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ORAL MUCOSAL HUMAN PAPILLOMAVIRUS AND EPSTEIN-BARR VIRUS RATES IN PATIENTS WITH DRY MOUTH AND/OR SJÖGREN'S SYNDROME

PhD thesis

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List of Abbreviations

ACPA – anti-citrullinated protein autoantibody
ACR/EULAR – American College of Rheumatology and the European League Against Rheumatism
ANA – antinuclear antibodies
BAFF - B-cell activating factor
BL – Burkitt's lymphoma
CMV – cytomegalovirus
CNS – central nervous system
COVID-19 – coronavirus disease 2019
DED – dry eye disease
DLBCL – diffuse large B cell lymphoma
DNA – deoxyribonucleic acid
E – early
EBV – Epstein-Barr virus
EGF – epidermal growth factor
FLS – focal lymphocytic sialadenitis
FS – focus score
GERD - Gastroesophageal reflux disease
Gy - Grey
HCV – hepatitis C virus
HHV 6 – human herpesvirus 6
HHV 7 – human herpesvirus 7
HAs – hisptopathological alterations
HIV – human immunodeficiency virus
HIV-SGD – HIV-associated salivary gland disease
HLA – human leukocyte antigen
HLA-DR - Human Leukocyte Antigen – DR isotype
HPV – human papillomavirus
HTLV-1 - human T-lymphotropic virus type 1
INF – interferon

L – late

MS – multiple sclerosis

NK/TL – Natural killer cell/T-cell lymphoma

OPSCC – oropharyngeal squamous cell carcinoma

OSCC – oral squamous cell carcinomas

PCR – polymerase chain reaction

pSS – primary Sjögren's Syndrome

RNA – ribonucleic acid

SLE – systemic lupus erythematosus

SS – Sjögren's Syndrome

SSA – Sjögren's syndrome-associated antigen A

SSB – Sjögren's syndrome-associated antigen B

TLR – toll-like receptors

UWS – unstimulated whole saliva

VZV – varicella-zoster virus

1. Introduction

Saliva is a complex biofluid that sustains oral and systemic health. Disruption of salivary quantity or quality (salivary hypofunction or xerostomia) therefore undermines mucosal integrity and host defenses, precipitating dysphagia, dysarthria, candidiasis, rampant caries, and substantial reductions in quality of life (1-4).

The aetiology of dry mouth is multifactorial, encompassing autoimmune disease, ageing, medications, radiotherapy and infectious agents. Viral pathogens are of particular interest because many show tropism for salivary gland tissue, can be shed in saliva, and may transform the oral cavity into both a reservoir and a conduit for transmission. Human papillomavirus (HPV) is implicated in a spectrum of oral lesions and is a major driver of oropharyngeal squamous cell carcinomas, while Epstein-Barr virus (EBV) is linked to oral and systemic lymphoid and epithelial malignancies and has been proposed as a cofactor in immune dysregulation. Emerging data also suggest associations between EBV and salivary dysfunction, including features overlapping with Sjögren's syndrome (SS) (5-10).

Despite these links, critical gaps remain. A diminished antimicrobial capacity and a decrease in the cleansing action of saliva may facilitate the colonisation of the oral mucosa by pathogenic organisms, viral and bacterial infections of the mucosa might occur more frequently. HPV and EBV are capable of infecting epithelial cells, and a reduction in saliva production may heighten mucosal susceptibility to these viral infections. It is unclear whether the prevalence of oral HPV and EBV is elevated among patients with orofacial sicca or Sjögren's syndrome in populations at high risk for vir6us-associated head and neck disease, nor is it known whether these viruses contribute to salivary gland histopathology or the initiation of autoimmune inflammation. Systematic investigation of HPV and EBV presence in the oral epithelium and salivary tissues of individuals with dry mouth could therefore clarify pathogenetic relationships, inform risk stratification in regions with high HPV-related oropharyngeal cancer burden, and identify novel avenues for diagnosis and intervention (11).

1.1. Production and role of saliva

Saliva, often referred to as whole or mixed saliva, is a continuous fluid secreted within the oral cavity, serving to bathe the teeth and mucosal surfaces. It comprises a complex

mixture predominantly produced by the major salivary glands (parotid, submandibular, and sublingual), alongside a large number of minor salivary glands (approximately 600–1000 distributed throughout the oral cavity), and contains gingival crevicular fluid (2, 3). The maintenance of oral health and the proper functioning of systemic physiological processes depend on the adequate secretion of saliva in both quantity and quality. This fluid is synthesised and released by the salivary system, which encompasses the major and minor salivary glands (3, 4, 12-14). Saliva is a biologically active fluid with diverse functions, broadly categorised as protective, nutritional, and related to speech. In the context of protection, its roles include hydration of oral tissues, mechanical cleansing, antimicrobial activity, maintenance of mucosal integrity, acid buffering, and facilitation of enamel remineralisation. The antimicrobial action of saliva is mediated by components such as lysozyme, peroxidase, secretory immunoglobulin A, and histatinss, which act collectively to inhibit microbial adhesion and proliferation. Furthermore, the presence of growth factors—most notably epidermal growth factor (EGF) and transforming growth factor—in saliva contributes significantly to epithelial regeneration, cellular differentiation, and wound repair (5, 15). In addition, saliva contains a range of components including microbial populations, desquamated epithelial cells, and residual food debris (1, 15). As the initial digestive secretion encountered in the alimentary tract, saliva is produced in response to gustatory, olfactory, and mechanical stimuli associated with food. It facilitates the oral phase of digestion by initiating enzymatic breakdown, enabling mastication, bolus formation, and deglutition, while also beginning the digestion of starches and lipids. (2) Beyond its digestive function, saliva serves as a solvent for tastants, thereby modulating taste perception. It also plays a critical role in the articulation of speech. (15)

Normal stimulated salivary flow ranges from 1.5–2.0 mL/min, while unstimulated flow typically measures between 0.3 and 0.4 mL/min (1, 5). Unstimulated saliva normally has an osmolarity of 50–70 mOsm/kg and a pH between 6.0 and 7.0, with a buffering capacity of 3.1 to 6.0 mmol H^+/L (3). Hyposalivation is defined as a stimulated flow rate of ≤0.5–0.7 mL/min or an unstimulated rate of <0.1 mL/min, assessed with the patient in an upright seated position (16, 17).

1.2. Histological structures of the minor salivary glands

Salivary glands are essential for maintaining oral homeostasis through the production of saliva (18). The salivary gland parenchyma (glandular secretory tissue) consists primarily of acinar cells, ductal cells, and myoepithelial cells (18). Saliva is secreted by the acinar lobules—also known as the end-pieces of the gland—composed of acinar cells that are classified according to the type of secretion they produce: serous or mucinous. These acini display distinct cellular architecture and intracellular components (3, 4, 19, 20). The ductal system plays an active role in modifying the initially hypotonic primary saliva into an isotonic fluid, a process achieved through ionic exchange between the ductal cells and the salivary fluid. This function is mediated by a highly branched ductal network, including intercalated, striated, and excretory ducts. Myoepithelial cells, associated with the glandular parenchyma, are contractile elements that play a crucial role in facilitating secretion. These cells encase the secretory units, lying between the acinar basement membrane and the surrounding mesenchyme. They contribute to structural stability, prevent back-pressure damage after secretion, and inhibit the back-diffusion of saliva under external mechanical stress (14). In addition to these core cell types, the salivary gland also contains fibroblasts, immune cells, neuroendocrine cells, endothelial cells, and pericytes (3, 18).

Nearly all cell types within the salivary glands receive innervation from both sympathetic and parasympathetic nervous systems. The major salivary glands are supplied by branches of the external carotid artery, while the vascular supply of the minor salivary glands derives from regional blood vessels (4, 19). A cross-section of a salivary gland is shown in **Figure 1.** (4)

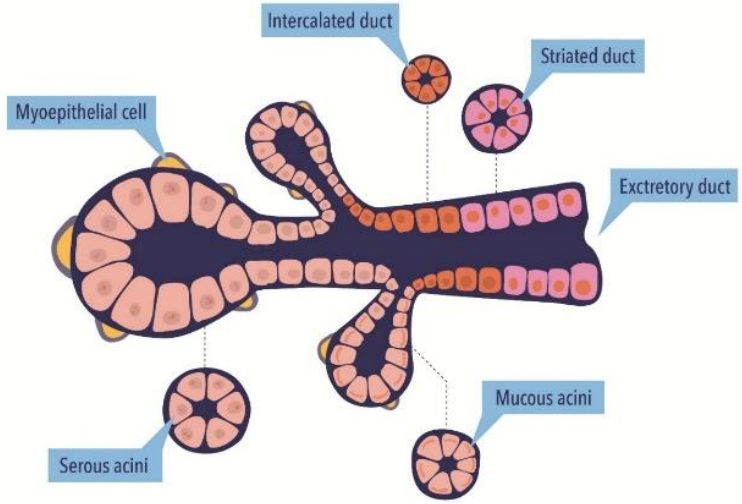

Figure 1. Cross-section of salivary gland with different acini and ducts

The serous secretory portion is composed of 8–12 pyramid-shaped cells, each with a round, centrally positioned nuclei. These cells are arranged into spherical structures that form a narrow central apical lumen. A large number of the molecular components of serous saliva are stored within secretory granules in the apical cytoplasm. Mucous acini consist of tubular secretory structures, with secretory cells whose flattened nuclei are displaced towards the basal surface due to the accumulation of mucin granules in the apical cytoplasm. The lumen of the secretory acini is continuous with the lumen of the intercalated ducts. Intercalated ductal cells have centrally located nuclei and limited cytoplasm containing small secretory granules apically. Microvilli project into the lumen, therby increasing the surface area for exchange. Striated ductal cells are characteristically columnar with centrally located nuclei. These cells control electrolyte secretion and reabsorption by mediating bidirectional transport between the lumen and the extracellular space, thereby modifying saliva from isotonic to hypotonic. This energy-dependent process requires a high number of mitochondria, which are concentrated along the basolateral membrane. The final collecting ducts are the interlobular excretory ducts, which are lined with pseudostratified epithelium comprising various types of columnar epithelial cells (4).

1.2.1. Minor salivary gland biopsy in diagnostics

Histopathological examination of minor salivary glands from the lower lip remains a cornerstone in the diagnostic evaluation of SS (21, 22). The histological features of the minor salivary glands are representative of those observed in major salivary glands. Owing to their superficial location beneath the oral mucosa, minor glands are particularly amenable to biopsy, enabling tissue sampling with minimal risk and trauma (13). The labial minor salivary glands are preferred for histological sampling due to their accessibility and their anatomical separation from the oral mucosa by a thin fibrous connective tissue layer. Following administration of local anaesthesia the lower lip is dried with sterile gauze, and the emergence of saliva droplets serves as a visual guide to identify the biopsy site. A histological sample is obtained using a specially designed biopsy instrument—an adapted Desmarres-Chalazion forceps commonly used in ophthalmological procedures for stye removal. The fenestrated side of the active end permits herniation of glandular lobules, while the solid opposing side compresses blood

vessels, reducing intraoperative bleeding. The retention screw in the handle stabilises the tissue and provides continuous compression. A 1–1.5 cm linear incision is made horizontally, followed by dissection through the connective and adipose tissues to expose the minor salivary glands. Between five and seven lobules are harvested and preserved in 10% formaldehyde solution within Eppendorf tubes. Wound closure is performed with 2–3 simple interrupted sutures. Sutures are removed after a one-week healing period, during which patients are advised to use chlorhexidine mouth rinse and maintain enhanced oral hygiene. During histopathological evaluation after fixation and haematoxylin-eosin staining, sections of 2–4 μm thickness are examined at magnifications ranging from 40x to 250x (21, 22).

1.3. Xerostomia and salivary hypofunction

Xerostomia, or the sensation of oral dryness, is inherently subjective. It becomes an objective clinical condition—termed hyposalivation—when the unstimulated whole saliva flow rate falls to 0.1 mL/min or below (23, 24). Nevertheless, some individuals may perceive their mouth as dry even when salivary secretion is within normal physiological limits. (17, 25)

Oral dryness may be symptomatic of, or secondary to, an underlying systemic disorder (1, 6, 23, 24, 26).

1.3.1. Evaluation of xerostomia

The diagnostic process begins with a detailed medical history. Key questions should address symptoms such as difficulty swallowing, chewing, or speaking, as well as any alterations in taste. Patients with dysfunctional salivary glands may also struggle with consuming dry, crunchy, acidic, or spicy foods. A comprehensive assessment should include a review of medications, prior medical diagnoses—particularly involving radiotherapy to the head or neck—and relevant lifestyle or social history (17).

A variety of questionnaires have been developed to detect and assess the severity of xerostomia (25, 27-31). However, evaluating this symptom poses challenges, not least because it relies on subjective patient reporting, and also due to the wide range of possible questions that may be employed. Among these tools, the Xerostomia Inventory is a widely adopted 11-item summated rating scale that provides a single score representing the chronic severity of oral dryness (17, 25). A shortened version, the Summated Xerostomia

Inventory Dutch Version, retains five key items and simplifies response options to three levels (17, 25, 32).

Saliva collection may be performed using gland-specific methods or via whole (mixed) saliva sampling, which reflects secretion from all salivary glands. The latter is more commonly used due to its non-invasive nature. Both unstimulated and stimulated samples can be obtained for diagnostic purposes (16, 32).

To investigate potential systemic causes of dry mouth, laboratory testing is often required. For suspected SS, serological evaluation should include anti-SSA/Ro and anti-La antibody testing (33). Imaging modalities—such as sialography, technetium-99m scintigraphy, computed tomography, magnetic resonance imaging, and ultrasound—can provide further diagnostic information on salivary gland structure and function (20, 34). In cases where autoimmune involvement is strongly suspected, a minor salivary gland biopsy, typically taken from the lower lip, may also be warranted (17, 22).

1.3.2. Epidemiology of dry mouth

Dry mouth affects between 5.5% and 46% of the population and is most prevalent among older adults. Impaired oral health is a component of the geriatric syndrome, characterised by multi-morbidity, polypharmacy, frailty, disability, and care dependency (3, 17, 26).

Dry mouth is also more common in women than men, with female sex recognised as a risk factor. Epidemiological studies consistently report a higher prevalence of dry mouth symptoms in women across all age groups. This remains true even when controlling for medication use: non-medicated women still report more dry mouth than their male counterparts (5, 26).

1.3.2.1 Epidemiology of dry mouth in Hungary

Márton et al. examined the prevalence of dry mouth and related oral and extraoral dryness symptoms in Hungary. Their aim was to explore associations between these symptoms and the unstimulated whole saliva (UWS) flow rate, and to determine correlations between UWS levels and oral health status. They found that 34% of respondents experienced some degree of subjective oral dryness or sicca symptoms, and 7.5% were severely affected—suggesting a significant public health concern in Hungary (23).

In a recent Hungarian study, Dézsi et al. collected data over a seven-year period. Among 1,076 patients presenting with sicca symptoms, 383 (35.6%) had hyposalivation (UWS ≤ 0.1 ml/min) (35). By comparison, Márton et al. found only 3.8% of randomly selected individuals with hyposalivation. The stark difference between the two studies highlights that objectively measurable dry mouth is much more prevalent in patients presenting with sicca symptoms, such as dry mouth and/or dry eyes (35).

