve Sparing (for neoplasm in level III or IV) the treatments with the best outcomes for WT. Only a few authors, considering the low risk of malignant transformation (0.3%) and the presence of a well-defined capsule, believe that enucleation is a sufficient surgical treatment for WT [13].

In patients with old age or comorbidity that contraindicate general anesthesia active monitoring, through periodic MRI (6–12 months), is the best solution for inoperable WT. The main features to evaluate are the increase in size, the changes in internal composition, and the extra-capsular extension. The onset of facial palsy or laterocervical lymphadenopathy are bad signs [13].

Oncocytoma, or monomorphic adenoma, is a rare benign salivary gland tumor composed of large epithelial cells known as oncocytes [14]. These are predominantly composed of oncocyctic cells lining the salivary ducts. The prefix onco is derived from the Greek word *onkoustai*, which means to swell [14]. The term “Oncocytoma” is ascribed to the eosinophilic granular cytoplasm of tumor cells, due to increased numbers of mitochondria. Oncocytoma is a rare tumor of SGs representing less than 2% of these tumors, and it presents a risk of malignant transformation of less than 1% [14].

Papillary cystadenoma is an uncommon benign epithelial neoplasm that shows a predominantly multicystic growth pattern with intraluminal papillary proliferation and areas of oncocyctic differentiation [15].

Canalicular adenoma (CA) is a rare benign tumor of the salivary glands, predominantly affecting elderly females, with a strong predilection for the upper lip [16].

The other benign tumors listed above are very rare, and they are too infrequent in order to establish the risk of malignant transformation.

It is mandatory to say that each surgical procedure for PG neoplasm must be preceded by a histological or cytological assessment through, respectively, FNAB (Fine Needle AgoBiopsy) or FNAC (Fine Needle Ago Cytology).

3. Traditional surgery of salivary glands and new technologies

3.1 Surgical approach to parotid gland

About 75–80% of parotid neoplasms are benign. PA and WT are the most frequent. When malignancy is encountered, adenoid cystic carcinoma and mucoepidermoid carcinoma are, approximately, equally represented [17].

Until the end of the eighteenth century, the SGs surgery was limited to the treatment of ranula and salivary calculi [17]. The concept of surgical excision of a parotid tumor has been attributed to Bertrandi in 1802, but the initial applications of this surgery included an extensive approach, which caused serious disfiguration and disability.

About in the 1850s, the focus shifted toward dissection and the intimate relationship between the FN and the PG. Attempts were made to perform the surgery with nerve preservation.

On October 16, 1846, in Boston, J.C. Warren performed the first surgery on a patient who underwent general anesthesia with ether practiced by W.J. Morton just on a patient with a PG tumor [17].

In 1892, Codreanu performed the first total parotidectomy with nerve preservation. Before the 1950s, enucleation of benign neoplasm procedures (e.g., for pleomorphic adenoma) was the most common extirpative procedure, but it was associated with an elevated recurrence rate. The reason to perform enucleation rather than removal of the gland was to avoid injury to FN [17].

In 1958, Beahrs and Adson described the procedure of superficial parotidectomy minimizing the risk of injury to FN and, at the same time, guaranteeing the radical excision of PG tumor [17].

Nowadays the gold standard for benign neoplasm of the superficial levels of PG (levels I and II) is ECD (ExtraCapsularDissection) when the tumor involves only one level (I or II); if it involves both levels, the Superficial Parotidectomy (SP) is necessary. For benign neoplasm involving one or both of the deep levels (III or IV), total parotidectomy with nerve sparing is indicated [2].

SP was first described by the State in 1949, and it is, nowadays, the surgical approach to the PG most performed [18]. The procedure consists of removing the entire superficial lobe of the gland (both level I and II). After the identification of the main trunk of FN, the course of its branches is followed, and the nerve is carefully dissected from the overlying parotid parenchyma.

The identification of the FN main trunk is mandatory for all types of parotid surgery (enucleation, ECD, SP, and TP) and it represents a true real phase of the procedure.

Total parotidectomy consists of the removal of both superficial and deep lobes of the gland. The procedure can be performed with “Nerve Sparing” or with “Nerve Sacrifice.” The first is indicated for benign or malignant tumors, but in the last case, only if the involvement of FN by the tumor was not acknowledged through clinical and/or radiological evaluation.

In consideration of the different surgical procedures that could be necessary, it is understandable that the identification of the histological nature of the neoformation before surgery is mandatory. That means radiological examination, rather through IMR with contrast medium, and at least cytological evaluation through Fine Needle

Aspiration Cytology (FNAC) or, if possible, through Fine Needle Aspiration Biopsy (FNAB) are indispensable assessments before surgery [19].

Surgical approach to PG was one of the most discussed topics of facial surgery in the past. Since the mid-nineteenth century, a large number of authors have described what each of them thought could be the best surgical approach to PG. In **Table 1**, the list of authors is reported (**Table 1**) [20].

The most famous approach was Blair's; it was described by the same author in 1920 [20]. He described a preauricular incision that extended inferiorly until reaching

Approach	Year
Weber	1845
Ferguson	1845
Dieffenbach	1848
Dieffenbach (Rhinoplasty)	1848
Endaural (Kessel)	1885
Trendelenburg (Rhinoplasty Mod.)	1886
Inverted V (Weir)	1892
Lateral rhinotomy (Moure)	1902
Gutierrez	1903
S Incision (Blair)	1920
Adson/Ott (Gutiérrez Mod.)	1923
Heermann (Endaural Mod.)	1930
Retroauricular (Axhausen)	1931
Lexer (Rhinoplasty Mod.)	1934
Rethi (Rhinoplasty Mod.)	1934
Bailey (Blair Mod.)	1941
Redon (Gutiérrez Mod.)	1955
Sercer (Rhinoplasty Mod.)	1956
Samengo (Gutiérrez Mod.)	1961
Tessier	1967
Appiani (Gutiérrez Mod.)	1967
Rowe (Preauricular Mod.)	1972
Al-Kayat/Bramley (Preauricular Mod.)	1979
Facelift (Hagan)	1980
Popowich/Crane (Preauricular Mod.)	1982
Starck (Endaural Mod.)	1993
Guerrero (Endaural Mod.)	2001
Thankappan (Lat. Rhinotomy Mod.)	2009

Table 1.

In chronological order, the authors who reported their contributions on surgical approaches to PG from the mid-nineteenth century to our days.

the earlobe, where it coursed posteriorly about one centimeter and then downward again. The downward extension could reach the clavicle, if needed, depending on the case. He stated that postoperative healing was acceptable and that the scars were minimal. Modified Blair incision (MBI) [20].

In 1941, Hamilton Bailey Modified Blair's approach. The author thought that Blair's technique was excellent, but, in some cases, it lacked exposure to the entire surgical field. The author proposed that the incision line should come as close as possible to the cartilage of the ear, and then it continues downward as in the original approach. He reported that, in this way, better access was achieved [20].

In 1955, Redon described a similar approach but more conservatively because it does not expect a post-auricular extension of the incision, which goes down immediately after going around the earlobe [20].

In 1980, Warren E. Hagan and Jack R. Anderson described the "facelift approach." The procedure was borrowed by the lifting one, and, thanks to an incision extended behind the ear and toward the scalp, it ensures better esthetic outcomes (**Figure 4**) [21].