1.3.3. Symptoms associated with dry mouth

Salivary hypofunction may have profound implications for oral and general health (5, 17). Individuals with dry mouth frequently present with symptoms such as impaired mastication, dysphagia, and dysarthria. Clinically, this condition is often accompanied by sensations of burning mouth, halitosis, dysgeusia, dry, shiny and glass-like appearance of the oral mucosa, atrophic or smooth gingiva, a depapillated, fissured or lobulated tongue, fissured lips. Increased incidence of oral candidiasis, and a heightened risk of dental caries, in the form of multiple cervical caries, debris accumulation on the palate; and the phenomenon of the dental mirror's adherence to the tongue or buccal mucosa can also be observed. Saliva is frothy, and minimal or absent salivary pooling in the floor of the mouth is present in this condition (17, 32). Over time, such manifestations may significantly impair the individual's quality of life (17). Clinical intra- and extraoral symp6toms in hyposalivation are summarised in **Table 1.** (1, 5, 15, 26, 36, 37)

Table 1. Dry mouth associated intraoral and extraoral symptoms

Intraoral symptoms	- furrowed, desiccated, sticky tongue, erythematous tongue - oral mucositis, denture stomatitis, atrophic, glazed, dry and red mucosa - mucosal ulcerations - cheilitis angularis, dry, cracked lips - candidiasis (erythematous/pseudomembranous) - adherence of food and dental plaque to dental surfaces - dental erosion - dental caries, development of atypical dental decay (cervical, incisal, cusps tips, radicular lesions); rapidly progressing (rampant) and aggressive

	- dental erosions - rapid formation of dental plaque and calculus, increased plaque index score, loss of alveolar bone, increased probing depth - burning mouth and tongue, oral soreness - halitosis - taste disturbances (dysgeusia, hypogeusia) - difficulty in wearing removable dentures
Extraoral symptoms	- stinging, burning or scratchy sensation in the eyes, sensitivity to light, eye redness, a sensation of having something in the eyes, stringy mucous in/around the eyes, decrease/lack of lacrimal gland function, severe tear deficiency - dry nose, dry throat, persisting hoarseness and dry cough, nose bleeding - pharyngitis, laryngitis - dysphagia, malnutrition, constipation/diarrhoea, weight loss - gastroesophageal reflux disease, heartburn, nausea - oesophagitis, oesophageal dysmotility - bronchitis - vaginal dryness - dry and itchy skin - psychosocial problems: impaired quality of life, depression, social isolation - difficulty in speech - sleeping disorders (insomnia, sleep deprivation, fragmented sleep)

1.3.4. Aetiology of dry mouth

The aetiology of oral dryness is multifactorial. Systemic conditions—such as endocrine or autoimmune diseases—alongside bacterial and viral infections, granulomatous diseases, other systemic causes and genetic disorders, neurological-, gastrointestinal diseases, ageing, polypharmacy, head and neck irradiation, and certain lifestyle factors have all been implicated in the onset of dry mouth and the associated reduction in the protective functions of saliva. A Hungarian study by Demeter et al. examined smoking

effects on salivary flow rates, and their results highlighted that high-intensity smoking may cause oral dryness and a reduction in unstimulated whole saliva (UWS) flow rate (38). Malnutrition, vitamin and mineral deficiencies, eating disorders such as bulimia and anorexia nervosa, and reliance on liquid diets also contribute to the development of oral dryness (1, 3, 5, 15, 17, 26, 36, 37) .Aetiology of dry mouth is detailed in **Table 2.** (3, 5, 7, 15, 17, 24, 26, 36, 38-51)

Table 2. Aetiology of dry mouth

Table shows the different ethiological factors: diseases and symptoms, drug interactions and conditions that may lead to dry mouth: either xerostomia or hyposalivation or both entities.

AETIOLOGY		**ORAL SYMPTOMS, (XEROSTOMIA AND/OR SALIVARY HYPOFUNCTION)**
ENDOCRINE DISEASES	***Diabetes mellitus***	xerostomia (dehydration, polyuria), burning mouth, taste disturbances and hyposalivation
	Hyper-and hypothyroidism	dry mouth
	Cushing's syndrome	
	Addison's disease	
AUTOIMMUNE DISEASES	***Autoimmune thyroid diseases***	dry mouth
	Sjögren's Syndrome	mononuclear cell infiltration of exocrine glands, salivary hypofunction, dry mouth
	Systemic lupus erythematosus	oral dryness, ulcers, mucosal pigmentation, glossodynia, cleft tongue, cheilitis

	Rheumatoid arthritis	*dry mouth, decreased peroxidase activity, reduced salivary flow and protein levels, and a lower specific content of secretory immunoglobulin A*
	Systemic sclerosis	*dry mouth, fibrosis affecting the excretory ducts, acini of the salivary glands, microstomia*
	Autoimmune myositis	*dry mouth, hyposalivation and dysphagia, fibrosis affecting the salivary glands*
	Primer biliary cirrhosis	*oral dryness, swallowing difficulty*
INFECTIOUS DISEASES	***Actinomycosis (Actynomyces)***	*invades the ducts of the parotid and submandibular glands, potentially leading to abscess formation*
	Human Immunodeficiency Virus (HIV)	*infiltration of $CD8^+$ cells in the salivary glands + the side effects of antiretroviral medications, such as didanosine and protease inhibitors*
	Hepatitis C Virus (HCV)	*5% to 55% of patients may experience dry mouth + HCV has also been found to be associated with Sjögren syndrome*
	Cytomegalovirus & EBV	*CMV: immunologic destruction of salivary*

		gland ductal cells leads to dry mouth + EBV is associated with many autoimmune conditions, including SS; the inflammatory effects on the exocrine glands are thought to be due to a combination of autoimmunity and EBV infection
GRANULOMATOSUS DISEASES	***Tuberculosis*** *(mycobacterium tuberculosis)*	*infiltrates salivary glands, resulting in oedema of the glands + oral dryness*
	Sarcoidosis	*parotid salivary gland enlargement, submandibular gland oedema + dry mouth*
OTHER SYSTEMIC CAUSES & GENETIC DISORDERS	***Graft-versus-host disease***	*fibrosis, lymphocytic infiltration, and destruction of the salivary gland tissue + dry mouth*
	End-stage renal disease	*oral dryness (dehydration, polyuria)*
	*E**ctodermal dysplasia, cystic fibrosis, salivary gland aplasia***	*hypoplasia/aplasia of the salivary glands; decreased salivary flow rate*
NEUROLOGICAL DISEASES	***Central nervous system trauma, stroke, cerebral- and Bell's palsy, Parkinson's-, Alzheimer's disease***	*dry mouth*

GASTROINTESTINAL DISEASES	***Crohn's disease, ulcerative colitis, coeliac disease***	*dry mouth*
AGING	***Aging***	*reduction in the amount of salivary gland tissue, acini decrease in the number, and adipose and fibrotic tissue accumulation; decrease in salivary function; a higher incidence of disease and therefore consume more medication*
	Menopause	*burning sensation, altered taste perception (dysgeusia), oral dryness, and shifts in saliva composition, buffering ability, and pH*
XEROGENIC MEDICATIONS	***Central-acting skeletal muscle relaxants, diuretics, antineoplastic, antiglaucoma, antipsychotic, antidepressant, analgesic, antihypertensive, anticholinergic, antimigraine, psychostimulants, antidementia, antiparkinsonian, antihistamine, anti-HIV, cytotoxic and sedative agents (including opioids***	*medication induced dry mouth*

	and benzodiazepines), bronchodilators, beta-blockers, retinoids, thyroid supplements, bisphosphonates	
HEAD AND NECK IRRADIATION	***Doses exceeding 60 Gy generally lead to irreversible salivary gland hypofunction and dry mouth,*** *while doses between 30 and 50 Gy may result in reversible damage*	*radiation-induced salivary hypofunction; decrease in salivary pH and buffering capacity, reduced amylase activity, and increased concentrations of lactoferrin, protein, sodium and chloride, along with changes in mucin and calcium levels; saliva becomes more viscous and ropier*
LIFESTYLE FACTORS	***Stress, anxiety, fear, tobacco use, alcohol consumption, caffeinated beverages, soft drinks, dehydration, heavy snoring; mouth breathing, malnutrition, vitamin and mineral deficiencies, eating disorders***	*oral dryness is typically reversible*

1.3.5. Treatment of oral dryness

Education of patients on the importance of consistent and appropriate oral hygiene is a cornerstone in both the prevention and management of dry mouth, often leading to excellent therapeutic outcomes. The available options for managing and treating dry mouth and salivary gland hypofunction are summarised in **Table 3.** (5, 17, 26, 32)

Table 3. Management of oral dryness

Modify behaviours	improve the temporary causes of dry mouth by avoiding/reducing intake of caffeine, alcohol, acidic, salty, hot and spicy food; avoiding mouth breathing and dehydration
Medication substitution or dosing changes	reducing the dosage or substituting the medication to a less xerogenic alternative
Chronic conditions	management strategies for long-standing symptoms, moistening oral mucosa (sucking on ice chips, adding moisture to the environment at night)
Various treatment modalities	intraoral topical agents: pastilles or chewing gums; artificial saliva/salivary substitutes, salivary stimulants: non-alcoholic mouthwashes, sprays, gels
Toothpastes	non-foaming, gel type toothpastes are less irritative
Prescription medications	sialogues (Pilocarpine-SALAGEN, Cevimeline), important to consider their contraindications and side effects

1.4. Sjögren's syndrome

SS is the most common chronic and heterogeneous autoimmune disease associated with hyposalivation and consequent dry mouth (52, 53). The disease is characterised by mononuclear cell infiltration of exocrine glands—primarily the salivary and lacrimal glands—resulting in immune-mediated damage (3, 54, 55). These infiltrates consist of macrophages, mast cells, B cells, T cells, and plasma cells. The plasma cells produce anti-Ro and anti-La autoantibodies, which target intracellular ribonucleoproteins (Ro60 and Ro52) involved in ribonucleic acid (RNA) maturation. Another key antigen is the muscarinic 3 receptor (M3R), a central element in parasympathetic regulation of the salivary and lacrimal glands. Anti-M3R antibodies may impair tear and saliva production (17).

SS predominantly affects women, with an average female-to-male ratio of 9:1. Although it can affect individuals of all ages, it typically becomes clinically apparent during the fourth or fifth decade of life (53, 55, 56). SS may affect virtually any organ system, resulting in highly diverse clinical manifestations. It has a considerable impact on quality of life, with many patients experiencing fatigue, depression, anxiety, and reduced physical performance (55).

1.4.1. Aetiology of Sjögren's Syndrome

The aetiology of SS remains unknown, though various environmental (e.g. infectious agents, hormonal changes, psychological stress) and genetic factors are believed to play a role. It is widely accepted that exposure to certain environmental triggers in genetically predisposed individuals can lead to immune system dysregulation and disease onset. A crucial factor in the early phase of SS pathogenesis is disruption of innate immune barriers, especially via activation of the interferon (IFN) pathway (52, 55). The adaptive immune system also plays a central role in SS development. Persistent B cell activation and proliferation of T helper cells (Th1 and Th17) are thought to contribute significantly to disease progression (55, 57). Genetic susceptibility includes polymorphisms in both human leukocyte antigen (HLA) and non-HLA genes (54, 56). SS is now recognised as an autoimmune epithelitis, with epithelial cells serving not only as the primary targets of autoimmune attack but also as key initiators of immune activation. Epithelial cells contribute to the pathogenesis of SS by: **(i)** expressing ribonucleoprotein complexes (Ro/SSA and La/SSB) during apoptosis, **(ii)** interacting with T cells via surface expression of costimulatory proteins such as CD86, **(iii)** producing cytokines (e.g. IL-21) that promote the development of T follicular helper cells, which regulate B cell activity; **(iv)** and secreting chemokines that recruit leukocytes (37, 55, 57). Local inflammation and the production of cytokines such as IFN-γ and TNF-α may contribute to secretory gland dysfunction by disrupting the epithelial cell tight junctions. These mechanisms likely underlie the qualitative and quantitative reduction of saliva, and the local inflammation observed in the salivary glands (55).

In genetically predisposed patients, a triggering event—often a viral infection—can initiate disruption of the salivary gland epithelium. This stimulates the IF pathway, induces the production of type I IFN, and elevates levels of B cell activating factor (BAFF). Autoantigens released from damaged cells create a pro-inflammatory

environment. Antigen-presenting cells then present both viral and self-antigens, activating autoreactive B and T cells. These, in turn, differentiate and activate autoantibody-producing plasma cells, which generate antibodies such as anti-nuclear antibodies (ANA), anti-SSA, and anti-SSB. Autoreactive T cells contribute to tissue damage by releasing cytotoxic granules that further disrupt epithelial integrity and increase exposure to autoantigens. The resulting immune complexes bind to receptors on plasmacytoid dendritic cells, boosting the production of type I IFN and perpetuating autoantibody production through continued B cell activation. This creates a self-sustaining autoimmune cycle (58, 59). In the early phase of primary SS, macrophages, dendritic cells, and CD4+ autoreactive T cells predominate. In later stages, autoreactive B cells form germinal centre-like infiltrates in the exocrine glands, especially the salivary and lacrimal glands, leading to glandular hypofunction. Similar infiltrates can be found in other organs and systems, including the lungs, respiratory and urinary tracts, central and peripheral nervous systems, and the gastrointestinal tract (56).

Recent research highlights the emerging role of the human microbiome in the aetiopathogenesis of SS (57). The pathogenesis of SS is illustrated in **Figure 2.** (58)

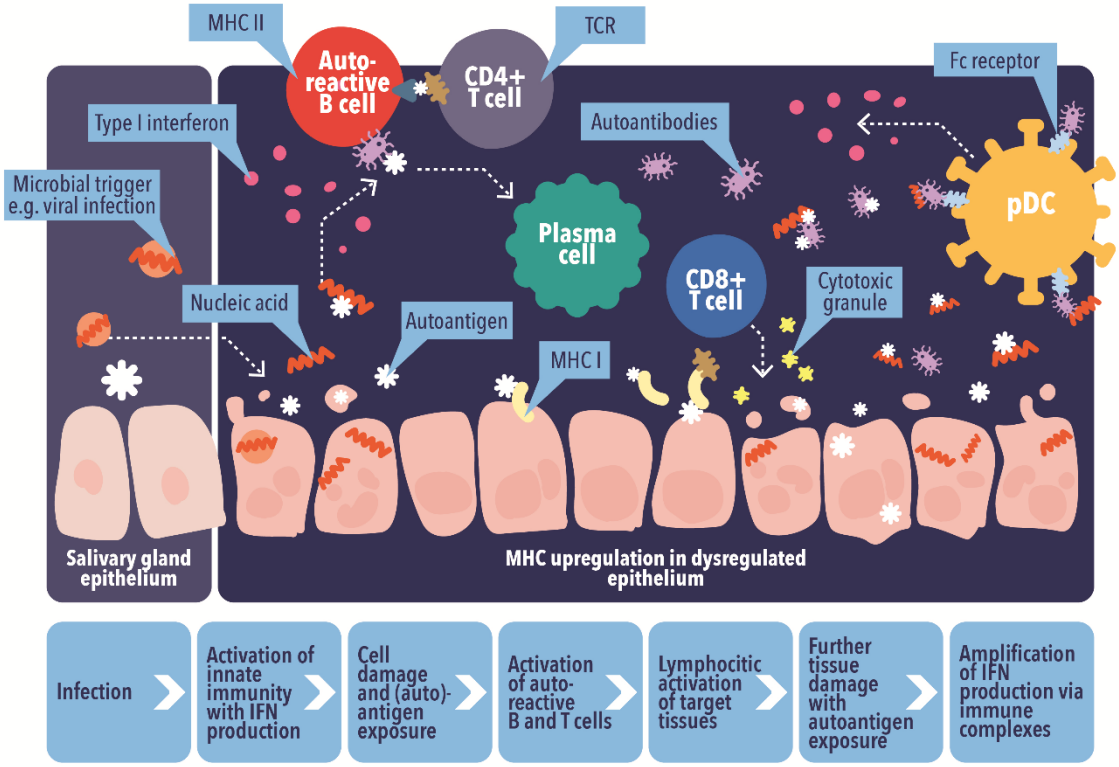

Figure 2. The pathogenesis of Sögren's Syndrome

1.4.2. Clinical manifestations of Sjögren's Syndrome

The clinical manifestations of SS can be divided into glandular manifestations and general symptoms. Ocular and/or oral dryness are the symptoms most frequently reported by SS patients. Among general symptoms, constitutional complaints are common; fatigue is reported by approximately 70–80% of patients. Other non-specific general symptoms include sleep disorders, anxiety, depression, and chronic widespread pain. Musculoskeletal involvement may present as myalgia, arthralgia, morning stiffness or arthritis. Dermatological manifestations include xerosis, erythema, dermatoses and cutaneous vasculitis. Respiratory tract involvement may be reflected in clinical abnormalities on pulmonary function tests, upper airway dryness, nasal crusting, epistaxis, rhinosinusitis, non-productive coughing, atelectasis, bronchiectasis, recurrent respiratory tract infections, tracheal dryness, bronchiolitis, interstitial lung disease, amyloidosis, bronchus-associated lymphoid tissue lymphomas, thromboembolic disease, pulmonary arterial hypertension, and pleural disease. Cardiac involvement may include an increased prevalence of valvular disease, left ventricular abnormalities, pulmonary hypertension, and pericardial effusion. Nervous system involvement often takes the form of immune-mediated sensorimotor neuropathies, typically due to mononeuritis simplex or multiplex. Renal involvement may manifest as distal renal tubular acidosis or glomerulonephritis. Gastrointestinal manifestations may include dysphagia, gastric and bowel motility disorders, subclinical pancreatic dysfunction, and abnormal liver function tests. Associated organ-specific autoimmune conditions may include autoimmune hepatitis, primary biliary cholangitis, hypothyroidism, Graves' disease, and the presence of antiphospholipid antibodies. SS is also associated with the occurrence of lymphomas and other haematological disorders (35, 55, 57).

1.4.3. Diagnosis of Sjögren's Syndrome

The diagnosis of SS is based on the 2016 American College of Rheumatology and the European League Against Rheumatism (ACR/EULAR) classification criteria. According to this system, the following must be assessed: **(i)** the presence of anti-SSA/Ro antibodies, which scores 3 points; **(ii)** focal lymphocytic sialadenitis based on minor salivary gland biopsy (focus score ≥1 focus/mm²), which also scores 3 points; **(iii)** corneal epithelial staining (OSS, ≥ 5 per eye), which is worth 1 point; **(iv)** Schirmer's test result (≤ 5 mm/5 min per eye), which scores 1 point; and (v) hyposalivation (unstimulated whole salivary

flow at rest ≤0.1 ml/min), which is also worth 1 point. If the total score is greater than 4, the diagnosis of SS can be established (33, 35, 60).

Sjögren's syndrome frequently coexists with other autoimmune conditions; in such instances, traditionally termed "secondary" SS, it accompanies underlying autoimmune connective tissue disorders like rheumatoid arthritis RA, systemic lupus erythematosus, or systemic sclerosis. However, the updated ACR/EULAR classification no longer differentiates between primary and secondary forms. It may now be more appropriate to refer to these presentations as overlapping syndromes within the spectrum of rheumatic diseases. A particularly severe complication of SS is an increased susceptibility to lymphoma, a factor that significantly contributes to mortality (61).

1.4.4. Sicca syndrome

When patients experience oral and ocular dryness in the absence of the characteristic serological or glandular inflammatory markers of autoimmune disease, the condition is described as Sicca Syndrome — a diagnosis made when SS cannot be definitively confirmed. Individuals with Sicca Syndrome often suffer from painful orofacial symptoms such as burning mouth syndrome, glossodynia, and difficulty in swallowing. These manifestations reflect both the damaging consequences of mucosal dryness and the heightened susceptibility of oral tissues to viral, bacterial, and fungal infections. Such infections may, in turn, instigate further local or systemic complications (15, 24).

1.5. Histopathological changes in minor salivary glands

Histological alterations of the minor salivary glands correspond with the major glands', and, they are aligned superficially under the mucosa, for they are suitable for sampling with less possible tissue damage (22). Histopathological findings in the minor salivary glands of patients with dry mouth reflect the wide range of underlying aetiologies. These range from morphologically normal glands—with no detectable structural lesions yet functional impairment—to various degrees of inflammatory change, including inflammatory foci that partially replace the glandular parenchyma (62).

One of the hallmarks of primary Sjögren's syndrome is the formation of mononuclear periductal infiltrates in the striated ducts. These foci, seen in both major and minor salivary glands, are primarily composed of T and B lymphocytes, along with a smaller number of macrophages and myeloid and plasmacytoid dendritic cells (57).

Given the diagnostic relevance of these foci, histopathological examination of minor salivary glands is a key step in the diagnostic process and a requirement for classification under the 2016 ACR-EULAR Classification Criteria. 4, 30 The diagnostic feature—focal lymphocytic sialadenitis (FLS)—refers to one or more lymphocytic foci within the biopsy, with surrounding tissue largely unaffected. A focus is defined as an aggregate of 50 or more lymphocytes, and the focus score (FS) refers to the number of such foci per 4 mm^2 of salivary gland tissue (33).

In a Hungarian study by Janka et al. (2023), 133 labial gland biopsies were evaluated at the Department of Pathology and Experimental Cancer Research between 2015 and 2022. Each biopsy included 2–6 lobules. Among the 66 patients with chronic oral dryness and/or xerophthalmia and clinical suspicion of SS, the minor salivary gland biopsies showed no diagnostic lymphoid foci. However, the following histopathological changes were observed: acinar loss (75.8%), fat infiltration (91%), fibrosis (97%), ductal ectasia (74.2%), non-specific chronic sialadenitis (75.8%) and normal histology (10.6%) (62).

1.6. Salivary glands and viral pathogenesis

The salivary glands are integral to the oral cavity, serving as key sites for viral entry, replication, and transmission, and acting as potential reservoirs for infectious viral particles. The host immune response to viral infection in these glands may lead to heightened inflammation, tissue damage, and glandular hypofunction (63).

Although genetic material from numerous viruses has been detected in saliva, less is known about direct viral infection of salivary gland tissues. Most viral infections in the salivary glands target acinar and ductal epithelial cells, facilitating viral shedding into saliva. Some viruses detected in saliva may originate from immune cells—such as lymphocytes—surrounding the glands (63)-

Salivary glands are immunologically active tissues, containing a variety of immune cells that play essential roles in both innate and adaptive immunity. These include B cells, T cells, macrophages, dendritic cells, and neuroendocrine cells (e.g. those expressing chromogranin A and glial cell-line neurotrophic factor receptor α3). Immunoglobulins

such as IgA—crucial in early virus neutralisation—and IgG are also found within salivary gland cells (18, 63).

While this immune activity offers protection against pathogens, abnormal or excessive immune responses can result in chronic inflammation, tissue destruction, and reduced glandular function—features common in many salivary gland diseases (63). **Figure 3** shows the viral infection sites in the different cell types of the salivary glands. (63)

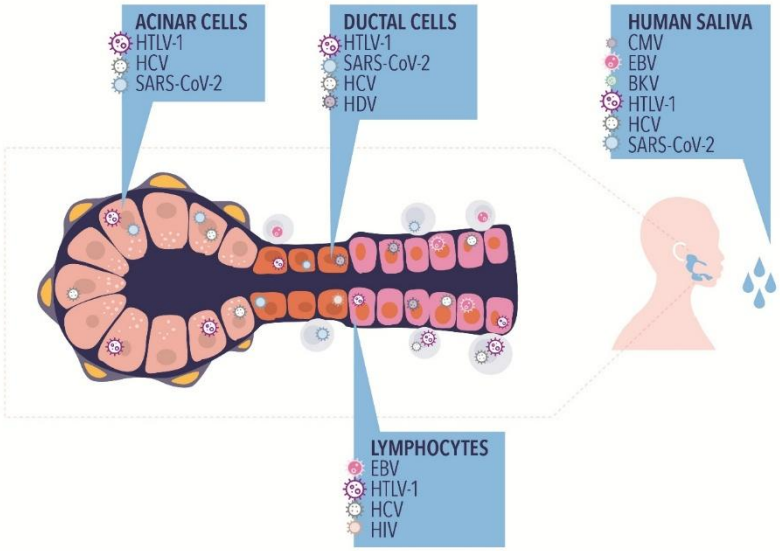

Figure 3. Viral infection sites in the salivary glands.

Most virus infections are associated with the acinar and ductal epithelial cells. Some viruses found in saliva may originate from surrounding immune cells such as lymphocytes.

In patients with chronic HCV infection, a variety of extrahepatic manifestations have been documented, including chronic lymphocytic sialadenitis and a sicca syndrome that clinically mimics primary Sjögren's syndrome (pSS). Oral dryness has been reported in 5% to 55% of individuals infected with HCV. Focal lymphocytic infiltrates resembling those seen in SS have been identified in the salivary glands of approximately half of HCV-infected patients, although these infiltrates generally result in less glandular tissue damage compared to pSS. In HCV-associated cases, the lymphocytic infiltrate within the salivary glands tends to be diffuse and predominantly comprised of CD8+ T cells. Nonetheless, some studies have noted a predominance of CD4+ T cells, although to a lesser degree than observed in pSS (64). Sialadenitis in HCV-infected patients is generally milder than

in primary Sjögren's syndrome (pSS), and these patients typically lack the SS-specific autoantibodies anti-Ro/SSA and anti-La/SSB (63). Sialadenitis observed in HCV-infected patients is generally milder than that seen in pSS, and patients typically do not present with SS-specific autoantibodies such as anti-Ro/SSA and anti-La/SSB (63). The 2016 American–European Consensus Criteria for the classification of pSS consider the presence of HCV infection to be an exclusion criterion for diagnosis (64) (33). Mechanistically, it has been suggested that HCV is directly involved in the pathogenesis of salivary gland dysfunction, although the exact mechanism driving oral dryness remains unclear. In clinical practice, the similarities between HCV-associated sialadenitis and pSS can lead to diagnostic confusion and complicate appropriate patient management (64). Perception of oral dryness in HCV-infected individuals may be influenced by viral infection of the salivary glands, which can reduce saliva production, alter its composition, cause glandular swelling, and/or result in diffuse infiltrative lymphocytic syndrome. However, the molecular mechanisms underlying dry mouth in this context are not well understood and are likely to be context dependent. HCV is capable of directly infecting salivary gland epithelial cells. Oral dryness in HCV-infected patients may result from inflammation of the glandular tissue, destruction of salivary gland epithelium, and dysfunction of the secretory and water-permeability pathways. In a study by Maldonado et al. 18 patients with active HCV infection and dry mouth were examined. Histopathological analysis of minor salivary gland biopsies revealed mild salivary gland atrophy, increased fibrosis, and diffuse lymphocytic infiltration predominantly composed of CD8+ cells (64). Salivary gland ductal epithelia play a key role in modifying salivary composition by secreting and reabsorbing ions as saliva is transported to the oral cavity. Their sialochemical analysis revealed that Na^+ concentration—normally reabsorbed by ductal cells—was significantly elevated in stimulated saliva from HCV-infected patients. Mass spectrometry also showed a reduction in mucin levels in stimulated saliva. Additionally, hepatitis D virus was detected in the mitochondria of both acinar and ductal cells (63).

SARS-CoV-2, the causative agent of coronavirus disease 2019 (COVID-19), primarily replicates in the upper respiratory tract, but viral genomic RNA is also readily detected in the saliva of both symptomatic and asymptomatic individuals. Single-cell RNA sequencing of minor salivary gland biopsies has identified SARS-CoV-2 within both

ductal and acinar cells. Moreover, postmortem biopsies of major and minor salivary glands from patients who died of COVID-19 revealed SARS-CoV-2 genomes in 75% of cases, accompanied by histopathological degeneration of acinar and ductal cells. Early studies in recovered COVID-19 patients suggest that SARS-CoV-2-induced oral manifestations—such as dry mouth and salivary gland swelling—may persist after viral clearance. Emerging evidence from post-acute COVID-19 patients also indicates symptomatic and histopathological overlap with pSS in the salivary glands (63, 65).

Human T-lymphotropic virus type 1 (HTLV-1) infection may induce an inflammatory phenotype in the salivary glands that is not autoimmune in origin (63). In a 2016 study by Lima et al., involving 272 HTLV-1-infected patients, 20% presented with sicca symptoms, yet none tested positive for SS-specific autoantibodies. Lymphocytic infiltration of the salivary glands in these patients with dry mouth suggests that salivary gland destruction is T-cell-mediated. However, the precise mechanism by which HTLV-1 causes dry mouth remains unknown, even though it was one of the most frequently reported oral manifestations in HTLV-1-infected individuals, according to Martins et al. (66).

Human immunodeficiency virus infection is linked to a spectrum of manifestations affecting the head and neck region. HIV-associated salivary gland disease (HIV-SGD) commonly presents as diffuse enlargement of the salivary glands—most notably the parotid glands—either unilaterally or bilaterally, sometimes accompanied by pain, oral dryness, or diminished salivary secretion. Multiple forms of salivary gland lesions related to HIV have been identified. These lymphoepithelial lesions arise due to lymphocytic proliferation within the glands alongside hyperplasia of adjacent lymph nodes. HIV-SGD is further associated with diffuse infiltrative lymphocytosis syndrome and the formation of lymphoepithelial cysts within the parotid glands. Histopathologically, HIV-SGD shares similarities with SS, such as lymphocytic infiltration and increased fibrosis and collagen deposition in the salivary tissues. However, it differs in that the inflammatory infiltrate is predominantly located in the periductal region, and autoantibodies typical of SS are absent. The infiltrating lymphocytes mainly comprise CD8+ T cells expressing CD29, characteristic of a memory T-cell phenotype (63, 67). In HIV-associated salivary gland disease (HIV-SGD), significant alterations in saliva composition are observed, including reduced secretory rates of sodium, calcium chloride, cystatin, lysozyme, and total

antioxidant capacity. These changes disrupt the oral cavity's homeostasis. Additionally, in HIV-positive individuals, infections with human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7) have been frequently documented (67). BK-polyomavirus has been described in relation to HIV-SGD and has been shown to infect and replicate in human salivary gland cells in vitro.

Histopathologically, HIV-SGD resembles pSS, with lymphocytic infiltration, increased fibrosis, and collagen deposition in the salivary glands. However, inflammation is typically localised to the periductal area and is not associated with SS-specific autoantibodies. The lymphocytic infiltrate predominantly comprises CD8+ T cells that are also CD29+, indicating a memory cell phenotype (63, 67). HIV-SGD is also marked by quantitative changes in saliva, including reduced secretory rates of sodium, calcium chloride, cystatin, lysozyme, and total antioxidant capacity—factors that disrupt oral cavity homeostasis. Furthermore, HHV-6 and HHV-7 have been detected in the salivary glands of HIV-positive patients (67). BK polyomavirus has also been implicated in HIV-SGD and has been shown to infect and replicate in human salivary gland cells in vitro (63).