In our School of Maxillofacial Surgery, the Modified Blair's incision is preferred. Our surgical approach to PG consists of a first phase of "Hydrodissection," which consists of the infiltration of a solution; we prefer Mepivacaine without adrenaline, using a long needle of 27 gauge. Alternatively, it is possible to use Saline Solution 0.9% [22]. The fluid has to be injected uniformly distributed under the skin plane. The aim of the infiltration is to make a dissection of the skin from the superficial subcutaneous fat (**Figure 5**).

The procedure continues with a Redon's incision, followed by the preparation of a cutaneous flap and the exposure of the underlying SMAS plane (Superficial MusculoAponeurotic System) (**Figure 6a**). The dissection is performed through the subcutaneous fat layer, so some lobules of superficial fat remain attached to the skin flap while others to the underlying SMAS (**Figure 6b**).

The intervention proceeds with the preparation of the SMAS flap below, where deep fat and the parotid-masseteric fascia are encountered (**Figure 6c–d**). The parotid gland is further exposed after the incision of the parotid-masseteric fascia.

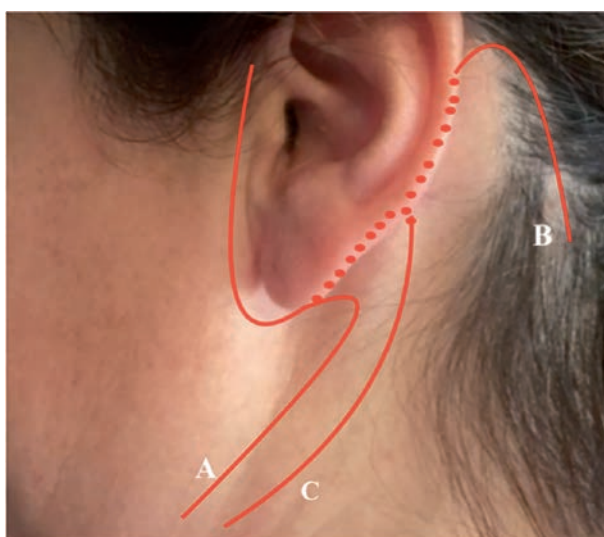


Figure 4.
Differences between Redon's incision (A), "facelift incision" (B), and Modified Blair's incision (C).

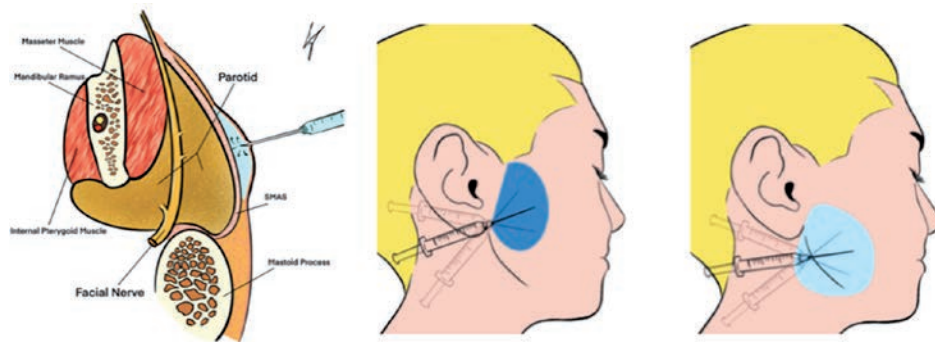


Figure 5.

Hydrosdissection: infiltration of a fluid solution, preferably local anesthetic without vasoconstrictor, under the skin plane.

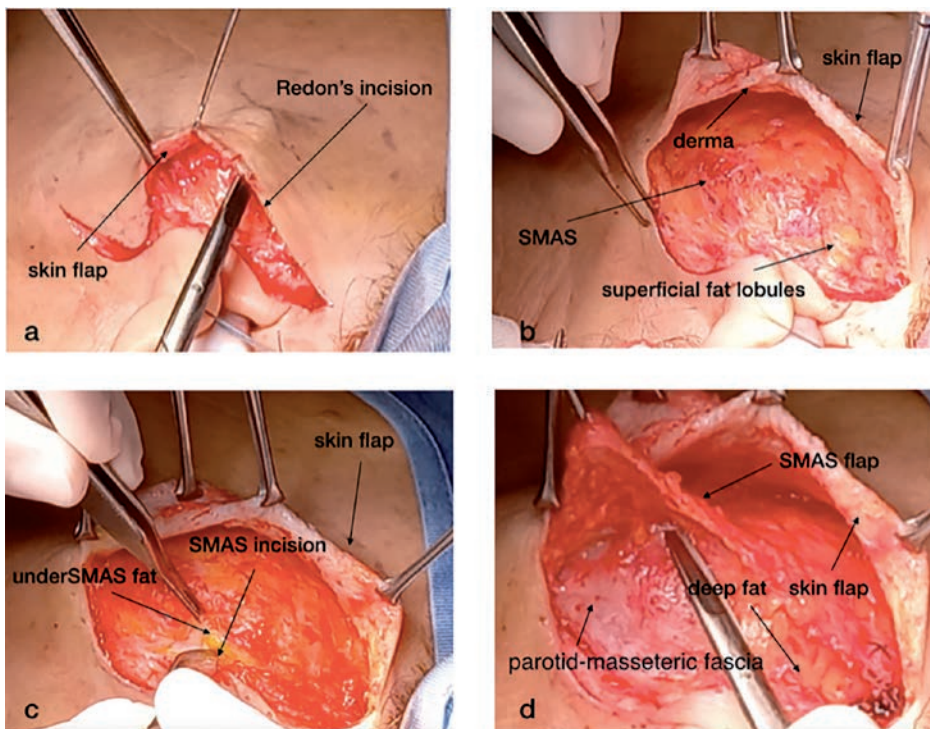


Figure 6.

Redon's incision (a); preparation of skin flap with the exposure of SMAS (b); SMAS incision (c); preparation of SMAS flap with the exposure of parotid-masseteric fascia and deep fat (d).

3.2 Surgical approach to submandibular gland

The history of the surgical approach to SG begins later than the PG one, about one century later. The reason for this delay is the rarity of the tumor frequency of these glands in comparison to the parotid ones. In fact, the SGs are generally interested in lithiatic pathology, and chronic sialadenitis-related (known as Kuttner tumors) is certainly more frequent than neoplastic pathologies. However, since the 1950s, even

chronic sialoadenitis has acquired surgical indication because of the possible complications related, particularly when a stone obstructs the proximal tract of Wharton's duct. When the calculus is near the end of the duct and it is palpable on the oral pelvis, it is indicated to remove the stone through endoscopy or ductotomy, without gland excision.

Postmortem studies suggest that sialolithiasis occurs in 1–2% of people, and 80–95% of them occur in SG (only 5–20% in PG) [23].

Even SG can be interested in tumors, but only 10% of salivary gland tumors are located in SG, and, unlike PG, half of the tumors (about 50%) are malignant [24], but both benign and malignant neoplasms submandibular excision is indicated. Even if histologically assessed, enucleation of benign tumors is not expected.