1.7. Viral infections and autoimmune diseases

Viral infections can trigger the development of autoimmune diseases; however, the precise mechanisms by which they do so remain poorly understood. In everyday medical practice, symptoms commonly associated with autoimmune and rheumatic diseases—such as arthritis, uveitis, and erythema nodosum—frequently appear following viral infections. Viruses are known to disrupt immune tolerance and induce the production of autoantibodies (68).

There are three primary mechanisms by which infections may lead to autoimmune disease: antigen mimicry, polyclonal lymphocyte activation and increased immunogenicity of organ autoantigens secondary to infection-mediated inflammation. Of these, molecular mimicry is considered the most significant in virus-induced autoimmunity. This mechanism involves viral antigens that structurally resemble self-antigens, thereby misleading B or T cells into mounting an immune response against the body's own tissues (69). Viruses also affect exocrine tissues, primarily through plasmacytoid dendritic cells and toll-like receptors (TLRs). Viral stimulation of dendritic

cells activates the innate immune system via TLR pathways. TLRs recognise conserved microbial molecular patterns, leading to epithelial cells producing chemokines—including type I interferons (IFN-I)—and upregulating co-stimulatory adhesion molecules. Once activated, epithelial cells may act as antigen-presenting cells (68). Numerous studies suggest that viral pathogens such as HTLV-1, Parvovirus B19, Rubella virus, HIV, Herpes Simplex Virus, Hepatitis B virus, HCV, Cytomegalovirus (CMV) and Epstein-Barr virus (EBV) play a key role in the pathogenesis of autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis (MS), and SS. However, the exact viral trigger remains elusive. Both viral and bacterial infections—such as *Mycobacterium* (non-tuberculosis types) and *Helicobacter pylori*—are considered significant environmental factors due to their chronic inflammatory effects (69, 70). Among these, HCV is widely regarded as a notable exogenous factor in the aetiopathogenesis of SS. According to Fox and Fox (59), viral particles may act as autoantigens, thereby stimulating the production of autoantibodies and triggering B-cell proliferation. Infected mucosal cells can also activate cytotoxic T-cells via the MHC class I antigen presentation pathway, potentially initiating autoimmune responses (11, 59, 68).

1.8. Human papillomavirus

1.8.1. General properties of Human Papillomaviruses

Papillomaviruses exhibit strict species-specificity and broad genetic diversity (71) They are responsible for the most widespread sexually transmitted infection globally (72). The manifestations of HPV infection vary widely, ranging from asymptomatic cases to benign warts, potentially malignant lesions, intraepithelial neoplasia, and invasive carcinomas. To date, approximately 220 HPV genotypes have been identified and fully sequenced. These are classified into five genera: *Alphapapillomavirus*, *Betapapillomavirus*, *Gammapapillomavirus*, *Mupapillomavirus*, and *Nupapillomavirus* (72). Among these, the alpha genus is of the greatest clinical importance, as it contains the majority of mucosal HPV types. These include both low-risk and high-risk strains, classified by the International Agency for Research on Cancer based on their carcinogenic potential (71-73). Low-risk mucosal HPV types, such as HPV 6 and HPV 11, are associated with benign lesions such as papillomas and condylomas (71). High-risk types—including HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59—possess strong oncogenic potential and are the principal aetiological agents of cervical, vulvar, vaginal, penile, and certain head and

neck squamous cell carcinomas. Notably, HPV plays a predominant role in the development of OPSCC (74). HPV 16 is the most frequently detected genotype in HPV-associated cancers and, together with HPV 18, is responsible for approximately 60–90% of cases. (72)

Human papillomavirus possesses a circular, double-stranded deoxyribonucleic acid (DNA) genome of roughly 8,000 base pairs, which is enclosed within a non-enveloped icosahedral capsid measuring approximately 55 nm in diameter (71, 75). The viral capsid is composed of 360 molecules of the L1 (L-late) protein, organised into 72 capsomeres, each consisting of five L1 subunits. Additionally, HPV particles contain a variable number of L2 proteins. The viral genome encodes several regulatory proteins that promote cell cycle progression and proliferation, including E5, E6, and E7 (early genes), as well as proteins involved in genome replication (E1, E2), virion assembly (E2, E4), and likely virus release and transmission (E4). While many of these genes are located in the early region of the genome, the L2 protein also plays a critical role immediately after infection by facilitating viral genome delivery and assisting in correct genome packaging. (76) Notably, only one DNA strand is transcribed, which comprises 9 to 10 open reading frames that potentially encode proteins (76).

The *long control region*—also known as the upstream regulatory region or non-coding region—houses the origin of replication and binding sites for both viral and host cellular proteins. It regulates viral transcription and is situated between the L1 and E6 regions (71, 76).

Among all known oncogenic viruses, HPV-16 (in species 9) is currently considered the most potent (71)

1.8.2. HPV infection, replication and transforming effects of papillomaviruses

Human papillomaviruses are epitheliotropic viruses, which can be subdivided based on their tropism for either mucosal or cutaneous keratinocytes (75). Infection occurs when viral particles gain access to the basal epithelial cells, typically through micro-abrasions or epithelial injury (**Figure 4**). (77)

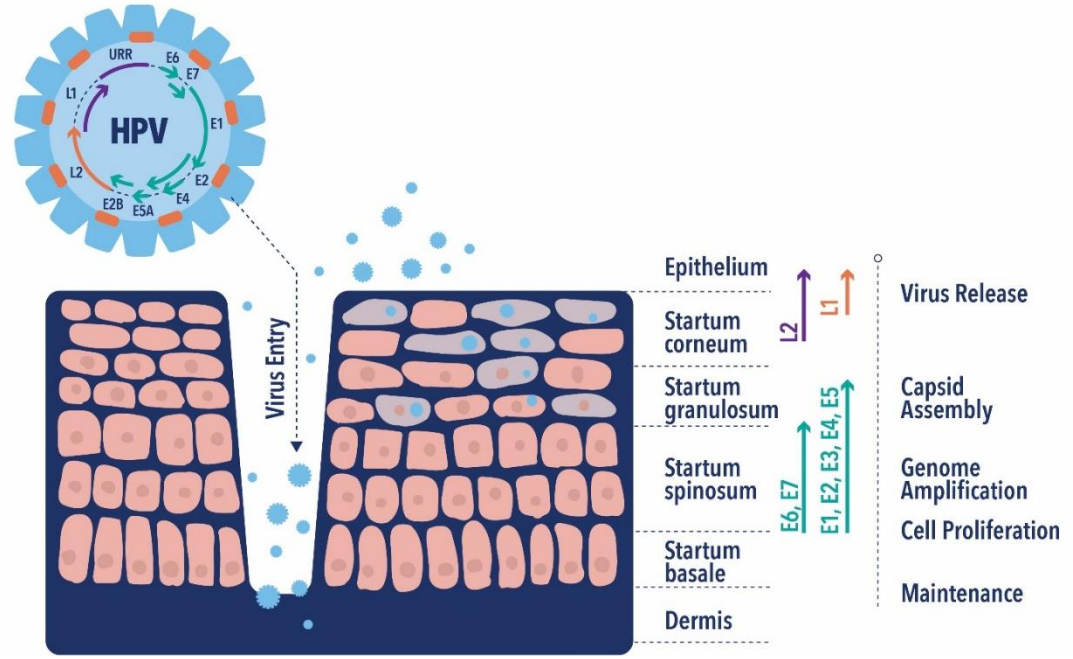

Figure 4. Mechanism of HPV infection

Minor abrasions allow HPV to reach the basal cells of the epithelium. Once inside, the virus depends on the host cell's DNA replication system, with the early viral proteins E1 and E2 facilitating this process. The viral genome replicates within infected basal and stem cells, establishing episomal HPV copies that are passed on as these cells divide. E6 and E7, both early viral proteins, help maintain cell proliferation. As infected cells undergo terminal differentiation in the upper layers of the epithelium, the expression of L1 and L2 is triggered, packaging the amplified viral genomes. The E4 protein breaks down cytokeratin filaments, enabling the release of viral particles when keratinocytes are shed from the epithelial surface.

The E6 and E7 viral oncoproteins have an essential role in the carcinogenesis and the E5 protein may also contribute to tumour formation. The E5 protein is a small, hydrophobic oncoprotein characterised by a single transmembrane domain. It interacts with receptor tyrosine kinases, including those for EGF and platelet-derived growth factor. E5 contributes to apoptosis regulation and helps the virus evade immune detection. Both E6

and E7 proteins promote cell cycle progression to support genome amplification in the stratified epithelium. The E6 proteins of high-risk HPV types exhibit oncogenic properties by binding to and promoting the degradation of host cellular signalling proteins that contain protein–protein interaction domains; additionally, they contribute to oncogenesis by activating telomerase. E7 proteins from high-risk HPV types bind and destabilise the tumour suppressor pRB, thereby promoting chromosomal instability and advancing carcinogenesis (71). HPV-infected cells maintain their proliferative ability after detachment, owing to the activity of E7. This allows differentiated cells in the upper layers to re-enter the S phase, supporting viral genome amplification and expression of late genes. These infected keratinocytes transition from the S phase to a G2-like phase, and subsequently complete terminal differentiation. L1 and L2 proteins are expressed only in fully differentiated cells, where they assemble into icosahedral capsids with the help of cellular chaperones (75, 78). During carcinogenesis, the HPV genome frequently integrates into the host's chromosomes. This integration occurs at random sites, with each instance being unique. Expression of the E6 and E7 genes is required to maintain the malignant phenotype of transformed cells. The oncogenic activity of these proteins stems from their capacity to bind and disrupt multiple cellular regulatory complexes, particularly those involving p53 and pRB. While E6 and E7 are capable of immortalising keratinocytes, such cells are not inherently tumour-forming, indicating that additional cofactors are essential for full malignant transformation in HPV-related carcinogenesis (71).

1.8.3. Oral aspects of HPV

In a comprehensive meta-analysis involving 4,500 individuals, Kreimer et al. (79) estimated the rate of subclinical oral HPV infection at 4.5%. In these asymptomatic cases, HPV DNA can be detected in the absence of cytological or histopathological changes. With a functioning immune response, about 90% of infections are naturally cleared within two years (8, 79). Potential reservoirs for HPV within the oral cavity include the inflamed gingival sulcus, ductal epithelium of the salivary glands, crypt epithelium of the tonsils, the oral–oropharyngeal junction, and the oropharynx itself. Latent infection may also persist in the basal epithelial cells, where certain stimuli, such as local inflammation or irritation, may trigger a shift from stable to vegetative viral DNA replication (71). A Japanese study by Shigeishi et al. examined the link between oral HPV type 16 (HPV16)

and periodontal inflammation in older women. Oral rinse samples from 46 participants were analysed, with HPV16 DNA detected in 4 individuals (8.7%). The study found a statistically significant association between HPV16 positivity and bleeding on probing. These findings suggest that oral HPV16 infection may be linked to periodontal inflammation in this demographic. However, additional studies are needed to further investigate the relationship between oral HPV infection and the composition and role of the oral microbiome (80). HPV has been implicated in the development of various benign and malignant lesions in the oral cavity. These conditions are summarised in **Table 4**. (8, 71, 81-83)

Table 4. Oral aspects of HPV

HPV is involved in the pathogenesis of several benign lesions, potentially malignant disorders and malignant neoplasms that develop in the oral cavity.

Clinical Infection	**Caused disesase**
Benign lesions (HPV 1, 2, 4, 6, 11, 13, 32)	*Oral (squamous cell) papillomas*
	Oral condylomas
	Verruca vulgaris
	Focal epithelial hyperplasia (FEH/Heck's disease)
Potentially malignant disorders (HPV 11, 16)	*Oral leukolpakia*
	Proliferative verrucous leukoplakia
	Oral erythroplakia
	Oral lichen planus
Malignant lesions (HPV 16, 18)	*Oral squamous cell carcinoma (OSCC)*

1.8.4. HPV and autoimmune diseases

Kim et al. hypothesised that immunosuppressed individuals are more likely to experience persistent HPV infection, a prerequisite for malignant transformation. Accordingly, women with autoimmune diseases may be more vulnerable to HPV infection and the progression of cervical disease (84). Majewski and Jablonska found that HPV could act as a superantigen, activating polyclonal T-cells and thereby triggering autoimmune phenomena such as psoriasis (85).

Chen et al. followed 47,302 patients with a first HPV diagnosis and 189,200 non-HPV controls over a 12-year period. During follow-up, the adjusted hazard ratio for pSS was significantly higher in those with HPV infection than in the controls, regardless of age and gender (86). He et al., in a two-sample bidirectional Mendelian randomisation study, identified a positive causal association between the HPV16 E7 protein and systemic lupus erythematosus (SLE), but found no such association between the HPV16 or HPV18 E7 proteins and RA (87). Yang et al. conducted a retrospective analysis of 15,677 and 8,944 subjects. Their findings suggested that HPV infection was positively associated with increased RA prevalence in adults aged 18–59, and that HPV immunisation may reduce the prevalence of RA in this age group (88). Dönmez et al. investigated the relationship between HPV and autoimmune disorders in 62 HPV DNA-positive women. Patients were divided into two groups based on autoimmune disorder status and compared in terms of single vs multiple HPV DNA types, and high-risk vs low-risk HPV types. They concluded that the prevalence of autoimmune disorders was high among HPV-positive women, but the rates of high-risk HPV and multiple infections were similar between the autoimmune-positive and autoimmune-negative groups (89).

1.9. Epstein-Barr Virus

1.9.1. General properties of EBV

The virus is a member of the Herpesviridae family, which comprises eight viruses grouped into three subfamilies: Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae. EBV belongs to the Gammaherpesvirinae, genus *Lymphocryptovirus* (90). Its circular double-stranded DNA genome is approximately 172 kilobases in length, encoding over 100 genes responsible for about 85 proteins and around 50 non-coding RNAs. The virus has an outer lipid envelope derived from the host cell, into which viral and host-derived membrane proteins are embedded. Inside the envelope is the tegument layer, which surrounds the capsid and contains the viral genome and associated proteins (91-93).

1.9.2. EBV infection, replication and transforming effects

Epstein–Barr virus (EBV) primarily infects epithelial cells and B lymphocytes. Transmission occurs via saliva, initially targeting epithelial cells. Subsequently, the virus infects B cells upon accessing the underlying tissue following its release from the

oropharyngeal epithelium (91, 92). The stages of the EBV life cycle include primary infection, establishment of latency, and reactivation; the lytic stage produces new virions and depends on the interplay between the virus and the immune system. EBV is lymphotropic and the causative agent of infectious mononucleosis. Most primary infections occur asymptomatically in infancy or early childhood. Teenagers and young adults who have not been exposed earlier are more likely to develop symptomatic infectious mononucleosis. After the primary infection, EBV establishes latency in circulating B cells, reducing viral gene expression to evade immune detection and persist with minimal impact (92). Originally discovered in cells from African Burkitt's lymphoma (BL), EBV was later found to be ubiquitous, infecting up to 95% of the global adult population and persisting for life in memory B cells (56, 92). It was the first human tumorigenic virus to be characterised (91-93).

EBV contributes to a broad spectrum of conditions, including benign diseases (e.g. infectious mononucleosis), oral conditions, immune dysfunctions, MS, systemic autoimmune diseases, and various malignancies (e.g. haematological and epithelial cancers) (90, 92, 93). During the lytic phase, periodic replication of the EBV genome occurs, producing infectious virions—a process observed in most asymptomatic carriers. The lytic cycle involves arresting the host cell cycle and promoting the S phase, which offers optimal conditions for viral replication. Lytic replication relies more heavily on EBV-encoded proteins than the latent cycle. During this phase, the viral DNA exists as a large concatemeric molecule, which is cleaved into unit-length genomes in the nucleus, packaged, and released from the cell in assembled virions (94).