The surgical procedure used for removing SG takes the name of submandibular sialoadenectomy. It begins with an incision made approximately two fingers below the inferior border of the mandible; it must be extended 3–4 cm anteriorly and posteriorly over the palpable poles of the gland. The incision should overlap a cutaneous fold of the neck for better esthetic outcomes. For accessing the digastric triangle, a dissection for planes is necessary. There are four structures that have to be dissected before achieving SG:

- Subcutaneous fat
- Superficial fascia
- Platysma muscle
- Deep cervical fascia.

The referent point for detecting SG is the “intermediate tendon” of the digastric muscle. Other structures that certainly will be met during an intervention of SG excision are the sternomastoid muscle, the posterior belly, and the anterior belly of the digastric muscle (mostly the posterior belly), and the stylohyoid muscle. Care must be taken for the cervical branch of the FN, while the mandibular marginalis branch should not be encountered. The retromandibular vein must be identified and tied with suture thread, while the facial artery should only be tied if necessary. Care is necessary for the ansa of the Hypoglossal Nerve too [25, 26].

3.3 New technologies in surgical approach to PG and SG

The complexity of the anatomy of PG and SG makes their surgical approach challenging. Many critical structures are inevitably encountered during these procedures. FN is certainly the most important structure to pay attention to; in fact, identification of FN is mandatory for all procedures to PG, even for enucleation. Other relevant structures for PG procedures are the AuriculoTemporal Nerve, the retromandibular vein, the External Carotid Artery, the Internal Maxillary Artery, the Superficial Temporal Artery, the Superficial Temporal Vein, the Middle Temporal Vein and the Transversal Facial Artery, the Facial Artery, and the External Jugular Vein, without forgetting that inside the parotid bed the carotid sheath and its contents (Internal Carotid Artery, Internal Jugular Vein, and the last four Cranial Nerves) run. However, the FN main trunk and all its branches are the most important structures involved during a PG surgical approach; generally, the nerve injury is transitory (stupor); sometimes it is irreversible.

Many critical structures are involved in the SG approach too. First of all, the RetroMandibular Vein and the Facial Artery but the Ansa of the Hypoglossal Nerve and the cervical and marginalis branches of FN are also relevant critical structures that deserve particular attention.

Just to reduce the risk of injury to these structures as low as possible, the new technologies are more and more diffused. Navigation-assisted and microscope-assisted are certainly the most diffused ones, but recently the use of exoscopy in salivary gland surgery has been proposed [27].

Exoscopy is a magnified vision system, similar to microscopy, but with the relevant difference of allowing a 3-D view thanks to a camera maneuvered by the surgeon. He moves a lens on the surgical field that is able to enlarge the field of view to 8–30x. Everything captured by the camera is aired on one or more monitors, and it can be visualized in a 3D view thanks to some special polarized glasses worn by the operators and all assistance staff.

The exoscope is equipped with two cloches that the operator holds in his hands, like the controls of an airplane. In the center between the right and the left cloches, there is a camera that the operator moves above the surgical field. The camera captures the images while the operator watches the procedure on a monitor. Two monitors are available so that the second and the third operators also can watch the procedure on the monitor in 3-D view. The machine is equipped with an autofocus system too, so that the operator only has to frame the surgical field, and the machine will focus the image by itself. In addition, the machine can record the coordinates of a certain position above the surgical field and can reposition itself at that selected point even after the camera has been moved.

In **Figure 7** we propose an exemplary clinical case of a surgical approach to the parotid gland performed with exoscope guidance. Unfortunately, it is impossible to reproduce the tridimensional view without special polarized glasses.

In **Figure 7A–C**, all phases of the surgical approach to PG are reported. In **Figure 7A**, the hydrodissection (**Figure 7A/a**) and Redon's incision (**Figure 7A/b**) can be seen. In **Figure 7A/c**, the skin flap with the exposure of superficial fat lobules (partly on the derma and partly on the SMAS) is reported. In **Figure 7A/d**, the incision of the SMAS.

In **Figure 7B/e** the preparation of the SMAS flap is reported; in **Figure 7B/f** the SMAS flap has been elevated and the parotid-masseteric fascia with deep fat lobules is evident.

In **Figure 7B/g**, the operator tastes the pointer, one of the reference points for searching the FN main trunk.

In **Figure 7B/h**, the FN has been identified.

In **Figure 7C** most of the details of anatomical structures are magnified: the cervical branch of FN (**Figure 7Ci–l**), the retromandibular vein (**Figure 7C/m**), and the distinction between the tumor mass and the gland parenchyma is well evident.

Unfortunately, the imaging reported in the text cannot reproduce the 3-dimensional images seen by the operators because it should be necessary to wear the special glasses supplied.

Of course, the possibility to see the critical anatomical structures in magnification makes the possibility to damage it very low, and this is the same for SG. In **Figure 8**, the details of SG excision are evident.

The advantages of exoscopy are numerous. In our experience, this technology is associated with a lower risk of complications, both intra and postoperative, such as bleeding and infections; it is associated with better esthetic outcomes and better control of the surgical radicality in oncology.

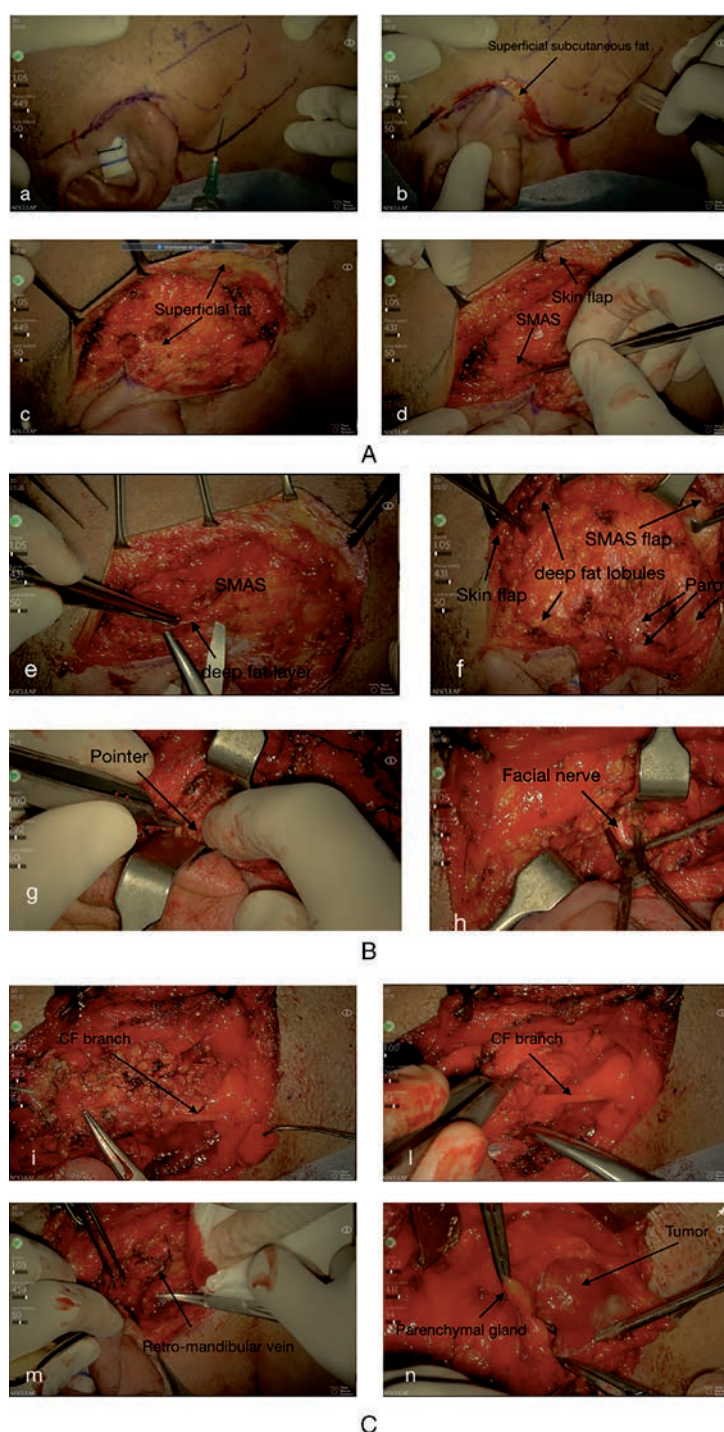


Figure 7.
 A. Hydrodissection (a); Redon's incision (b); exposure of SMAS plane with lobules of superficial fat partly on the derma and partly on the SMAS. B. Preparation of SMAS flap (e); elevation of SMAS flap with exposure of parotid-masseteric fascia and deep fat (f); tasting of pointer (g); FN main trunk (h). C. The details of anatomical structures are well evident through magnification: cervical branch of FN (i-l), retromandibular vein (m), tumor mass, and parenchyma gland (n).

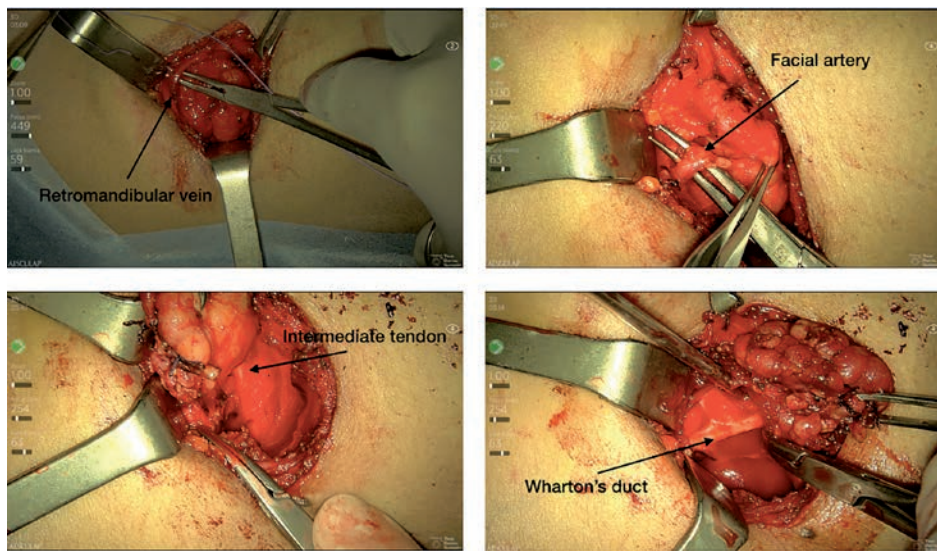


Figure 8.
Details of SG excision. Retromandibular vein (a), facial artery (b), intermediate tendon (c), Wharton's duct (d).

In **Table 1**, our casuistry is reported. We treated 14 patients with benign tumors of the parotid gland between 2023 and 2024, 9 women and 5 men (W/M ratio 1:0.5), ages 37–72 y.o. (average 56.7 y.o.). All patients had benign tumors of the parotid gland, and all patients underwent surgery after cytological examination through FNAC (but one case with cystic lesion appearance). The lesions found by the pathologist were seven Pleomorphic Adenomas (PA), four Warthin's tumors, and one lipoma. In one case, a suspected node was noticed in the MRI but FNAC was not decisive, so the patient underwent excisional biopsy without a cytological indication. In another case, both MRI and CT documented the presence of a lesion with a cystic appearance, so the patient underwent surgery without previous cytology. In **Table 1** are reported the diameters of each lesion measured in the axial and coronal planes. The surgical procedure (enucleation, ECD, superficial parotidectomy, deep parotidectomy, or total parotidectomy) was decided based on the measures of each lesion and on its location: small lesions (less than 25–30 mm) underwent enucleation (7 cases) or extra-capsular dissection (3 cases); larger lesions or those sited between two levels of the parotid gland underwent partial parotidectomy (2 superficial, 1 deep). Just one total parotidectomy was performed for a patient with a large pleomorphic adenoma located in the deep lobe.

All the procedures were performed by the same expert surgeon, who, at the end of each procedure, reported the entity of the intraoperative bleeding in comparison to traditional procedures performed without an exoscope. The bleeding could be reported as minimal, moderate, high, or very high. As we can see in **Table 1**, most of the procedures were conducted with minimal bleeding (11 out of 14); just in three cases was the entity of bleeding moderate. No case of high or very high bleeding was reported.

Unfortunately, we have no data about our previous experience without an exoscope, but a study conducted in our department reported that its use is associated with better preservation of the functionality of the FN.

In **Table 2**, the results of our study are reported. We followed fourteen patients with a series of checks at the immediate postoperative period (T0), at 1 month (T1),

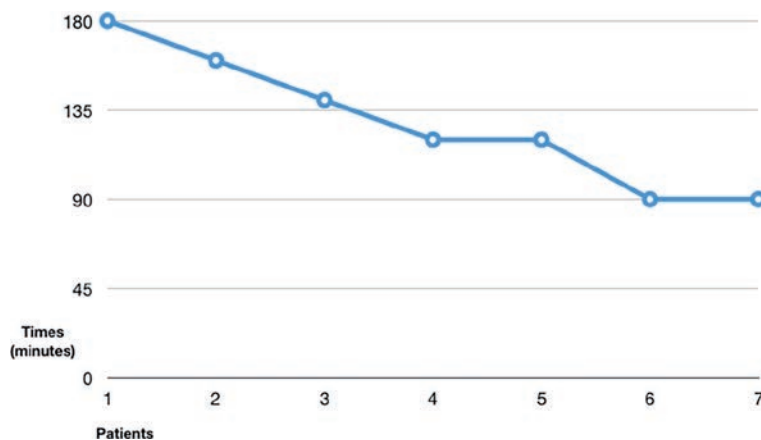
Patient	Age	Sex	Pathology	Dimensions (mm)	Surgery	Operative times	Intraoperative bleeding	FN palsy	Other complications
1	60	M	WT	17 × 15	Enucleation	180'	Minimal	Transient (buccal)	NO
2	54	W	PA	19 × 22	Enucleation	160'	Minimal	Transient (frontal)	NO
3	62	M	PA	27 × 25	ECD	160'	Minimal	NO	Sialoceles
4	46	W	PA	32 × 35	Superficial parotidectomy	200'	Moderate	Transient (buccal)	NO
5	37	W	WT	16 × 15	Enucleation	140'	Minimal	Transient (frontal)	Salivary fistula
6	57	M	Lipoma	20 × 22	Enucleation	120'	Minimal	NO	NO
7	72	W	Intra-parenchymal node	16 × 12	Enucleation	120'	Moderate	NO	NO
8	54	M	PA	34 × 33	Superficial parotidectomy	180'	Minimal	Transient (frontal)	NO
9	43	W	Cyst	24 × 22	ECD	140'	Minimal	NO	NO
10	64	W	W	18 × 15	Deep parotidectomy	200'	Moderate	Persistent (buccal)	NO
11	53	W	PA	22 × 19	ECD	120'	Minimal	NO	Sialoceles
12	63	M	PA	35 × 34	Total parotidectomy	220'	Minimal	Persistent (frontal + buccal)	NO
13	70	M	WT	22 × 24	Enucleation	90'	Minimal	NO	Salivary fistula
14	59	W	PA	25 × 24	Enucleation	90'	Minimal	Transient (buccal)	NO

Table 2.
Patients treated for benign neoplasm of PG between 2023 and 2024.