During latency, the EBV genome remains in the nucleus as a closed circular plasmid, replicating in synchrony with host DNA. Infected naïve B cells are stimulated to differentiate into memory B cells. After a brief proliferation phase, the cells pass through the germinal centre and become dormant memory B cells with minimal viral expression (95). After infecting naïve B cells, EBV stimulates their differentiation into memory B cells. The latently infected cells initially undergo a phase of rapid proliferation, pass through the germinal centre, and subsequently adopt a more restricted latency programme as they mature into dormant memory B cells. In this state, the virus can persist lifelong with minimal or no gene expression (95). Resting memory B cells may later differentiate into plasma cells in response to specific stimuli, reactivating the virus into the lytic phase

and generating infectious virions. Once epithelial cells are infected, EBV replicates, lyses the host cells, and releases B-cell–tropic virions into the saliva, initiating a new replication cycle (95). Spontaneous reactivation of EBV in memory B cells can occur following impairment of the cellular immune response and may take place at any mucosal site (94).

1.9.3. Oral aspects of EBV

Epstein–Barr virus (EBV) is known to cause infectious mononucleosis and oral hairy leukoplakia and is implicated in the development of various lymphoid and epithelial malignancies. Its involvement has also been suggested in the pathogenesis of advanced periodontal disease. Notably, EBV DNA has been identified in 60–80% of lesions associated with aggressive periodontitis and in 15–20% of cases involving gingivitis or clinically healthy periodontal tissues. The presence of EBV in the periodontium is commonly linked to an increased prevalence of periodontopathic anaerobic bacterial species. Endodontic inflammation arises from a broad spectrum of infectious agents and is mediated through both cellular components—such as macrophages, lymphocytes, and leukocytes—and molecular mediators, including cytokines and chemokines. Anaerobic bacteria dominate in acute periapical abscesses and within root canals associated with asymptomatic periapical lesions. Among viral pathogens, herpesviruses appear to contribute significantly to the pathogenesis of symptomatic periapical lesions. These symptomatic and radiographically large lesions demonstrate a markedly higher prevalence of active EBV and cytomegalovirus (CMV) infections than do asymptomatic lesions of equivalent or smaller radiographic size. Although CMV is generally considered the more pathogenic herpesvirus in endodontic contexts, co-infection with EBV is frequently observed in severe periapical disease. (9) EBV encodes proteins that enhance the expression of cytokines and growth factors, which are considered central to the proliferative activity of tongue epithelial cells in oral hairy leukoplakia and to the oncogenic transformation processes associated with EBV-related malignancies, including oral lymphomas and carcinomas (9). EBV-positive oral lymphomas commonly localise within the oral cavity or Waldeyer’s ring. While EBV functions as a powerful transforming agent in B cells, lymphomagenesis likely results from a combination of viral gene expression and host genomic alterations. The principal EBV-associated B-cell lymphomas include BL, Hodgkin’s lymphoma, and diffuse large B-cell lymphoma (DLBCL), with the latter two being most frequently observed within the oral region. Oral

manifestations of EBV-linked non-Hodgkin lymphomas, particularly DLBCL and BL, may present clinically as swelling or ulceration of the gingiva, tonsils, buccal mucosa, palate, or tongue. These may be accompanied by tooth mobility due to alveolar bone loss and pain—features which are often mistaken for periapical abscesses or other forms of endodontic inflammation (10). A rarer EBV-associated subtype, natural killer/T-cell lymphoma (NK/TL), generally originates in the nasal cavity but may extend to involve the oral cavity. Despite the critical need for early diagnosis, recognition of NK/TL remains difficult (96). EBV is also frequently detected in oral carcinomas, including OSCC and in a subset of HPV-positive oropharyngeal squamous cell carcinomas (OPSCCs). However, its inconsistent association with histologically similar tumours has led to uncertainty regarding its role in OSCC pathogenesis. The majority of HPV-positive OPSCCs occur in lymphoid-rich areas such as the tonsils and the base of the tongue—regions where EBV also tends to reside. Acting as a potential co-factor, EBV has been found in approximately 5–20% of HPV-positive OPSCC cases (10). Oral aspects of EBV are summarised in **Table 5**. (9, 10, 97, 98)

Table 5. Epstein-Barr virus in oral diseases

EBV is involved in the pathogenesis of several benign lesions, lymphomas of the head- and neck region

<table>
<tr><th>Clinical infection</th><th colspan="2">Caused disease</th></tr>
<tr><td rowspan="5">EBV caused benign disorders</td><td colspan="2">Periodontal disease</td></tr>
<tr><td colspan="2">Endodontic diseases (pulpitis, apical periodontitis)</td></tr>
<tr><td colspan="2">Mononucleosis infectiosa/“kissing disease”</td></tr>
<tr><td colspan="2">Follicular lymphoid hyperplasia</td></tr>
<tr><td colspan="2">Oral hairy leukoplakia</td></tr>
<tr><td>EBV caused malignant disorders</td><td>EBV-associated oral lymphomas</td><td>- Burkitt’s lymphoma
- diffuse large B cell lymphoma
- natural killer/T-cell lymphoma</td></tr>
</table>

1.9.4. EBV and autoimmune diseases

Compelling epidemiological data have recently reinforced the association between EBV and MS, while also indicating a broader connection with various rheumatic and autoimmune conditions. These include RA, SLE, SS, autoimmune thyroiditis, autoimmune liver disease, inflammatory bowel disease, myasthenia gravis, and insulin-dependent diabetes mellitus (68, 99, 100). Compared with healthy individuals, patients affected by several autoimmune disorders typically exhibit elevated serum titres of EBV antibodies, increased circulating EBV DNA loads, and diminished cytotoxic T-cell responses specific to EBV (68).

Multiple lines of evidence point to EBV's involvement in the pathogenesis of reduced salivary flow, culminating in the development of SS. Firstly, EBV has been detected throughout the salivary glands, whose epithelial cells serve as sites for viral latency, implying that an augmented immune reaction to EBV may contribute to tissue damage. Secondly, salivary gland biopsies from individuals with SS contain significantly higher EBV DNA copy numbers than those from healthy controls, indicating a failure of immune regulation to control viral replication. Thirdly, increased expression of human leukocyte antigen (HLA)-DR in the epithelial cells of affected glands, alongside the presentation of EBV-like antigens to T cells, has also been observed. Collectively, these findings suggest that persistent EBV infection, a suboptimal virus-specific immune response, and disturbances in host immune regulation—such as antigenic mimicry, the influence of EBV microRNAs, viral interleukin-10, and alterations in epigenetic processes—are likely contributors to glandular damage in SS. (61) Further support for the EBV–SS link comes from two independent research groups, both of which conducted seroprevalence analyses comparing patients with SS to healthy controls (101, 102). Barcelos et al. additionally included patients with RA in their comparison, finding that IgG antibodies against EBV early antigen (EA) were significantly more prevalent among patients than in healthy subjects, with an overall seropositivity rate near 80%. Xuan and colleagues (2020), who performed a meta-analysis of relevant literature, similarly identified elevated positivity rates not only for EBV anti-EA IgG but also for anti-viral capsid antigen IgM among patients with SS, suggesting active viral reactivation (102).

Barcelos and his team also explored T-cell dynamics in EBV-related autoimmune diseases. Their findings indicated that CD8+ T-cell counts specific to EBV increased

during B-cell transformation and the productive replication phase in patients suffering from EBV-associated RA and SLE. (101) EBV's contribution to the aetiology of RA appears multifactorial and may involve mechanisms such as molecular mimicry at disease onset, bystander activation, or persistent infection of synovial and joint epithelial B cells. Chronic EBV infection mirrors key pathological features of RA, and serum EBV DNA levels have been shown to correlate with disease activity. Moreover, EBV has been directly identified within the synovial tissue of RA patients (91).

In RA, strong genetic associations exist with the HLA-DRB1 shared epitope (HLA-DRB1SE) and the presence of anti-citrullinated protein autoantibodies (ACPAs), which frequently emerge prior to clinical or subclinical manifestation of the disease. Among the infectious agents implicated as potential autoimmune triggers in RA, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans—both periodontal pathogens—and EBV are prominent. P. gingivalis expresses arginine gingipains, enzymes that cleave proteins at arginine residues, and also produces peptidylarginine deiminase, which converts these residues into citrulline, generating neoantigens that elicit ACPA production. In fact, peripheral blood plasmablasts from ACPA-positive RA patients predominantly generate antibodies reactive to P. gingivalis. Similarly, A. actinomycetemcomitans releases leukotoxin A, a pore-forming toxin targeting neutrophils, leading to the citrullination and extracellular release of autoantigens within gingival tissues. EBV, which establishes latent infection in B cells and epithelial cells, induces the production of autoantibodies against citrullinated peptides derived from EBV nuclear antigens. These antibodies, which can be detected years before clinical onset, cross-react with citrullinated human fibrin, highlighting citrullinated proteins as plausible arthritogenic autoantigens in RA (103).

Environmental factors known to exacerbate the risk of developing SLE include exposure to silica dust, ultraviolet radiation, tobacco use, vitamin D deficiency, and EBV infection. The underlying mechanisms likely involve molecular mimicry and bystander activation. Impaired immune surveillance of chronic EBV infection has emerged as a contributory element. Additionally, various other infections are believed to influence both the onset and exacerbation of SLE (91).

2. Objectives

The primary objective of the present study was to assess the prevalence of oral mucosal infections with human papillomavirus and Epstein-Barr virus through the collection of exfoliated cells from the full extent of the oral mucosal surface of individuals suffering from xerostomia and/or hyposalivation, and/or SS. A further aim was to compare these findings to the detection rates of healthy control subjects and, to explore potential associations between viral infections and the aforementioned clinical conditions.

Additionally, the study sought to examine correspondence between oral mucosal viral infections and the histopathological characteristics observed in minor salivary gland biopsies from the same patient cohorts.

The first null hypothesis is that HPV and EBV are not more frequent in the oral epithelial cells of patients with xerostomia and/or hyposalivation, and/or SS compared to healthy controls.

The second null hypothesis is that mucosal HPV or EBV infection causes no histopathological alterations in the salivary glands.

3. Methods

3.1. Patient selection

A total of 60 patients (54 females and 6 males; mean age 56.7 ± 15.6 years), referred with symptoms of oral dryness and/or xerophthalmia to the "Xerostomia Clinic Working Group" at the Teaching Centre of the Faculty of Dentistry, Semmelweis University (Budapest, Hungary), between January and December 2021, were included in the analysis for the purpose of confirming a diagnosis of pSS. In parallel, 32 healthy individuals (29 females and 3 males; mean age 49.7 ± 8.7 years) served as a control group.

Participants had been referred from various healthcare institutions and practices across Central Hungary and Budapest, including the National Institute of Locomotor System and Disability, the Buda Hospital of the Hospitaller Order of Saint John of God, and several departments of Semmelweis University (Ophthalmology, Internal Medicine and Haematology, and Dermatology), as well as from Medicover Eiffel Clinic, regional general dental practices, and general medical practitioners. The predominant complaints among patients included dryness of the eyes and/or mouth, joint inflammation of rheumatic origin, and arthralgia. The majority had at least one underlying systemic condition (e.g., hypertension, cardiovascular disease, diabetes mellitus) and were receiving pharmacological treatment for these comorbidities. Diagnosis of pSS was established according to the 2016 ACR-EULAR classification criteria (33). Individuals with a history of radiotherapy, chemotherapy, immunosuppressive therapy, or previous HPV vaccination were excluded from the study. Healthy control subjects were matched for age and sex, reported no systemic illnesses, were not taking any medications at the time of inclusion, and attended the Teaching Centre solely for routine dental care.

Ethical approval for the study was granted by the Regional Institutional Scientific and Research Ethics Committee of Semmelweis University (SE RKEB 132/2020). All participants provided informed written consent prior to enrolment.

3.2. Assessment of subjective orofacial sicca symptoms

Following comprehensive general and dental histories and a stomato-oncological examination, participants completed a structured questionnaire designed to elicit detailed information regarding their subjective orofacial and ophthalmological symptoms. In

accordance with ACR-EULAR diagnostic requirements, the questionnaire addressed specific indicators of dryness and discomfort.

1. Have you had daily, persistent troublesome dry eyes for more than 3 months?
2. Do you have a recurrent sensation of sand or gravel in the eyes?
3. Do you use tear substitutes more than 3 times a day?
4. Have you had a daily feeling of dry mouth for more than 3 months?
5. Do you frequently drink liquids to aid in swallowing dry foods? (33)

If the patient answered 'yes' to any of the questions, the examination continued with the assessment of the objective symptoms.

3.3. Ophthalmological examination

The tests were outlined in the Department of Ophthalmology, Semmelweis University, Budapest. The Schirmer test is a widely used standard method for evaluating aqueous tear production. During the procedure, a narrow strip of filter paper is placed in the lower eyelid, after which the patient closes their eyes. The extent of paper moistening is measured in millimetres after five minutes. A moisture level exceeding 10 mm is regarded as normal. This test variant is recommended for inclusion in the classification criteria for pSS. Although the procedure may cause brief discomfort for the patient, it is considered safe, easy to administer, and cost-effective. Van Bijsterveld's ocular staining score involves the use of rose bengal dye to identify pathological areas on the corneal and conjunctival surfaces, typically associated with mucin layer disruption. Notably, this scoring system is the only staining method explicitly referenced in the classification criteria developed by the American-European Consensus Group. However, in contemporary clinical practice, rose bengal is frequently replaced by lissamine green for staining the conjunctiva and fluorescein for the cornea, owing to their superior patient tolerability. Studies have demonstrated high inter-rater reliability in scoring by trained ophthalmologists when using the ocular staining score. The most recent classification criteria for pSS rely solely on objective ocular findings in at least one eye, including an ocular staining score of ≥5, a van Bijsterveld score of ≥4, or a Schirmer test result of ≤5 mm over five minutes. Tear breakup time (TBUT), an established parameter for evaluating tear film stability, is routinely employed in the diagnosis of dry eye disease. The test involves the instillation of 5–10 µl of fluorescein dye into the conjunctival sac,

after which the patient is instructed to gaze steadily ahead. The tear film is then examined under cobalt blue light, and the interval between the final blink and the emergence of the first dry spot is recorded as the TBUT. While tear breakup time exhibits considerable inter-individual variability in healthy subjects, a duration greater than 10 seconds is deemed normal, 5–10 seconds is considered borderline, and a time of less than 5 seconds is indicative of an abnormal result. It has been proposed that the most effective method for differentiating SS from other forms of DED is a composite approach that integrates the Ocular Surface Disease Index with TBUT and corneal fluorescein staining (104, 105).

3.4. Measurement of unstimulated whole saliva flow rate

The UWS flow rate was determined by collecting saliva over a five-minute period into pre-weighed vessels, with participants seated in an upright position. To minimise potential confounding factors, patients were instructed to refrain from eating, drinking, brushing their teeth, or smoking for at least two hours prior to the assessment. Additionally, they were advised to avoid swallowing or moving their lips and tongue, and to remain relaxed and as motionless as possible throughout the procedure. Saliva collection vessels were weighed both before and after the test using an electronic balance (Sartorius BA 110 S; Sartorius AG, Göttingen, Germany). The salivary flow rate was expressed in mL/min, a unit that approximates grams per minute due to the similar density of saliva to water. A secretion rate of ≤0.1 mL/min was indicative of salivary hypofunction (106).