Patient	T0 (p-o)	T1 (1 month)	T2 (3 months)	T3 (6 months)
1	2 H-B	2 H-B	0 H-B	0 H-B
2	3 H-B	2 H-B	1 H-B	0 H-B
3	0 H-B	0 H-B	0 H-B	0 H-B
4	4 H-B	2 H-B	2 H-B	0 H-B
5	2 H-B	1 H-B	0 H-B	0 H-B
6	0 H-B	0 H-B	0 H-B	0 H-B
7	0 H-B	0 H-B	0 H-B	0 H-B
8	5 H-B	3 H-B	2 H-B	0 H-B
9	0 H-B	0 H-B	0 H-B	0 H-B
10	5 H-B	4 H-B	4 H-B	3 H-B
11	0 H-B	0 H-B	0 H-B	0 H-B
12	5 H-B	5 H-B	4 H-B	4 H-B
13	0 H-B	0 H-B	0 H-B	0 H-B
14	3 H-B	2 H-B	1 H-B	0 H-B

Table 3.

Evaluation of FN function based on House-Brackmann score (H-B) at the immediate postoperative period (T0), at 1 month (T1), at 3 months (T2), and at 6 months (T3).

**Table 4.**

Learning curve of exoscopy that reports the progressive reduction of the operative times.

at 3 months (T2), and at 6 months (T3). Each patient was evaluated for the FN functionality through the House-Brackmann scale. As **Table 2** reports, only two patients presented a residue of FN deficit 6 months after surgery, but they correspond to patients who underwent deep parotidectomy and total parotidectomy. Other patients were subjected to less invasive procedures (extra-capsular dissection, enucleation, or superficial parotidectomy) and nobody presented an FN deficit 3 months after surgery. These results are certainly better in comparison to our surgical practice before the availability of the exoscope in our department.

Many pieces of information have been required of us about the maneuverability of the exoscope. In our experience, the machine is very easy to use. Obviously, a better familiarity is acquired with time, but its functionality is intuitive also for the less experienced operators.

In **Table 3**, the learning curve of the exoscope is reported. It is evident the repeated use of an exoscope leads to a reduction of the operative times. This curve has been realized by reporting the operative times of the same types of operation (ECD) performed by the same operator (expert surgeon) (**Table 4**).

In conclusion, new technologies are quickly increasing in surgery. It is undeniable that they are very charming, and their use is often spectacular, even if more experience is necessary to allow their total inclusion in surgical practice, they are certainly part of the future of surgery.

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Nanoparticle-Based Biosensors for Salivary Analysis in Dentistry

Ayşe Nur Dogan, Soley Arslan, Mustafa Nisair and Ismail Ocsoy

Abstract

We have focused on nanoparticles (NPs)-based biosensors to detect various biomarkers, including pathogens in saliva, for the determination of disease activities in dentistry. Recently, NPs-based studies related to dentistry research have been receiving growing interest from researchers. The NPs have the potential to give rapid and efficient responses for the detection of targets owing to their unique and enhanced physico-chemical and biological properties. We have discussed NPs-based biosensors consisting of plasmonic NPs (silver, gold NPs, etc.), metallic NPs (quantum dots, etc.), the carbon family (graphene oxide, single-walled carbon nanotubes, multi-walled carbon nanotubes, etc.), and hybrid NPs.

Keywords: biomarkers, biosensors, dentistry, pathogens, nanomaterials, salivary analysis

1. Introduction

The biomarkers or their derivatives existing in the human body play critical roles in the early detection of potential diseases [1]. In contrast to symptoms, which frequently represent the final stages of a disease, biomarkers can emerge, diminish, or fluctuate in concentration from the disease's inception, making them valuable for early detection [2]. The utilization of biomarkers enhances diagnostic reliability, facilitating the provision of more effective and safer treatments for patients [3]. In recent years, saliva has emerged as an alternative to traditional biofluid assays due to its accessibility and dependability in assessing several biomarkers [4, 5]. Saliva is collected using simple and non-invasive methods, facilitating convenient storage and transit of samples, and many disease biomarkers in saliva may accurately reflect both physiological and pathological states in humans [6]. Unlike blood samples, saliva can be collected without specialized equipment or trained personnel in phlebotomy, thereby minimizing or eliminating the risk of cross-contamination among patients and significantly reducing the exposure of medical staff to bloodborne pathogens such as HIV and hepatitis [7, 8]. Additionally, working with saliva carries advantages for individuals, such as hemophiliacs, babies, elderly patients, and those with disabilities, who encounter challenges with blood sampling [9]. Despite these advantages, there are also problems such as matrix effects, sample interferences, and viscosity.

There is a high demand for the rapid and reliable quantification of salivary biomarkers through biosensing technology (**Figure 1**) [10, 11].

NPs exhibit remarkable properties and have been actively used in designing various biosensors [12, 13]. The working principle of NPs-based biosensors can rely on colorimetric, optical, electrochemical, and piezoelectric responses, which may be directly or indirectly correlated with biomarker levels [14–17]. NPs-based transducer systems into electrochemical biosensing devices have lately garnered significant interest from researchers due to their capacity for rapid, straightforward, and precise assessment of biomarkers contained in minimal concentrations within bodily fluids [18, 19]. For instance, the integration of graphene into these advancements may enhance the signal owing to its intrinsic properties [20]. Among plasmonic nanoparticles (NPs), gold nanoparticles (Au NPs) have gained popularity owing to their unique optical and electronic properties with biocompatibility [21]. Several more NPs [22–25] have been used for the fabrication of various biosensors [20].

The mouth cavity has a multitude of commensal bacteria, referred to as the oral microbiome. Bacteria present on various surfaces within the oral cavity are organized into extensive colonies that flourish and mutually support each other, referred to as biofilm. Within the biofilm, several bacterial species engage in communication, facilitating host colonization, providing defense against competing bacteria, and responding to environmental changes. The pathological impact of the oral microbiota arises when the host's defensive mechanisms are compromised or overwhelmed by underlying conditions, such as autoimmune disorders or diabetes [26, 27]. The direct identification of pathogens, including bacteria and viruses, is crucial for both the early diagnosis of diseases and the assessment of treatment efficacy [4].

In this book chapter, we focused on NPs-based biosensors to detect various biomarkers, including pathogens in saliva, for the determination of disease activities in dentistry. Recently, NPs-based studies related to dentistry research have been receiving growing interest. The NPs have the potential to give rapid and efficient responses

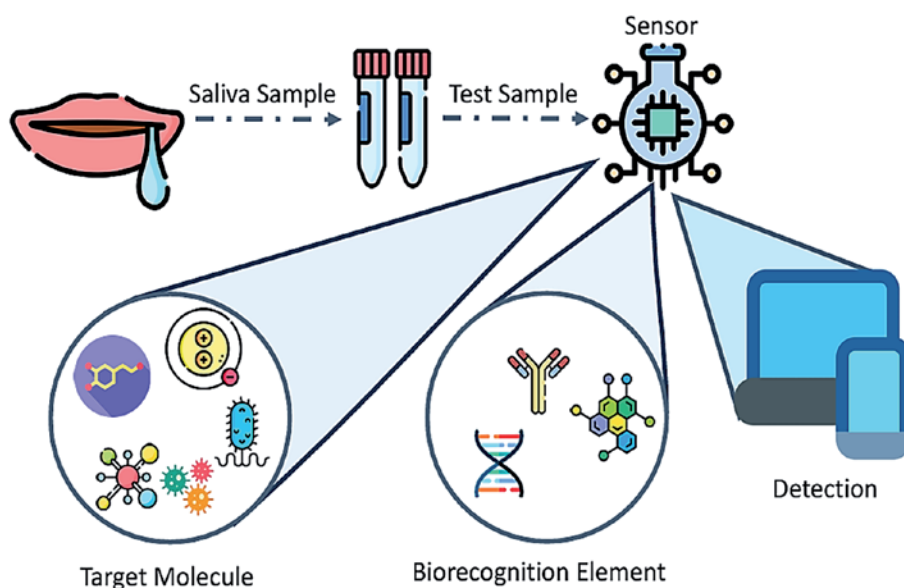


Figure 1.
Illustration of detection of pathogen biomarkers in saliva by the nanoparticle-based biosensor.

for the detection of targets owing to their unique and enhanced physico-chemical and biological properties.

2. Nanoparticles-based biosensors for oral pathogens

2.1 Nanoparticles-based biosensors for bacteria in saliva

Recently, molecular techniques such as polymerase chain reaction [28] and enzyme-amplified methods (e.g., enzyme-linked immunosorbent assay) have progressively supplanted traditional plating and culture [29] owing to their superior sensitivity and specificity. Traditional techniques for bacterial identification, such as colony counting, fluorescence microscopy, PCR-based methods, and immunological assays, are highly specific and accurate; nonetheless, they are regarded as time-consuming (>24 hours), need specialist technical equipment, and involve considerable sample preparation [30]. However, the requirement for costly instruments and specialized operation constrains their practical utilization. Consequently, the straightforward, rapid, and direct identification of oral infections is a critical concern for public health. This non-invasive diagnosis of pathogen biomarkers from saliva samples is simple, easy, and low-cost.

The oral microorganisms create intricate, surface-adhered aggregates known as biofilms, which initially sustain a homeostatic balance [31]. However, disruption of the microbiome causes dysbiosis, which may lead to oral pathogenic diseases [32, 33]. Dental caries is the most prevalent dental disease [34]. Advanced caries can lead to pulpitis, apical periodontitis, and potential tooth loss [35]. As caries is a bacteriogenic infection, identifying cariogenic bacteria is significant for prevention and diagnosis. In most cases, acidic substances can inflict irreparable harm on dental tissue, preventing its restoration to its previous condition by reparative treatment. Consequently, the early prevention of dental caries is essential for preserving oral health and alleviating patient discomfort [36]. The World Health Organization's 2022 report estimates the global average prevalence of irreversible dental caries at 29% [37]. Extensive epidemiological and biochemical research has established *Streptococcus mutans* (*S. mutans*) as a primary causal agent of human dental caries [38, 39]. Reports indicate that when *S. mutans* levels in saliva exceed 10^5 CFU mL⁻¹, there is a considerable risk of substantial harm to dental hard tissues, perhaps hastening the progression of dental caries [40–42].

Li et al. [43] developed a portable carboxyl-modified multi-walled carbon nanotube (c-MWCNTs)-based biosensor for the swift detection of *S. mutans* in saliva with exceptional sensitivity, selectivity, and robust stability for prolonged practical use. *S. mutans*-specific DNA aptamers and *S. mutans* imprinted polymers (SIPs) served as the primary sensing elements for the creation of *S. mutans*-specific cavities and recognition surfaces. Methylene blue served as redox probes and was integrated into the SIP networks to directly generate electrical signals during the *S. mutans* binding reaction for sensitive label-free amperometric detection. Research [44] indicates that DNA-functionalized nanozymes enable swift, label-free, and highly sensitive direct colorimetric detection of *S. mutans* with superior differentiation from other dental bacteria. In the experimental design of the study, the peroxidase-like activities of three Fe₃O₄ bioconjugates were optimized by evaluating the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of H₂O₂, with absorbance recorded at 652 nm. The effect of ssDNA concentrations (0–2 µM) and pH levels (4–8) on catalytic activity

was evaluated to determine ideal settings for subsequent research. For the quantitative assessment of *S. mutans*, bacterial suspensions (10^9 to 10 CFU mL⁻¹, including a negative control) were treated with bioconjugates ($10\text{ }\mu\text{g mL}^{-1}$) in HAc-NaAc buffer (pH 4) for 30 minutes. For selectivity tests, bioconjugates were incubated with *Fusobacterium nucleatum* (*F. nucleatum*), *Porphyromonas gingivalis* (*P. gingivalis*), and *Lactobacillus acidophilus* (*L. acidophilus*) (10^9 CFU mL⁻¹) under identical conditions. For application experiments, *S. mutans* was resuspended in artificial saliva at high (10^9 CFU mL⁻¹), medium (10^6 CFU mL⁻¹), and low (10^3 CFU mL⁻¹) concentrations, with saline used as a negative control. After incubation, reactions with TMB and H₂O₂ were performed at 37°C and 140 rpm for 15 minutes in the absence of light. After centrifugation, the supernatants were assayed for absorbance at 652 nm using a microplate reader, with all measurements performed in triplicate.

A high quantity of hydrogen sulfide (H₂S) in the oral cavity is related to the advancement of oral diseases, including halitosis, gingivitis, and periodontitis [45]. The elevation of H₂S levels due to the proliferation of pathogens and anaerobic and proteolytic bacterial metabolism can cause periodontal disorders [46]. A study [47] introduced a paper-based colorimetric test for the detection of H₂S molecules. This H₂S-sensing paper demonstrated superiority compared to lead acetate paper. The H₂S concentrations are generally generated by some oral bacteria, including *Eikenella corrodens* (*E. corrodens*), *Streptococcus sobrinus* (*S. sobrinus*), *Streptococcus mutans* (*S. mutans*), and *Lactobacillus casei* (*L. casei*).