3.5. Minor salivary gland biopsy

Minor salivary gland biopsies were obtained in 46 cases (Dézsi et al.). Histopathological evaluation was carried out at the Department of Pathology and Experimental Cancer Research, Faculty of Medicine, Semmelweis University. Following fixation and hematoxylin-eosin staining, tissue sections of 2–4 μm thickness were examined under various magnifications ranging from 40x to 250x (22).

3.6. Cytology sampling of exfoliated cells of oral mucosa

Screening for exfoliated HPV and EBV was conducted via brush biopsy, following the technique described by Mensch et al. For the cytological sampling, a brush specifically designed for this purpose—Cervical Rambrush Type 1 (Jiangsu Yada Technology Group Co., Ltd., Yangzhou, Jiangsu, China)—was employed. Each brush was individually packaged in a sterile, disposable format and measured 190 mm in length (107). The sequence in which the various oral sites were sampled is detailed in **Figure 5**. (107)

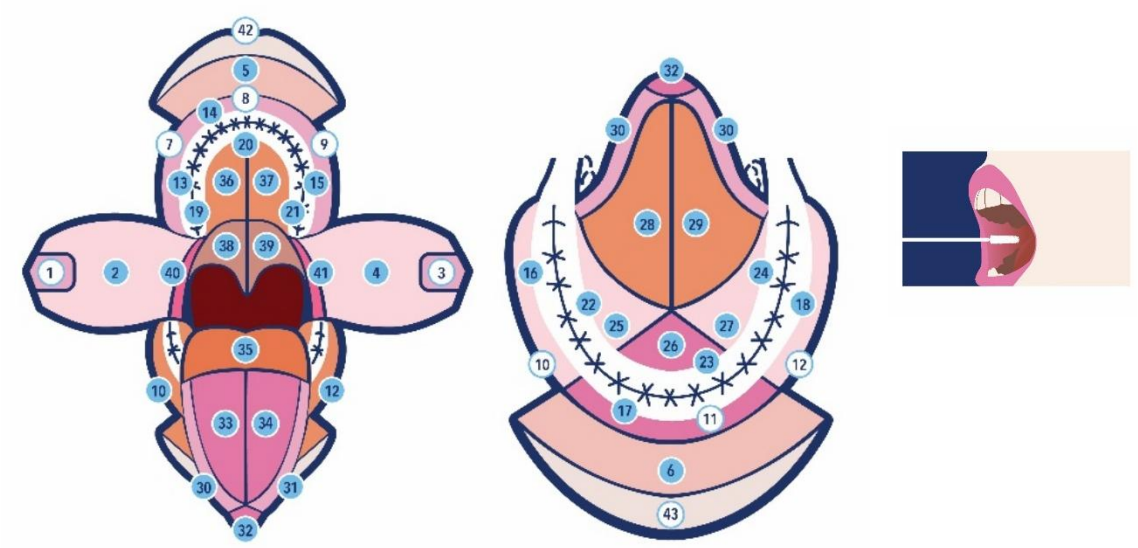

Figure 5. Order of exfoliative sampling from representative areas of the oral cavity according to Mensch et al.

3.7. HPV and EBV infection detection with polymerase chain reaction

Sample processing and detection of HPV- and EBV-specific sequences were performed at the Institute of Metagenomics, University of Debrecen, Hungary.

To detect HPV infection, exfoliated cells obtained using the cytological brush were placed in ThinPrep Pap Test vials containing PreservCyt solution (Hologic Inc., Marlborough, Massachusetts, USA). These specimens were stored at −20 °C prior to analysis. For DNA extraction, the 20 mL of sampling solution containing mucosal cells was centrifuged at 2,500 rpm for 10 minutes at room temperature. After removal of the supernatant, the resulting pellet was resuspended in 200 µL of phosphate-buffered saline. The DNA was then isolated from this 200 µL suspension using the Viral DNA/RNA Extraction Kit (BioTeke Corporation, Wuxi, China), in conjunction with the BioTeke automated magnetic isolation system, following the manufacturer's protocol.

The integrity of the extracted DNA was verified using β-globin polymerase chain reaction. Detection of HPV-specific sequences was carried out using MY09/MY11–GP5+/GP6+ consensus nested polymerase chain reaction (PCR), which targets highly conserved regions within the L1 open reading frame, specifically amplifying fragments approximately 450 base pairs in length that are characteristic of mucosal HPV types (82).

Genotyping of HPV was achieved through sequencing of PCR amplimers generated using the MY09/11 and/or GP5+/6+ primer sets. Amplification reactions were performed with Phusion High-Fidelity DNA Polymerase (Thermo Scientific, Waltham, USA). The resulting PCR products were purified using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany), and bidirectional sequencing was subsequently conducted in duplicate using the dideoxy chain termination method at Macrogen (Amsterdam, The Netherlands). Sequence data were analysed against reference genomes available in the Papillomavirus Episteme (PaVe) database (108) using CLC Main Workbench version 7.9.1 (Qiagen, Aarhus, Denmark). HPV16 positivity was further assessed through a type-specific PCR employing oligonucleotide primers targeting the E7 open reading frame, the region characteristic of the most prevalent HPV genotype (109).

Analogous to the approach used for HPV detection, the presence of Epstein–Barr virus (EBV) DNA was confirmed using a nested PCR protocol designed to amplify a 97-base pair segment within the internal repeat region of the BamH1-W fragment of the EBV genome. In the initial PCR round, the primers EBV-F (5′-GAGACCGAAGTGAAGGCCCT-3′) and EBV-R (5′-ACAGCTCCTAAGAAGGCACC-3′) were used to generate a 171-base pair amplicon. This was followed by a second round of amplification using primers EBV B-F (5′-GCCAGAGGTAAGTGGACTTT-3′) and EBV B-R (5′-GAGGGGACCCTGAGACGGGT-3′), yielding a 97-base pair product. Each reaction was carried out in a 25 µL volume containing 1× DreamTaq Green Master Mix (Thermo Scientific, Waltham, USA), 25 pmol of each primer, and 2 µL of template DNA (containing 0.1–0.3 µg). The thermal cycling conditions for both PCR rounds were as follows: initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final elongation step at 72 °C for 5 minutes. As a positive control, DNA isolated from the EBV-positive B95-8 cell line was included (110).

3.8. Statistical analysis

Data for all groups were presented as mean ± standard deviation. The Kolmogorov–Smirnov test was applied to assess the distribution of variables. Comparisons of age were conducted using a two-sample t-test, whereas the Mann–Whitney U test was employed

for analysing differences in unstimulated whole salivary flow rates. To evaluate differences in HPV and EBV infection rates among the healthy control (group 1), xerostomia (group 2), hyposalivation (group 3), and Sjögren's syndrome (group 4) cohorts, Pearson's chi-square test and Fisher's exact test were utilised as appropriate. Statistical analyses were performed using SPSS software (version 15.0, SSP Inc.). A significance threshold of $p < 0.05$ was set for all tests. Statistical power was calculated using G*Power software version 3.1.9.2 (Heinrich Heine University, Düsseldorf, Germany).

4. Results

4.1. Questionnaire results

In the study, responses to the five-item questionnaire assessing sicca symptoms—used in the ACR-EULAR classification criteria—yielded the following results across the patient groups (33).

In the xerostomia group (n=28), 92.86% of patients (n=26) answered "yes" to the question *"Have you had a daily feeling of dry mouth for more than 3 months?"*, while 7.14% (n=2) responded "no". In the hyposalivation group (n=22), 90.90% (n=20) responded affirmatively, and 9.10% (n=2) negatively. Among patients with Sjögren's syndrome (n=10), 80% (n=8) reported experiencing persistent dry mouth for more than three months, whereas 20% (n=2) did not.

Regarding the question *"Do you frequently drink liquids to aid in swallowing dry foods?"*, 71.43% of the xerostomia group responded "yes" and 28.57% "no"; in the hyposalivation group, 90.90% answered "yes" and 9.10% "no"; while in the Sjögren's syndrome group, 70% responded affirmatively and 30% negatively.

To the question *"Have you had daily, persistent troublesome dry eyes for more than 3 months?"*, 75% of xerostomia patients responded "yes" and 25% "no"; 59.10% of hyposalivation patients answered affirmatively and 40.90% negatively; while in the Sjögren's syndrome group, 90% reported experiencing persistent dry eyes, and 10% did not.

Responses to the question *"Do you have a recurrent sensation of sand or gravel in the eyes?"* were as follows: in the xerostomia group, 60.72% answered "yes" and 39.28% "no"; in the hyposalivation group, 54.54% responded affirmatively and 45.46% negatively; while among those with Sjögren's syndrome, 90% reported this symptom, and 10% did not. Figure 6 compares the affirmative answers to the questions among the individual patient groups.

In response to the question *"Do you usually use tear substitutes more than three times per day?"*, 64.28% of individuals with xerostomia, 40.90% of those with hyposalivation,

and 80% of patients diagnosed with Sjögren's syndrome reported regular use of artificial tears. **Figure 6.** shows the ratio of 'yes' answers on ACR-EULAR specific questions.

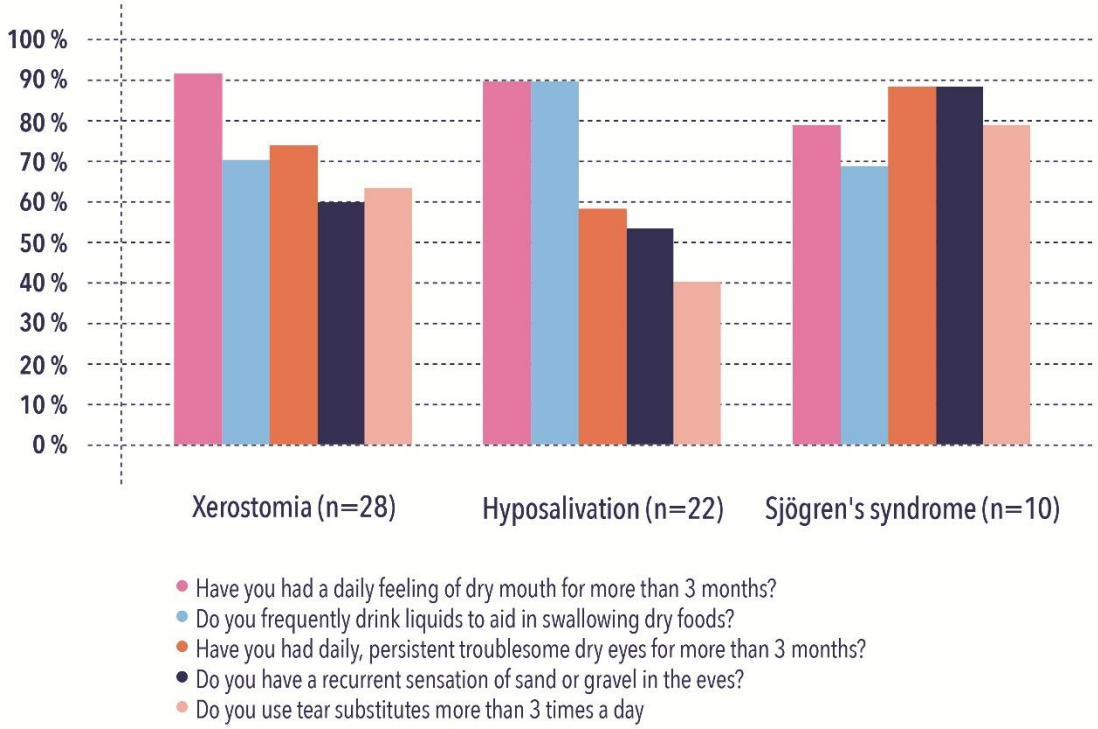

Figure 6. Ratio of 'yes' answers to the 5 questions asked based on the ACR-EULAR criteria

4.2. Sialometry results

The mean UWS flow rate among patients was 0.29 ± 0.31 ml/min, whereas the healthy control group exhibited a higher mean value of 0.46 ± 0.27 ml/min. Statistically significant differences were observed in UWS flow rates between the control group (Group 1) and the hyposalivation group (Group 3), which had a markedly lower flow rate of 0.09 ± 0.04 ml/min ($p < 0.001$). Additionally, a significant difference was found between the control group and the SS group (Group 4), which exhibited a mean UWS flow rate of 0.22 ± 0.21 ml/min ($p < 0.006$) (**Figure 7**).

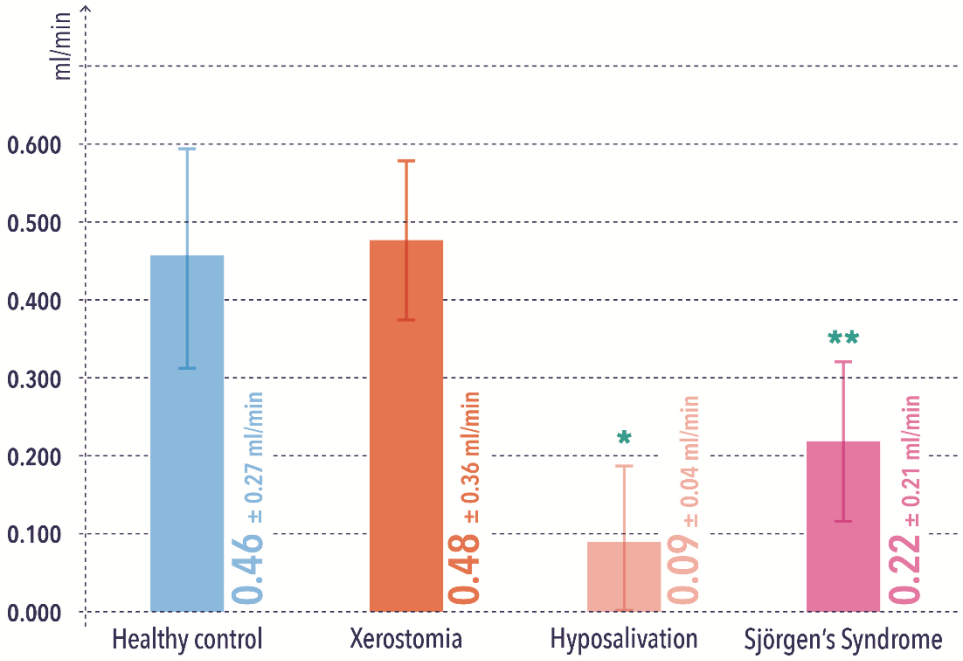

Figure 7. Unstimulated whole saliva flow rate of patients with dry mouth and/or primary Sjögren's syndrome (n = 60) compared to healthy controls (n = 32).

*A statistically significant reduction in unstimulated whole salivary flow rate was observed in both the hyposalivation group (*p < 0.001) and the Sjögren's syndrome group (**p = 0.006) (33) compared to the healthy control group. We obtained significant results with high power (87%) in the control group and the hyposalivation group. Due to the low number of cases in the Sjögren's group, the power is low (42%).*

4.3. Histopathological results of the minor salivary gland biopsy

Out of the total patient cohort, 46 individuals underwent lower labial minor salivary gland biopsy to support the diagnosis of pSS. Histopathological assessment revealed normal (intact) minor salivary gland tissue in 13 cases (28.2%). Chronic sialadenitis was identified in another 13 cases (28.2%), while stromal fibrosis and fibrous atrophy were each observed in 3 cases (6.5%). Lipomatous atrophy was reported in 4 cases (8.6%). A definitive diagnosis of SS was confirmed histologically in 10 patients (21.7%) (**Figure 8**). HPV infection was not associated with a significantly higher incidence of

histopathological abnormalities in the minor salivary glands. The distribution of histopathological findings is presented in **Figure 8**.

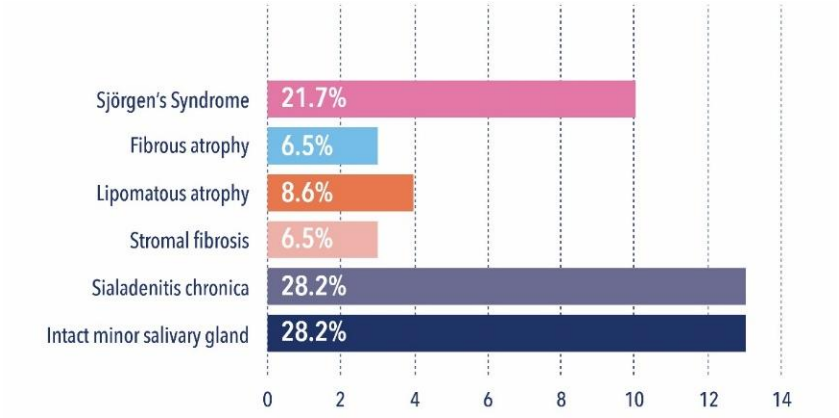

Figure 8. Histopathological diagnoses of minor salivary gland biopsy samples.

Chronic sialadenitis was the most frequently observed lesion. A positive focus score, characteristic of SS, was identified in 21% of the cases.

Typical histopathological picture of the most common findings is shown in **Figure 8**.

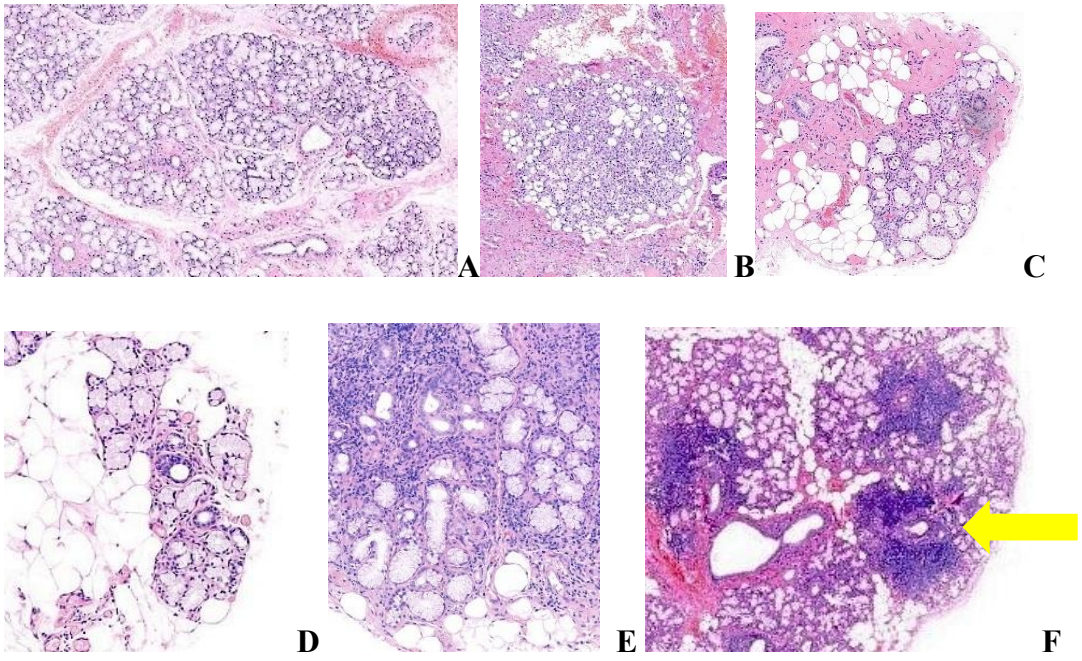

Figure 9: Histopathological picture of A: intact salivary gland, B: stromal fibrosis, C: fibrous atrophy, D: lipomatous atrophy, E: sialadenitis chronica, F: Sjögren's Syndrome.

In Sjögren's Syndrome the focus score (FS) quantifies the extent of lymphocytic infiltration by measuring the number of such foci per 4 mm² of salivary gland tissue ***(yellow arrow)*** *(The histopathological images are from Attila Zalatnai's archive, sialadenitis chronica: 100x, other images: 40x magnification.)*

4.4. The prevalence of HPV infection in the different patient groups

The presence of β-globin sequences in all DNA samples confirmed adequate DNA quality and absence of PCR inhibitors, allowing all specimens to be subjected to further virological analysis.

Among the 60 patients presenting with dry mouth symptoms, HPV-specific sequences were detected in five cases (8.3%). In the xerostomia group, one patient tested positive for HPV 16 using type-specific PCR, with general primers (GP) positivity. HPV genotyping via Sanger sequencing confirmed HPV 11 and HPV 33 in two additional cases. HPV 11 was detected in a patient within the hyposalivation group (MY/GP positivity), while HPV 33 was identified in a xerostomia patient (GP positivity). Both HPV 16 and HPV 33 are considered high-risk genotypes, whereas HPV 11 is classified as low risk.

Two further samples (3.3%) showed weak HPV positivity; however, due to a low viral copy number, genotyping by either virus-specific PCR or Sanger sequencing was unsuccessful. Notably, no HPV infection was detected among patients in the SS group. Within the healthy control cohort, a single individual (3.1%) tested positive for HPV 16. Statistical analyses revealed no significant differences in HPV positivity rates either between patient subgroups or when compared with the healthy controls, as assessed using Fisher's exact test (Table 10). Samples with inconclusive genotyping due to low viral load were classified as weakly positive but not typeable. All tested samples yielded positive results for β-globin-specific PCR, confirming sample integrity.

4.5. The prevalence of EBV infections in the different patient groups

Epstein–Barr virus (EBV) DNA was detected in the oral mucosal samples of 29 patients out of 60 (48.3%) presenting with dry mouth and/or confirmed SS. Among these, 17 were from the xerostomia group, 6 from the hyposalivation group, and 6 from the SS group. In the healthy control group, EBV-specific sequences were present in 14 of 32 individuals (43.8%). A breakdown of EBV positivity across patient groups and its relationship with

histopathological alterations (HAs) from minor salivary gland biopsies is shown in **Table 6** and **Figure 10**. Statistical analysis using Pearson's chi-square test revealed no significant differences in the prevalence of EBV infection between patient groups or in comparison with the healthy controls.

Table 6. Percentage positivity for HPV and EBV infections among healthy controls and various patient cohorts.

No statistically significant differences were observed between any patient group and the healthy control group regarding the prevalence of HPV or EBV infections in oral mucosal epithelial cell specimens.

Subjects (n=92)	**Ratio of HAs in the non-HPV and non-EBV positive cases (n=86)**	**Number and ratio of HPV positive cases (n=6)**	**HPV - genotypes (numbers of cases)**	**Number and ratio of HAs in the salivary gland samples of the HPV positive cases (n=6)**	**EBV positive cases (n=43)**	**Ratio of HAs in the salivary gland samples of the EBV positive cases (n=43)**
Controls (n=32)	-	1 (3.12%)	HPV-16+ (1)	-	14 (43.7%)	-
Xerostomia (n=28)	atrophy:7 (28%) chronic sialadenitis: 2 (8%)	3 (10.7%)	HPV-16+ (1) HPV-11 MY+, GP+ (1) HPV-33 GP+ (1)	atrophy:1 (33%) chronic sialadenitis :1 (33%)	17 (60.7%)	atrophy:3 (17.6%)

Hyposalivation (n=22)	atrophy:4 (21.0%) chronic sialadenitis :5 (26.3%)	2 (9.09%)	MY+, GP+ (2)	0	6 (28.5 %)	fibrosis:2 (33,3%) atrophy:1 (16.6%) chronic sialadenitis :1 (16.6%)
Sjögren's syndrome (n=10)	positive focus score: 10 (100%)	0	-	-	6 (54.5 %)	positive focus score: 6 (100%)

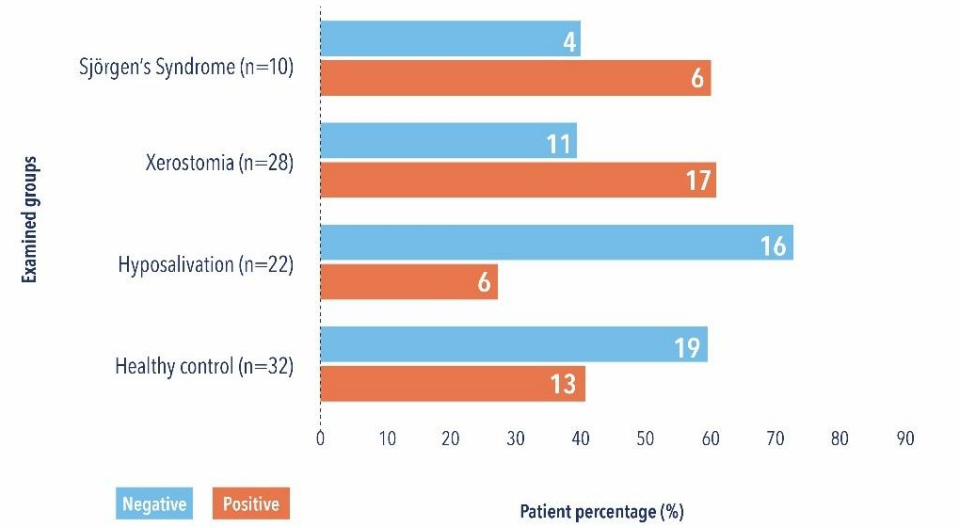

Figure 10. Prevalence of Epstein-Barr viral infections in the different patient groups with dry mouth and/or Sjögren's syndrome compared to healthy controls

Neither the positivity between the patient groups was significant, nor was there a significant difference in EBV prevalence between the patient groups and the healthy control group.

Neither xerostomia nor hyposalivation, nor the presence of SS, was associated with a significantly higher frequency of alterations in salivary gland biopsy specimens from subjects testing positive for HPV or EBV.

5. Discussion

5.1. HPV and EBV sampling methods

The present thesis investigated the prevalence of oral HPV and EBV infections in the examined patient and control cohorts. Particular attention has been given in recent years to the stomato-oncological implications of HPV infection, which stands as the most prevalent sexually transmitted infection globally. Among high-risk HPV genotypes, HPV-16 has emerged as the principal risk factor for oropharyngeal tumour development. While HPV has long been recognised as a significant risk factor for cervical neoplasms—where its prevalence is markedly higher in the genital tract than in the oral cavity—one hypothesised but insufficiently explored explanation for this discrepancy may lie in the non-specific and specific protective properties of saliva. From a stomato-oncological perspective, our research group sought to determine whether reduced saliva production might increase susceptibility to oral HPV infection.

EBV infection, another widespread viral condition, is implicated not only in infectious mononucleosis but also in the pathogenesis of non-Hodgkin lymphoma and nasopharyngeal carcinoma. For similar reasons as with HPV, we examined EBV prevalence in patients exhibiting reduced salivary flow. Given that SS is an autoimmune disorder, we also investigated whether HPV and EBV infections in SS and in individuals with hyposalivation would be associated with abnormalities in salivary gland biopsy specimens.

A total of sixty patients presenting with orofacial sicca symptoms and/or pSS were evaluated and compared with 32 age- and sex-matched healthy controls in this cross-sectional study. It is important to highlight that the study was designed to identify whether orofacial mucosal viral infections of HPV or EBV could be implicated as causative factors in dry mouth or SS.

Currently, there is no consensus gold standard for oral sample collection to detect HPV. Various sample types have been utilised: saliva, oral rinse-and-gargle, tonsillar washings, mucosal scraping or brushing, tissue biopsies, and even self-sampling kits (73, 111). The quantity and quality of material collected via these techniques vary considerably, contributing to the wide range in detection rates of asymptomatic HPV infection. This variability can be principally attributed to three factors: sampling site, sampling method, and HPV testing technique. Sampling from the keratinised mucosal surface often yields

insufficient nucleated cells for analysis. Moreover, the sensitivity and specificity of HPV testing methods, including genotype coverage and transport media, are critical considerations (71).

Some studies advocate oral rinse techniques, which sample both the oral cavity and oropharynx—an area predisposed to HPV infection—while others favour brush biopsy, which accesses deeper, particularly basal, mucosal cells. However, brush biopsies are generally performed on specific mucosal regions. Our method involved brush biopsy sampling of representative oral cavity areas, thereby combining the advantages of both approaches. Importantly, due to the predilection of HPV for oropharyngeal sites, the sequence of sampling is crucial; oropharyngeal areas such as the lingual root, soft palate, tonsils, and oropharynx should be sampled last to minimise potential loss of material.

Castañeda-Avila et al. examined 346 Hispanic adults who provided oral rinse and cytobrush self-samples for HPV detection. Compared to cytobrush samples, oral rinse identified a greater proportion of HPV-negative individuals, though self-collection may have introduced variability in sampling quality (112). In the Oromouth Cohort Study (Hillier et al.), involving 945 participants, samples from oral rinse, oropharyngeal brushings of the tongue base and posterior pharyngeal wall, tonsil tissue, and blood were collected. Among the four oral sites, oral rinse yielded the highest detection rates (113). Termine et al., in a hospital-based study of 83 adults with OSCC, compared HPV genotype prevalence in paired cytological brushings and histological biopsy samples. Biopsies were taken from non-necrotic tumour areas under local anaesthesia, while brushings sampled oral mucosal cells from the lesion. Their results indicated that HPV was detected more frequently via brushing than biopsy, though this difference was not statistically significant. In malignant lesions, biopsies were more accurate for detecting high-risk HPV than superficial brushings (73). Similarly, Della Vella et al. collected cytobrush and biopsy specimens from oral lichen planus lesions at identical sites. Their findings showed biopsy to be more reliable for identifying HPV in oral epithelium. Although brush biopsy remains the standard procedure for cytological HPV detection, providing samples predominantly from superficial epithelial layers, it may be less sensitive for detecting basal infection (114). Castillo et al. evaluated the accuracy of liquid-based brush cytology for HPV detection in oropharyngeal and oral cancer diagnosis. Their study, involving 75 patients, found that brush cytology samples were

reliable for HPV DNA detection and showed fair morphological diagnostic utility. This technique may benefit early diagnosis and follow-up management of OPSCC. Given the critical role of HPV status in OPSCC staging, brush cytology offers a non-invasive, repeatable diagnostic tool. Between 2015 and 2018, seventy-five patients, either newly diagnosed with or suspected of having squamous cell carcinoma (SCC) of the oropharynx or oral cavity, were enrolled in the study. Brush cytology was performed, and two distinct study groups were analysed based on the timing of sample collection: samples obtained during the initial endoscopic examination prior to treatment, and samples collected from the tumour site during the first post-treatment control endoscopy in patients with SCC. Following treatment, paired cytology and biopsy samples were taken from lesions with a high suspicion of carcinoma, whereas lesions deemed to have a low suspicion were evaluated by cytology alone. In all cases, cytobrush heads were rotated several times over the lesional surface to ensure adequate sampling. The study concluded that brush cytology samples are reliable for the detection of HPV DNA, and that cytological morphology provides reasonably accurate results for diagnosing OPSCC. Furthermore, patients could benefit from cytology procedures not only for early diagnosis but also as a valuable follow-up diagnostic tool. This study thus assesses the utility of liquid-based brush cytology and HPV detection both for the diagnosis of clinically suspected oropharyngeal and oral carcinomas and for identifying tumour persistence following treatment (115). Bräutigam et al. investigated the distribution of HPV subtypes across diverse anogenital and oral sites in women and examined the correlation between these infections and cervical neoplasia. Samples were simultaneously collected from five anatomical regions—cervical (cervix), vaginal (vagina), vulvar (between the major and minor labia), anal (perianal), and oral (cheek)—from 509 patients using flocked swabs by a physician. Their findings revealed that high-risk HPV positivity ranged from 60.4% to 64.3% in anogenital samples, while 14.6% of oral samples tested positive. HPV 16 demonstrated the highest prevalence across all sampled sites. HPV 31 was the second most common subtype in cervical and vaginal samples, whereas HPV 35 ranked second in vulvar, anal, and oral samples. The authors concluded that testing for multiple specific HPV subtypes is valuable in elucidating the associations between particular HPV strains and lesions or their precursors across different anatomical regions (116). Napolitano et al. investigated the genotype-specific prevalence of HPV in urine and oral samples from young Italian