P. gingivalis is considered a significant pathogen that proliferates during bacterial dysbiosis [48, 49]. *Aggregatibacter actinomycetemcomitans* (Aac) contributes virulence factors, including leukotoxin and lipopolysaccharide, which induce proinflammatory mediators. Aac is known as a primary contributor to endocarditis, soft tissue infections, abscess development, and periodontitis [50]. *Actinomyces naeslundii* (*A. naeslundii*) and *Streptococcus oralis* (*S. oralis*) contribute to the maintenance of a healthy oral cavity and respiratory tract. Both bacterial species are regarded as early colonists in oral biofilms [51]. Au NPs demonstrate unique localized surface plasmon resonance (LSPR) based on their morphology (size and shape). The LSPR shift is observed as a color change in samples, and it is influenced by the aggregation state of Au NPs, particularly in colorimetric detection. Consequently, the accumulation of smaller Au NPs at the surface of bacteria can result in a visible color change that is discernible to the naked eye and can be quantitatively assessed using a camera [52]. The sizes, shapes, functionalizations, and surface charges of Au NPs play a critical role in reacting with negatively charged bacterial surfaces [52]. For instance, (1-hexadecyl) trimethylammonium bromide (CTAB) and mercaptoethylamine (MEA) capped Au NPs were employed to identify some oral bacteria, including Aac, *A. naeslundii*, *P. gingivalis*, and *S. oralis*. The optical responses were quantitatively examined via UV-visible absorbance measurements and RGB analysis. The other study created a swift and economical smartphone-based dual-emission bacterial sensor utilizing nano-peptide conjugates, capable of selectively identifying two oral bacterial species *Streptococcus salivarius* (*S. salivarius*) and *Streptococcus Sanguinis* (*S. sanguinis*) in actual samples with commendable sensitivity [53]. A metal-NP-supported nanozyme (MNN) sensor array was also employed to identify five oral bacteria with distinct characteristics [53].

Due to its extensive surface area and superior physico-chemical characteristics, two-dimensional (2D) molybdenum disulfide (MoS₂) demonstrated potential as an effective substrate for the incorporation of metal NPs, owing to its appropriate surface diversity [48, 49]. In light of this, they devised a straightforward approach to synthesize a range of nanozymes, wherein diverse metal NPs were affixed to the hierarchical

MoS₂ surface on polypyrrole (PPy) nanotubes as encoders through *in situ* reduction, attributed to the inherent reducibility of MoS₂. Au, Ag, and Pd NP were deposited on the PPy@MoS₂ nanostructures to serve as colorimetric probes in the development of a three-component cross-reactive sensor array. The periodontal infections associated with *P. gingivalis* and gingipains were detected with gingipain-specific peptide-conjugated magnetic nanobeads and anchored to an Au NP-based biosensing platform. This assay exhibits great sensitivity and specificity with the detection of 49 CFU mL⁻¹ of *P. gingivalis* within 30 seconds [54]. Wignarajah et al. identified *P. gingivalis* proteases with multiple colorimetric assays for the detection of chronic periodontal disease. The limit of detection (LOD) was obtained as 1 pg/mL for Human Neutrophil Elastase (HNE) and 100 fg/mL for cathepsin G in less than 30 seconds [54]. Hoyos-Nogués et al. [55] employed a peptide-integrated assay to detect *S. sanguinis*, a bacterium associated with inflammatory disorders related to dental implants. The assay underwent testing in artificial saliva with various amounts of *S. sanguinis* (10 to 10⁵ CFU mL⁻¹) with an LOD of 8.6 × 10² CFU mL⁻¹ bacterial concentration.

A study [56] presented a matrix-insensitive sensor array method for detecting bacteria, fungi, and viruses through the separation of signal peptide-aggregated NPs using thiolated polyethylene glycol (HS-PEG) polymers. Research demonstrated that HS-PEGs of varying sizes had distinct capabilities in segregating citrate-coated AuNPs from silver NPs (Ag NPs) assemblies. This sensor array utilized the separation capabilities of HS-PEGs to differentiate *P. gingivalis*, *Fusobacterium nucleatum* (*F. nucleatum*), and *Candida albicans* (*C. albicans*) in water and saliva with a 90% confidence level by linear discriminant analysis (LDA).

Pseudomonas aeruginosa (*P. aeruginosa*) is an opportunistic pathogen implicated in numerous nosocomial infections [57]. *P. aeruginosa* was recognized as the second most common pathogenic bacteria isolated from patients with hospital-acquired pneumonia (HAP) [58]. A fast, precise, and specific colorimetric biosensor utilizing magnetic nanoparticles (MNPs) was developed to detect clinical *P. aeruginosa* strains. These biosensors relied on quantifying the proteolytic activity of *P. aeruginosa* upon proteolysis of the corresponding substrate. *Mycoplasma pneumoniae* (*M. pneumoniae*) is a contagious bacteria and the primary etiological agent of pneumonia, particularly in school-aged children [59]. A study employed Au NPs-based colorimetric biosensing to detect the specific target DNA of *M. pneumoniae* [60]. Single-stranded DNA was combined with ideal target DNA to form double-stranded DNA, which did not adsorb on the surface of Au NPs; then Au NPs were not stabilized and were prone to aggregation at elevated ionic strength (from 100 mM). This method facilitated straightforward, efficient, and precise detection of *M. pneumoniae* target DNA with enhanced discrimination and without the necessity for intricate equipment.

The graphene-integrated biosensor has been developed for near-field communication (NFC) data transmission [61]. Graphene is applied to bio-resorbable silk used as a substrate via transfer printing, succeeded by e-beam evaporation of Au to fabricate the interdigitated electrode and for NFC data transmission. The 100 cells of *Helicobacter pylori* (*H. pylori*) were detected while retaining a linear response within the range of 10² to 10⁶ cells.

2.2 Nanoparticles-based biosensors for viruses in saliva

The prevention and treatment of viral infectious diseases rely on promptly and accurately detecting viruses. Various molecular methods are available for the detection of viruses in clinics, including polymerase chain reaction (PCR), enzyme-linked

immunosorbent assay (ELISA), and fluorescence-based serological tests [62–64]. Nonetheless, the utilization of the majority of these strategies is constrained by intricate operations, elevated costs, and diminished sensitivity.

Jin et al. introduced a microfluidic system (SLIM) in order to detect bacteria and viruses. The technique is evaluated using 10 saliva samples that have previously received a positive diagnosis of infection via real-time PCR caused by herpes zoster (78.6% sensitivity) [65]. Xue et al. developed a multicapillary glass plate immunoassay to both rapidly and sensitively detect viral antibody infections. Comparable outcomes were achieved by utilizing the technique with swabs and plasma samples [66]. The peptide-based biosensor with Zaitouna et al. employed an electrochemical property that was discussed to detect antibodies of anti-HIV with an LOD of 1 nM [67].

The extraordinary coronavirus 2019 (COVID-19) has resulted in significant disruptions to public health and economies in the world [68]. Various studies have been introduced in the fabrication of biosensors for the detection of SARS-CoV-2 [69, 70]. For instance, Ag NPs used as a surface-enhanced Raman Spectroscopy (SERS) substrate were combined with acetonitrile and calcium ions for the swift identification of the new coronavirus. Acetonitrile can amplify the calcium-induced hotspots of Ag NPs and markedly improve their stability [70]. It also disclosed the extremely sensitive SERS signals of the virus. The other study reported N-acetyl neuraminic acid (sialic acid)-functionalized Au NPs (SA-Au NZs) as a novel and effective nano(e)zymes rapid, precise, and colorimetric detection of the SARS-CoV-2 virus [71]. The SA-Au NZs were also utilized for the detection of influenza A (H1N1), influenza A (H₃N₂), and influenza B with unique peroxidase-like activities. UV-visible spectroscopy was employed to observe and document the results, alongside visual detection. They displayed a color change from red to purple in the presence of SARS-CoV-2, which is well consistent with a redshift in the absorbance spectrum. A study developed a straightforward and highly sensitive technique for detecting SARS-CoV-2 in human saliva following concentration with MNPs attached to polyethylene glycol (PEG) [72]. This novel approach facilitates the efficient and dependable concentration of the virion in under 60 minutes, hence enhancing the aggregated sensitivity of the SARS-CoV-2 assay. The spike antigen of SARS-CoV-2 was also detected with the aggregation state of Au NPs via antibody-antigen interactions. Both color changes from red to purple were observed with the naked eye and peak shift in absorbance spectrum recorded by UV-Vis spectrophotometry were exhibited owing to aggregation of Au NP [73].

Furthermore, electrochemical detection was accomplished by a commercial and disposable screen-printed gold electrode, eliminating the need for any electrode preparation or modification. Sialic acid (SA) on the surface of lung epithelial cells is regarded as a primary binding target for numerous respiratory viruses via hemagglutinin (existing on the virus surface as a viral surface protein) [74]. The SA-Au NPs were prepared for detection of SARS-CoV-2, influenza B virus, and Middle East respiratory syndrome-related coronavirus (MERS) with their optical properties [75]. The experimental strategy of the thesis involved the preparation and characterization of sialic acid-gold nanoparticles (SA-Au NPs) and their use for virus detection. SA-Au nanoparticles were synthesized by mixing sialic acid with chloroauric acid in an alkaline solution, followed by stirring at elevated temperatures until a color shift to dark red indicated nanoparticle formation. SA-Au NPs were evaluated by UV/VIS spectroscopy to confirm surface plasmon resonance (524–531 nm), dynamic light scattering to assess particle size and zeta potential, FTIR to verify conjugation, and SEM/TEM

to determine morphology and size. Nasopharyngeal swab specimens from patients diagnosed with influenza B, MERS-CoV, or SARS-CoV-2 (confirmed by RT-PCR) were immersed in solutions of SA-Au nanoparticles. After 20 minutes of incubation, visual inspections and recordings of color changes in the solution and swabs were performed, indicating the potential of the nanoparticles for colorimetric detection of viral particles. The influenza virus attacks the mucosa of the respiratory system, occasionally resulting in severe disease and elevated mortality, often accompanied by complications like pneumonia [76]. A study created a very sensitive and convenient approach for identifying the influenza virus utilizing modified end-point melt curve analysis of an RT-qPCR SYBR Green technique and influenza virus-binding sugar chain-immobilized Au NPs (SC Au NP). The Checkin group created [77] an rGO/MoS₂-modified glassy carbon electrode (GCE) platform in order to detect human papillomavirus type 16 (HPV-16 L1) serving as a marker for genital cancer. This biosensor exhibits a detection limit of 0.1 ng/mL, facilitating precise and accurate screening of saliva samples. The recovery values for human saliva testing range from 97% to 105%, demonstrating effective performance. The sensor exhibits a mere 5% reduction in performance following 1 month of dry storage. Nonetheless, it exhibits an inter-assay reproducibility of 9.3% in the relative standard deviation (RSD), indicating inadequate consistency during fabrication and the possibility of imprecise results. The sensor demonstrated selectivity by detecting HPV-16 L1 without interference from ovalbumin (OVA) or human serum albumin (HSA). A biosensor created by Yoo et al. [78] employed shellac-based thermally reduced graphene oxide (TrGO) for the detection of viral agents. The sensor demonstrated similar detection limits in both real saliva and buffer solutions, retaining 90% of its performance after a 2-week period. The method demonstrated selectivity against adenovirus, influenza B virus, and MS₂ bacteriophage, resulting in minimal interference during electrochemical impedance spectroscopy (EIS) analysis.

3. Conclusion and future perspectives

This chapter provides a thorough overview of nanoparticle-based biosensors for detecting bacterial and viral infections in saliva. Saliva is a viable bodily fluid for assessing illnesses and overall health status. Human saliva is utilized for biomarker detection owing to its accessibility, non-invasive collection method, and richness in diverse analytes. Portable technologies and non-invasive diagnostic tests for diseases are an increasingly prominent emphasis in the therapeutic domain. They are distinguished by their simplicity and efficiency in delivering data promptly and at a lower cost than conventional laboratory testing. These characteristics render them an optimal substitute for remote or inaccessible areas where establishing a conventional laboratory is not economically viable. They are easily transportable and suitable for mobile diagnostic locations due to their portability and low energy requirements. Conversely, the potential of current development, like smartphones equipped with high-resolution cameras for processing the information presents a precious resource that remains underutilized and possesses significant potential. This procedure will significantly streamline the workflow for both patients and professionals, as it eliminates the necessity for frequent in-person hospital visits. These biomarkers assist physicians in the early detection and diagnosis of diseases, facilitate therapeutic decision-making, and enable the monitoring of disease progression and treatment efficacy. Moreover, both parties will reduce expenses related to travel, labor, and

system administration, while physicians will be equipped to deliver prompt diagnoses and subsequent treatment informed by previously gathered data during routine operations.

Nonetheless, many analytes in saliva have a poor correlation with blood or urine due to factors such as partitioning rates, chemical transformations, and the effect of food consumption. Bleeding into the oral cavity can cause misleading signals due to contamination from microvascular leakage or biomolecular transfer via gingival crevicular fluid. The presence of protein and food debris in saliva affects the functionality of wearable biosensors by causing non-specific adsorption, biofouling, and reduced device reliability. Further research is needed to integrate saliva-based diagnostics into routine practice to overcome these challenges. Standardization and validation of saliva and biomarker-collecting methodologies are essential. Furthermore, innovative tests and tools must be developed at a commercially viable standard. The anticipated introduction of saliva analytical equipment with enhanced sensitivity and specificity, together with the establishment of definitive protocols, will render saliva diagnostics a tangible reality in the forthcoming years.

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Conflict of interest

The authors declare no conflict of interest.

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Saliva, as an innovative and accessible diagnostic fluid, is transforming research and clinical practice in both dentistry and medicine. This volume offers a comprehensive overview of advances in the use of salivary biomarkers and the evolving characteristics of this fluid, emphasizing their value in early diagnosis, monitoring, therapeutic response, and the prevention of a wide range of oral and systemic diseases. Key topics include the oxidant–antioxidant balance, xerostomia, hyposalivation, periodontal disease, glandular disorders, and the detection of potentially malignant lesions. The book also explores emerging approaches such as nanoparticle-based biosensors and novel technologies in salivary gland surgery. In addition, it presents new perspectives on the role of neuropeptides and cytokines in assessing stress and resilience, underscoring the significance of saliva as a window into complex biological processes. By bringing together the most up-to-date evidence and practical applications in a single volume, this book highlights the non-invasive, safe, and increasingly reliable nature of saliva as a source of clinical information. Aimed at researchers, health professionals, and students, this book serves as an essential guide to understanding the expanding potential of saliva as a diagnostic fluid and as a cornerstone in the future of personalized medicine and preventive dentistry.

Sergio Alexandre Gehrke, Dentistry Series Editor

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