adults, comparing unvaccinated and vaccinated individuals. A total of 1,002 participants completed a self-administered questionnaire and provided self-collected urine and saliva samples following detailed instructions. The prevalence of HPV in urine was significantly higher among females, older participants, those with a history of sexual intercourse, individuals reporting one or more occasional partners, inconsistent condom use, oral sex experience, and current smokers. HPV DNA was detected in 81 participants; 46 were infected with a single genotype, while 35 harboured multiple genotypes. Oral HPV infection was identified in eight participants, six of whom also tested positive for urinary HPV, with four showing concordant genotypes in both urine and oral samples. The low prevalence of genital HPV infections supports the effectiveness of current HPV vaccination strategies, while oral HPV infections were found to be relatively uncommon among these young adults (111). A retrospective Hungarian study by Mensch et al. explored the relationship between genital and oral HPV infections. The investigation was conducted in a private practice in Budapest between 2012 and 2015, screening 34 female patients and their partners, along with 14 single female patients. Following brush-biopsy sampling, HPV DNA identification and typing were performed using PCR techniques. Gynaecological screening, clinical, and cytological examinations were conducted alongside HPV typing, with vaccinated patients excluded from the study. A single practitioner performed all genital examinations, while a second practitioner conducted the oral examinations and collected HPV samples. The oral examination included clinical screening for mucosal lesions, followed by brush biopsy sampling. Genital and oral samples from partners were collected simultaneously or within a week. Analysis of the results indicated that genital HPV infection is associated with a potentially higher risk of oral HPV infection in both sexes compared to HPV-negative cases. Moreover, HPV-positive males exhibited a greater risk of concurrent genital and oral infections than partners of HPV-positive females (107).

Walling et al. evaluated a non-invasive brush biopsy technique as an alternative to surgical biopsy for the study of oral Epstein–Barr virus (EBV) infection and associated disease. Paired samples from the same site on the tongue epithelium were collected from research participants, first using the brush technique and then via surgical biopsy. Both brush cell pellets and surgical specimens were fixed for histological sectioning and/or processed for nucleic acid extraction. Histological examination of brush cell pellet sections proved

equivalent to surgical tissue sections for the diagnosis of hairy leukoplakia using routine staining, EBV immunohistochemistry, and in situ hybridization. This study was the first to directly compare surgical biopsy with a non-invasive brush biopsy technique in the context of oral EBV infection. The brush biopsy was found to be safe and well tolerated by participants, consistent with results from larger studies evaluating oral lesions. Unlike exfoliative cytology, the brush biopsy technique collected epithelial cells down to the basal layer, thus closely mimicking surgical biopsy in the depth and quality of epithelial samples. In conclusion, Walling et al. demonstrated that brush-collected epithelial cells are at least equivalent—and potentially superior—to surgical specimens for detecting oral EBV infection. The safety, tolerability, and molecular diagnostic accuracy of this method support its use in large, prospective studies on oral EBV infection and pathogenesis. The authors further recommended extending the brush biopsy technique to research on oral epithelial diseases caused by human papillomaviruses and other human herpesviruses (117).

5.2. HPV-infections in dry mouth, Sjögren's syndrome and healthy controls

The results showed that among the 92 patients, 5 (5.4%) had HPV infection of the orofacial mucosa. This prevalence did not differ significantly from the findings of a previous Hungarian study that investigated concurrent HPV infection of both the orofacial and genital mucosa (118). In that study the positivity rate was 5%.

In this study's four patient groups, the oral mucosal HPV infection rate was 3% among healthy controls, 10% in the xerostomia group, 8% in the hyposalivation group, and 0% in patients with SS. Thus, patients exhibiting subjective or objective orofacial sicca symptoms showed a slightly higher HPV infection rate in the oral mucosa compared to healthy controls and those with SS. Nevertheless, neither xerostomia, hyposalivation, nor SS was significantly associated with an increased HPV infection rate relative to healthy subjects from the central region and the capital of Hungary. To date, no studies investigating the relationship between dry mouth and HPV carriage have been reported in the literature.

One relevant study examined the association between HPV carriage and SS. Chen et al., in a 12-year follow-up study using healthcare data from Taiwan, compared subjects with HPV viral infections to HPV-free controls. The prevalence of HPV carriers in the Taiwanese population was 6.3%. All subjects were free from SS at baseline. By the end

of the follow-up, their results indicated a twofold increased risk of developing SS in the HPV-infected population by the twelfth year (86). In the present Hungarian investigation, all subjects were newly diagnosed with either sicca syndrome (xerostomia and/or hyposalivation, xerophthalmia and/or keratoconjunctivitis sicca), SS, and/or HPV and/or EBV viral infections. Consequently, the results are only partially comparable to those of other studies. It should also be noted that Chen et al. did not specify the anatomical source of viral carriage identification—whether samples were taken from the head and neck region or the genital tract.

5.3. EBV-infections in dry mouth, Sjögren's syndrome and healthy controls

A more precise understanding of EBV infection status in patients with dry mouth can be achieved through detection of viral genetic material and EBV-specific antigens. An increase in viral DNA load or the presence of EBV-specific proteins clearly indicates viral reactivation.

In a study conducted by Saito et al. (1989), EBV DNA was detected in 18% of peripheral blood mononuclear cell samples from patients with SS and in 13% of salivary gland biopsies from healthy controls. However, virus-specific sequences were found at a significantly higher rate (78%) in tissue samples from patients with SS (119).

Several studies have reported significantly higher EBV DNA positivity in salivary gland biopsy specimens from patients with SS compared to controls. Depending on the sensitivity of the detection method employed, the EBV genome has been identified in 50 to 86% of salivary gland biopsies from affected individuals. Conversely, the low EBV DNA positivity observed in the serum of patients with SS suggests a stringent systemic immune control of viral infection within the bloodstream (61). Pflugfelder et al. similarly detected a high rate of EBV positivity in lacrimal gland samples from patients with SS compared to controls (80% versus 32%) (120). As evident from the literature, most investigations have focused on blood samples and salivary gland biopsies to assess EBV infection. To our knowledge, no previous studies have analysed oral mucosal cell HPV or EBV infections as potential factors contributing to oral dryness or the development of salivary gland damage and pSS.

Our recent findings demonstrated that EBV viral DNA was detectable in oral mucosal cells in 28% to 60% of samples from patients with dry mouth and/or SS, compared to 43% in healthy controls. Overall, the EBV positivity rate in mucosal cells did not differ significantly from that of the control group across the four patient cohorts examined. This contrasts with previous studies that have established a role for EBV in pSS. It should be emphasised that, in our study, viral DNA was detected in mucosal cells rather than in salivary gland tissue samples.

The discrepancy in outcomes may be attributable to the nucleic acid extracted from oral mucosal exfoliated cells potentially under-representing the true EBV positivity within the salivary glands or serum. This limitation should be considered in interpreting our results. Further research analysing both EBV and HPV across oral mucosa, blood, and salivary gland tissues is warranted to clarify how the presence of these viruses in the oral mucosa corresponds with systemic and glandular infection. It remains possible that viral DNA may be absent from the oral mucosa while persisting in blood or salivary glands, thereby contributing to systemic autoimmune responses, as suggested by Barcelos et al. (101) and others (61, 102).

5.4. Histopathological alterations in the minor salivary glands related to mucosal HPV and/or EBV viral infections

Histopathological analysis of minor salivary gland biopsies in patients with SS most frequently revealed atrophic changes—both fibrous and lipomatous—alongside focal lymphocytic infiltration. The data obtained in this study indicated that neither oral mucosal HPV nor EBV positivity had a statistically significant impact on the number or nature of these histopathological alterations in patients with dry mouth and/or SS (see Figures 8 and Table 6).

A prior Hungarian study conducted in 2009 investigated the prevalence of Epstein–Barr virus in oral squamous cell carcinoma and in potentially malignant oral disorders within an Eastern Hungarian population. That study reported a 19.1% EBV positivity rate in exfoliated oral mucosal cells (110). In the present Central Hungarian patient cohort, EBV-specific sequences were detected at a rate 1.5 to 2 times higher than that reported in the Eastern Hungarian population, even though all samples were processed and evaluated by the same laboratory and expert team. The authors suggest that this disparity may reflect

regional differences in lifestyle: Central Hungary is more industrialised and densely populated, whereas the Eastern region remains largely agricultural with a lower population density. It is, therefore, plausible that individuals in the eastern region have reduced exposure to infection. Nonetheless, this hypothesis warrants further investigation to substantiate any regional epidemiological variation.

It also remains an open question whether dry mouth could serve as an initial factor contributing to increased susceptibility to mucosal viral infections, which might, in turn, play a role in the development of autoimmune disease. However, the present data did not demonstrate a higher prevalence of oral mucosal viral infections in any of the dry mouth groups compared to the healthy controls. Consequently, no elevation in oral mucosal viral infection rates was observed at any stage of dry mouth or SS. This study, therefore, did not support a direct role for either HPV or EBV in the aetiology of pSS within the examined Hungarian cohort. Moreover, the findings of the present investigations suggest that HPV and EBV infections of the oral mucosal cells were not likely harboured as secondary consequences of increased mucosal vulnerability due to dry mouth or SS. The lack of a clear epidemiological association, i.e. a similar prevalence of HPV and EBV in oral epithelial cells of patients with newly diagnosed xerostomia and SS does not rule out either of the two aforementioned possibilities. Salivary gland destruction leading to oral dryness is a multi-factorial process. Even more complex is the development of autoimmune diseases, including SS. Both of the two investigated viruses may potentiate several cellular and molecular mechanisms contributing to salivary gland damage and SS (121). It is important to emphasise that this investigation included the complete population of newly diagnosed dry mouth and SS cases from the Central Hungarian Region (population: 2.9 million) over the course of the one-year study period. It may take longer for patients with vulnerable oral mucous membranes and characterized by a negative oral epithelial HPV or EBV status at diagnosis to become infected. A longer follow-up time involving a larger patient cohort may be necessary to reveal potential associations between oral mucosal HPV or EBV infections and the development of dry mouth and/or SS.

5.5. Limitations

One of the main limitations of this study was the relatively small number of patients diagnosed with SS. It is important to acknowledge, however, that although SS ranks

among the most prevalent autoimmune disorders, it nonetheless qualifies as a rare disease within the Hungarian population. As such, the number of newly diagnosed cases was inherently constrained by the total number of individuals who presented to the Xerostomia Clinic Working Group during the investigation period. Moreover, the restrictions imposed by the COVID-19 pandemic posed significant challenges to in-person clinical assessments and the conduct of clinical trials, further limiting the number of cases that could be included

6. Conclusions

This is the first study to analyse infection of oral mucosal cells by HPV or EBV in patients with xerostomia, hyposalivation and/or SS. The presence of HPV and EBV viral DNA was demonstrated in a substantial proportion of patients; however, the prevalence of oral epithelial HPV and EBV infection did not differ significantly from that of control subjects with healthy oral mucosa. Therefore, the first null hypothesis, that HPV and EBV are not more frequent in the oral epithelial cells of patients with xerostomia and/or hyposalivation, and/or SS compared to healthy controls can be confirmed.

Results suggest that viral infection of oral mucosal epithelial cells with either HPV and EBV might not be essential in causing either isolated salivary gland damage or as part of SS. The second null hypothesis, that mucosal HPV or EBV infection causes no histopathological alterations in the salivary glands might also be confirmed.

It should also be emphasised, that in our study viral DNA was detected in the mucosal epithelial cells, not in salivary gland tissue samples or lymphocytes. Thus, the results do not rule out the possible contribution of either HPV or EBV infection as potentiators of salivary gland damage and of the development of autoimmune conditions, including SS. To decide this, it is necessary to outline further histopathological studies of the salivary gland biopsies. This could be pursued in a subsequent phase of the study.

Oral HPV screening on the other hand, may be conveniently incorporated into routine stomato-oncological examinations, given its non-invasive nature. Furthermore, the use of PCR—already a standard tool in laboratory diagnostics—may prove particularly effective for oral HPV detection due to the typically lower viral DNA copy number in oral samples compared to those from the genital region.

The novel findings of this investigation are as follows:

1. Reduced salivary secretion does not correspond with an increased prevalence of oral HPV infection.
2. The presence of high-risk HPV type 16—clinically significant in the context of oropharyngeal carcinogenesis—is not elevated among individuals with diminished saliva production.
3. Decreased salivary output does not lead to an increased rate of oral EBV infection.

4. Among patients with xerostomia, oral HPV infection was not associated with histopathological abnormalities in minor salivary gland biopsy specimens, suggesting that this infection does not constitute a risk factor for glandular damage or dry mouth.
5. Similarly, oral EBV infection did not coincide with histological alterations in minor salivary gland tissues in patients experiencing dry mouth.
6. Neither xerostomia nor hyposalivation, nor the presence of SS, was associated with a significantly higher frequency of alterations in salivary gland biopsy specimens from subjects testing positive for HPV or EBV. These clinical manifestations are therefore unlikely to be directly related to HPV or EBV infections within the mucosal epithelial cells, given that such histopathological changes were also commonly observed among virus-negative individuals.

7. Summary

Dry mouth is a common clinical symptom that can significantly compromise oral comfort, function, and quality of life. Despite extensive research into its aetiology, important gaps remain—particularly concerning the role of persistent viral infections. Among the potential contributors, human papillomavirus and Epstein–Barr virus have received little attention in relation to oral dryness and salivary gland dysfunction.

This doctoral thesis investigated the prevalence of HPV and EBV infections in the oral mucosa of patients with xerostomia, hyposalivation, or primary Sjögren's syndrome, compared to healthy controls. It also examined whether these viral infections correlate with histopathological alterations in the minor salivary glands of affected patients.

A total of 60 patients reporting oral and/or ocular dryness and 32 healthy control subjects were enrolled. All participants underwent comprehensive clinical evaluation, including unstimulated whole saliva (UWS) measurement, symptom questionnaires, and viral detection from exfoliated oral mucosal cells. Minor salivary gland biopsies were performed in 46 cases and examined histologically.

HPV DNA was identified in 8.3% of patients and in 3.1% of controls, with both low- and high-risk genotypes observed. EBV was more prevalent, detected in 48.3% of patients and 43.8% of controls. Although UWS flow rate was significantly lower in patients with hyposalivation and SS, no statistically significant associations were found between the presence of either virus and salivary dysfunction, histopathological abnormalities, or SS diagnosis.

In summary, although HPV could be detected in higher ratio in the xerostomia and hyposalivation groups compared to the healthy controls (10 and 8% vs 3% respectively) this virus is not a significant agent in provoking either dry mouth or SS. EBV can be frequently detected in the oral mucosal cells in every patient group (controls: 43%, xerostomia: 60%, hyposalivation: 28%, SS:54%), but its presence did not appear to contribute meaningfully to the pathogenesis of dry mouth or salivary gland damage in this study population.

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9. Bibliography of the candidate's publications

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